Towards a modular approach for heparin synthesis

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Trisaccharide **23** and sulfated disaccharide **28** have been prepared by a strategy whereby the glucuronic acid moieties were introduced at a late stage of a synthetic sequence by selective oxidation of primary hydroxy groups with TEMPO and NaOCl. During the oxidation step, the primary hydroxy groups of glucosamine moieties were efficiently protected as TBDPS ethers. The oxidation procedure and removal of the TBDPS groups proved to be compatible with the presence of sulfate esters.

Introduction

Heparins and heparan sulfates (HS) are naturally occurring polydisperse linear polysaccharides that are heavily *O*- and *N*-sulfated.[1–6] The interactions of these anionic polysaccharides with proteins play critical roles in the regulation of many physiological processes such as hemostasis, growth-factor activity, anticoagulation, cell adhesion and enzyme regulation. Currently, more than one hundred heparin-binding proteins have been identified. Some of the proteins have been extensively characterized, but the oligosaccharide structure that mediates a particular interaction has been defined in only a few cases. This problem is mainly due to the structural complexity of HS which, in turn, arises from a complex biosynthetic pathway.

The biosynthesis of heparins and HS involves the initial formation of a simple glycosylaminoglycan (GAG) composed of alternating α-D-glucuronic acid (GlcA) and β-*N*-acetyl-D-glucosamine (GlcNAc) units joined by 1→4 anomeric linkages. This structure is then modified by a series of enzymatic transformations involving C-5 epimerization of GlcA to give L-iduronic acid (IdoA), *N*-deacetylation followed by *N*-sulfation and *O*-sulfation. Ultimately, these modifications result in the formation of an IdoA(2-OSO_3)-Glc*N*SO_3(6-OSO_3) sequence. Detailed structural studies have, however, shown that heparins and HS contain eighteen other disaccharide sub-units arising from incomplete or additional enzymatic modifications. Combining the nineteen different disaccharides into larger structures results in an enormous structural diversity and, for example, 6859 (19^3) different hexasaccharide sequences can in principle be formed from the sub-structures.

It is to be expected that screening of a relatively large panel of well-defined synthetic heparin fragments will offer the most productive approach to identify oligosaccharide structures that can interact with heparin-binding proteins. Although elegant synthetic approaches for heparin synthesis have been described,[7–10] no efficient strategy for the synthesis of a wide range of HS structures has been reported.

We are developing a modular approach for HS synthesis, whereby nineteen disaccharide building blocks, resembling the naturally occurring disaccharide units, are employed for oligosaccharide synthesis. The attraction of this strategy is obvious: this set of disaccharide building blocks can repeatedly be used for the preparation of all targeted HS fragments. An important aspect of our synthetic approach will be the formation of uronic acids by selective oxidation of the C-6 hydroxy groups of glucosides and idosides after the assembly of an oligosaccharide is completed. This feature is important because uronic acids are prone to epimerization, have low glycosyl-donating properties, and complicate protecting-group manipulations.

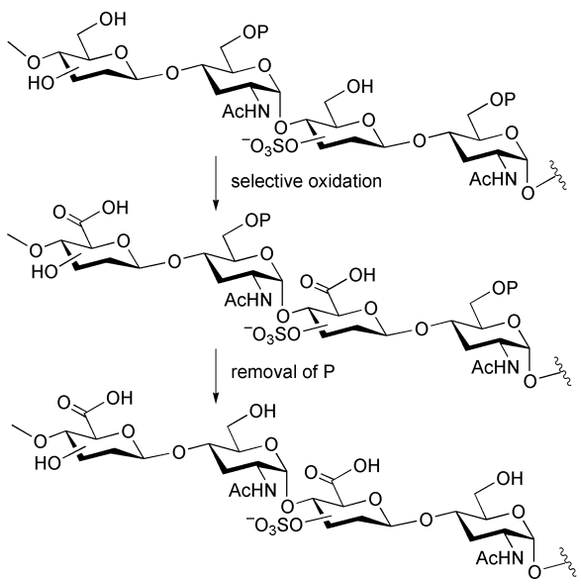

Fig. 1

Our proposed synthetic strategy is schematically illustrated in Fig. 1. A sulfated oligosaccharide will be assembled using modular building blocks. All protecting groups will be removed except the ones at C-6 of glucosamine units. Next, the C-6 hydroxy groups of the glucosides and idosides will be selectively oxidized to carboxylic acids and finally, the remaining C-6 protecting groups will be removed to give the target HS fragment. The selective oxidation will be performed with a catalytic amount of 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO) and sodium hypochlorite as co-oxidant[11] and a critical aspect will be the compatibility of this procedure with the presence of sulfate groups. Another important feature will be the selection of a permanent protecting group (P), for the C-6 positions of glucosamine residues, which is compatible with all protecting-group manipulations and the oxidation procedure but which can be removed without affecting sulfates. Here, we report the synthesis of trisaccharide **23** and disaccharide **28** to examine these two important requirements.

Results and discussion

The trisaccharide **23** and disaccharide **28** were synthesized from the readily available allyl glycosyl acceptor **5** and vinyl glycosyl donors **7** and **14** (Schemes 1 and 2). These derivatives can be used in a latent-active glycosylation strategy[12] whereby an

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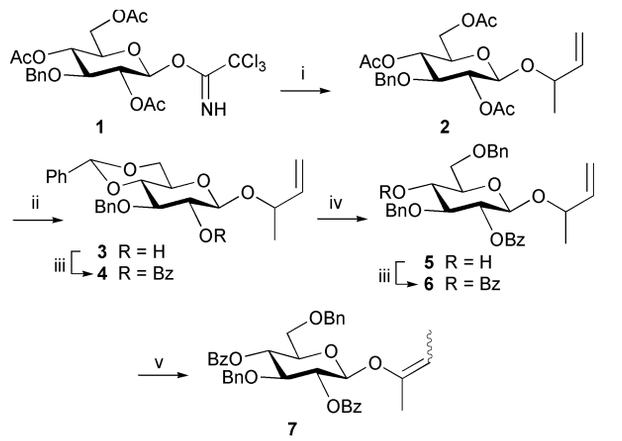
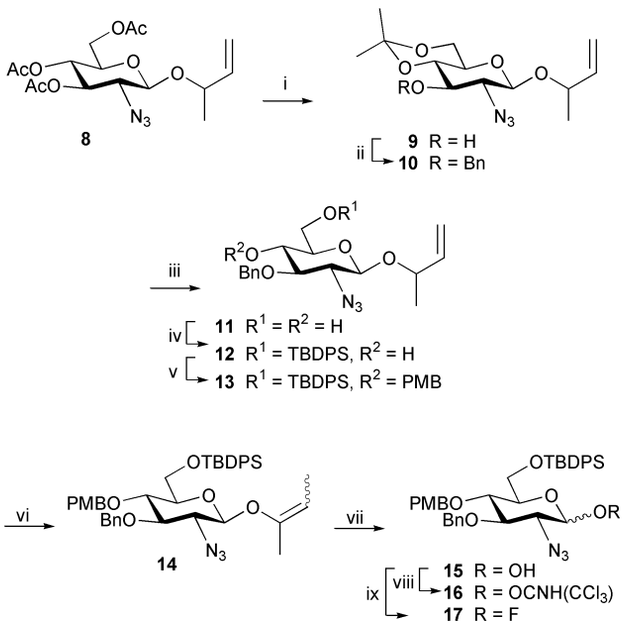


Scheme 1 *Reagents*: i) but-3-en-2-ol, TMSOTf, DCM; ii) NaOMe, MeOH; then $PhCH(OMe)_2$, CSA, acetonitrile; iii) BzCl, pyridine; iv) Et_3SiH, TFA, DCM; v) $(Ph_3P)_3RhCl$, BuLi, THF.



Scheme 2 *Reagents*: i) NaOMe, MeOH; then DMP, *p*-TsOH, acetone; ii) BnBr, NaH, DMF; iii) AcOH, water; iv) TBDPSCl, imidazole, DMF; v) PMBCl, NaH, DMF; vi) $(Ph_3P)_3RhCl$, BuLi, THF; vii) HgO, $HgBr_2$, aq. acetone; viii) CCl_3CN, DBU, DCM; ix) DAST, THF.

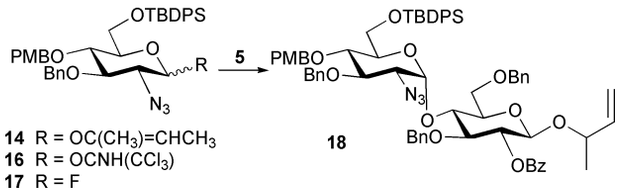
anomeric substituted allyl moiety is isomerized to a vinyl glycoside, which in turn can be employed in a Lewis acid-catalyzed glycosylation. The use of these anomeric functionalities in combination with a set of orthogonal protecting groups allows one common building block to be converted into a range of glycosyl donors and acceptors. Furthermore, a disaccharide obtained from a coupling of an active vinyl glycoside with a latent allyl glycoside can be elongated at either the reducing end by isomerization followed by glycosidation or at the non-reducing end by selective deprotection followed by glycosylation. These features make the latent-active glycosylation strategy attractive for the assembly of libraries of complex oligosaccharides. The *tert*-butyldiphenylsilyl (TBDPS) group at C-6 of glycosyl donor **14** will function as a permanent protecting group. This functionality should offer efficient protection when the glucoside moieties are oxidized to glucuronic acid units and, furthermore, its removal should not interfere with the presence of sulfate esters. The C-4 functionality of glycosyl donor **14** is protected as a *p*-methoxybenzyl (PMB) group. This functionality will be removed after disaccharide formation to give a glycosyl acceptor, which can be coupled with mono- or disaccharide glycosyl donors.

Glycosyl acceptor **5** and donor **7** were prepared as follows. Coupling of the known trichloroacetimidate **1**[13] with but-3-en-2-ol in the presence of a catalytic amount of trimethylsilyl trifluoromethanesulfonate (TMSOTf) gave the allyl glycoside **2** exclusively as the β-anomer in 93% yield. The acetyl protecting groups of **2** were cleaved under Zemplén conditions and the 4,6-*trans* diol functionality of the resulting derivative was protected as a benzylidene acetal by reaction with dimethoxytoluene in the presence of a catalytic amount of camphor-10-sulfonic acid (CSA) to give **3** in 88% overall yield. The C-2 hydroxy group of **3** was benzoylated with benzoyl chloride in pyridine to give **4**, which was converted into glycosyl acceptor **5** by ring opening of the benzylidene acetal by treatment with triethylsilane and trifluoroacetic acid (TFA) in dichloromethane (DCM).[14] This reaction proceeded with high regioselectivity and only the derivative with a benzyl ether-protecting group at C-6 was isolated. Compound **5** was the starting material for the preparation of glycosyl donor **7**. Thus, benzoylation of the C-4 hydroxy group of **5** with benzoyl chloride in pyridine, followed by isomerization of the anomeric but-3-en-2-yl moiety of the resulting compound **6** with Wilkinson's catalyst that was treated with *n*-BuLi,[12b] gave the active vinyl glycoside **7** in a good yield of 70%.

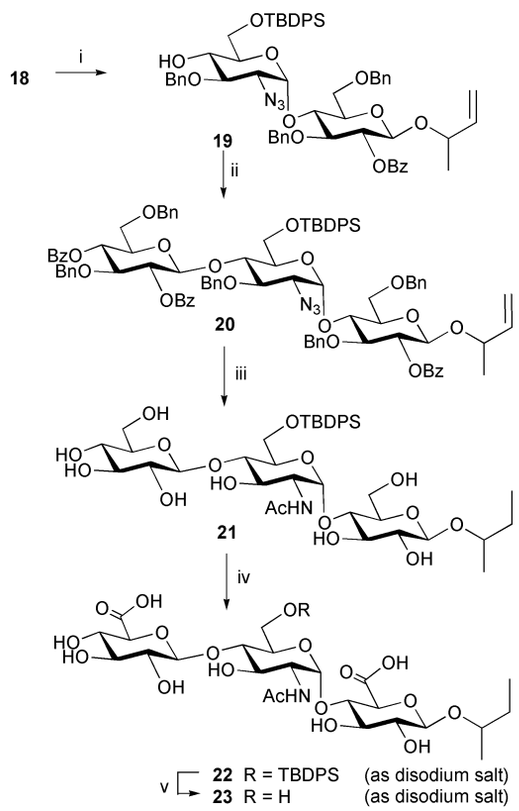
Glycosyl donor **14** was easily synthesized from known but-3-en-2-yl 2-azido-2-deoxyglucoside **8**[12e] (Scheme 2). The 4,6-diol of this compound was protected as an isopropylidene acetal by treatment with 2,2-dimethoxypropane (DMP) and a catalytic amount of *p*-TsOH in acetone to give **9**. Benzylation of the C-3 hydroxy group of **9** under standard conditions, followed by removal of the isopropylidene ketal of the resulting compound **10** with aq. acetic acid gave diol **11**. The primary hydroxy group of **11** was protected as its TBDPS ether by reaction with TBDPSCl in the presence of imidazole in *N*,*N*-dimethylformamide (DMF) to give **12** in 88% yield starting from compound **9**. Finally, treatment of **12** with *p*-methoxybenzyl chloride and NaH in DMF gave fully protected **13**, and the allyl moiety of this derivative was isomerized under standard conditions to give glycosyl donor **14**.

Having the requisite glycosyl donors and acceptor in hand, attention was focused on the preparation of trisaccharide **23**. TMSOTf-mediated coupling of glycosyl donor **14** with glycosyl acceptor **5** in diethyl ether–dichloromethane gave, after a reaction time of 2 hours, disaccharide **18** in a modest yield of 40% as a separable mixture of anomers (α : β = 3 : 1) (Scheme 3). The application of *N*-iodosuccinimide (NIS)–TMSOTf as the activator, or of toluene–1,4-dioxane as the solvent system,[15] did not improve the yield or anomeric selectivity. These disappointing results led us to explore another avenue to disaccharide **18**. An attractive feature of vinyl glycosides is that they can be hydrolyzed to lactols, which in turn can be easily converted into glycosyl donors such as anomeric fluorides, trichloroacetimidates or thioglycosides (Scheme 2). Thus, **14** was treated with HgO–$HgBr_2$ in acetone–water to give **15**, which was converted into trichloroacetimidate **16** by reaction with trichloroacetonitrile in the presence of 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU).[16] Glycosyl donor **16** was isolated as an α/β mixture in 78% overall yield. The glucosyl fluoride **17** was easily obtained by treatment of **15** with (diethylamino)-sulfur trifluoride (DAST).[17] Coupling of **5** with **16** in the presence of TMSOTf[18] gave the disaccharide **18** in an improved yield of 78% as mainly the α-anomer (α : β = 3 : 1). When the fluoride **17** was used as the glycosyl donor and $Zrcp_2Cl_2$–AgOTf as the promoter system,[19] disaccharide **18** was obtained as exclusively the α-anomer but in a modest yield of 50%.

Compound **18** is an attractive derivative for conversion into a glycosyl donor by isomerization of the allyl into a vinyl glycoside or into a glycosyl acceptor by removal of the PMB protecting group. In this case, **18** was treated with 2.5% TFA in dichloromethane[20] to give glycosyl acceptor **19** in quantitative



Scheme 3

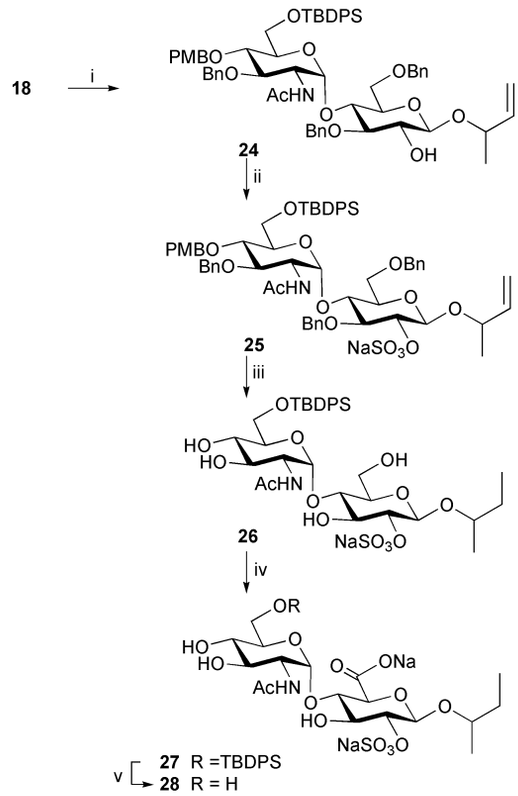


Scheme 4 *Reagents*: i) 2.5% TFA in DCM; ii) **7**, TMSOTf, MS 4 Å, DCM; iii) K_2CO_3, MeOH; then CH_3COSH, pyridine; then $Pd(OAc)_2$, H_2, EtOH; iv) TEMPO, NaOCl, NaOH, NaBr, water; v) HF·pyridine, aq. CH_3CN.

yield (Scheme 4). TMSOTf-mediated glycosylation of **19** with vinyl glycosyl donor **7** in DCM gave the trisaccharide **20** exclusively as the β-anomer in 75% yield. The anomeric selectivity of this glycosylation was due to neighboring-group participation of the C-2 benzoyl ester of **7**.

Next, the azido functionality of **20** had to be converted into an acetamido moiety and the C-6 hydroxy group of the glucosyl moieties selectively oxidized to glucoronic acid units. The latter step requires removal of all protecting groups except the primary TBDPS ether of the glucosamine moiety. Thus, the benzoate esters of **20** were cleaved by treatment with potassium carbonate in methanol, the azido group of the resulting compound was converted into an acetamido group by reaction with thioacetic acid,[21] and finally the benzyl ethers were removed by catalytic hydrogenation over $Pd(OAc)_2$ to give **21**. The primary hydroxy groups of **21** were oxidized with catalytic TEMPO and NaOCl as co-oxidant.[11] This procedure allows oxidation of primary alcohols to carboxylic acids in the presence of secondary ones. The best selectivities are, however, achieved when the reaction is performed under basic conditions (pH = 10). Compound **22** was obtained in 89% yield after purification by reversed phase column chromatography and no partially or over-oxidized products were isolated. Finally, the TBDPS group of **22** was removed by treatment with HF·pyridine to give the target trisaccharide **23**.

Next, attention was focused on the preparation of disaccharide **28** (Scheme 5). This compound was prepared to explore the compatibility of the oxidation procedure and removal of the TBDPS group with the presence of sulfate esters. Thus, reduction of the azido moiety of **18** with K_2CO_3 in methanol followed by treatment with thioacetic acid[21] to remove the benzoyl ester gave **24**. The C-2 hydroxy group of **24** was sulfated with SO_3·NEt_3 in DMF[22] to give the sulfate ester **25**, which was subjected to catalytic hydrogenation over $Pd(OAc)_2$ to give the partially deprotected derivative **26**. The primary unprotected hydroxy group of **26** was selectively oxidized with catalytic TEMPO and NaOCl,[11] and it was found that the pH of the reaction mixture had to be carefully monitored to avoid cleavage of the sulfate ester. No sulfate cleavage was, however, observed when the pH was maintained below 10. Finally, the TBDPS group of **27** was removed by treatment with HF·pyridine to give the sulfated disaccharide **28**. Other reaction conditions, such as tetrabutylammonium fluoride (TBAF) in tetrahydrofuran (THF) or HF in acetonitrile, resulted in cleavage of the sulfate ester.



Scheme 5 *Reagents*: i) K_2CO_3, MeOH; then CH_3COSH, pyridine; ii) SO_3·NEt_3, DMF; iii) $Pd(OAc)_2$, H_2, EtOH; iv) TEMPO, NaOCl, NaOH, NaBr, water; v) HF·pyridine, aq. pyridine.

Conclusions

It has been demonstrated that heparin fragments can be synthesized by a strategy whereby the glucuronic acid moieties are introduced at a late stage of a synthetic sequence by selective oxidation of primary hydroxy groups with TEMPO and NaOCl. This strategy avoids problems such as epimerization of the C-5 position, low glycosyl-donating properties of uronic acid derivatives, and problems with protecting-group manipulations. During the oxidation step, the primary hydroxy groups of glucosamine moieties are efficiently protected as TBDPS groups. It has been shown that the oxidation procedure and removal of the TBDPS groups are compatible with the presence of sulfate esters. The strategic features explored in this study will be important for the development of an efficient modular synthetic approach for the synthesis of a large number of heparin fragments.

Experimental

All reactions except where water was used as solvent were conducted under argon atmosphere. Column chromatography was performed on silica gel 60 (EM Science, 70–230 mesh), size-exclusion column chromatography was performed on Sephadex LH-20 (methanol–dichloromethane, 1 : 1, v/v) or Sephadex G-25 (water elution). Reversed phase column chromatography was performed using C_{18} silica gel from Waters Corp. with a methanol–water gradient as eluent. Reactions were monitored by TLC on Kieselgel 60 F_{254} (EM Science) and the compounds were detected by examination under UV light and charring with 5% sulfuric acid in methanol. Solvents were removed under reduced pressure at <40 °C. All organic solvents were distilled from the appropriate drying agents prior to use: acetonitrile, DCM, diethyl ether, 1,4-dioxane, DMF, pyridine and toluene were distilled from CaH_2. THF was distilled from sodium directly prior to use. Methanol was dried by refluxing with magnesium methoxide, distilled, and stored under argon. Molecular sieves were crushed and activated *in vacuo* at 390 °C for 3 hours prior to use. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Varian 300 spectrometer and a Varian 500 spectrometer equipped with Sun workstations. Assignments were made by standard gCOSY, gHSQC and gHMBC. MALDI-TOF spectra were recorded on a LD-TOF system, equipped with a HP G2025 A Series 4 5/166 computer.

(*R*/*S*)-But-3-en-2-yl 2,4,6-tri-*O*-acetyl-3-*O*-benzyl-β-D-glucopyranoside 2

To a cooled solution (−40 °C) of 2,4,6-tri-*O*-acetyl-3-*O*-benzyl-α,β-D-glucopyranosyl trichloroacetimidate[13] **1** (5.1 g, 9.4 mmol) and but-3-en-2-ol (5.7 ml, 94 mmol) in DCM (10 ml) was added dropwise TMSOTf (400 µl, 1.4 mmol). After 30 minutes, TLC analysis showed complete consumption of the starting material. The reaction was quenched by addition of solid $NaHCO_3$, the resulting suspension was filtered and the filtrate was concentrated *in vacuo*. The residue was dissolved in DCM (300 ml), and the solution was washed subsequently with saturated aq. $NaHCO_3$ (2 × 50 ml) and brine (1 × 30 ml), dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (eluent 2% acetone in DCM) to yield compound **2** (3.9 g, 92.6%) as a white solid, R_f 0.31 (3% acetone in DCM); ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.26–7.19 (m, 5H, ArH), 5.81 (ddd, 0.5H, C*H*=CH_2, $^3J_{trans}$ = 16.5 Hz, $^3J_{cis}$ = 9.9 Hz, ^3J = 6.6 Hz), 5.55 (ddd, 0.5H, C*H*=CH_2, $^3J_{trans}$ = 16.5 Hz, $^3J_{cis}$ = 9.6 Hz, ^3J = 7.5 Hz), 5.16–4.93 (m, 6H, H-2, -4, CH=CH_2, ArCH_2), 4.39 (d, 1H, H-1, $^3J_{1,2}$ = 7.5 Hz), 4.20–3.98 (m, 3H, H_2-6, OCH), 3.65–3.58 (m, 1H, H-3), 3.50–3.46 (m, 1H, H-5), 1.99, 1.93, 1.89 (3s, 9H, Ac), 1.19, 1.16 (2d, 3H, CH_3CHCH=CH_2, ^3J = 7.2 Hz, ^3J = 6.6 Hz); ^{13}C NMR (300 MHz; $CDCl_3$) δ_C 171.0, 169.7, 169.5 (CO), 140.0, 139.1 (*C*H=CH_2), 138.0 (Ar-C_q), 128.8–128.1 (Ar-CH), 117.0, 115.4 (CH=*C*H_2), 99.8, 98.5 (C-1), 80.4, 80.3 (C-3), 75.9, 73.9, 73.0, 72.9, 72.2, 72.1, 70.1, 70.0 (C-2, -4, -5, ArCH_2), 62.7, 62.6 (C-6), 21.8, 21.1, 21.0, 20.9, 20.6 (*C*CH_3, CH*C*H_3). (Calc. for $C_{23}H_{30}O_9$: C, 61.32; H, 6.71. Found: C, 61.16; H, 6.90%).

(*R*/*S*)-But-3-en-2-yl 3-*O*-benzyl- 4,6-*O*-benzylidene-β-D-glucopyranoside 3

Compound **2** (6.7 g, 15 mmol) was dissolved in methanol (50 ml) and MeONa (81 mg, 1.5 mmol) was added. After 2 h, the mixture was neutralized with Dowex H^+, filtered and concentrated *in vacuo*. The residue was dissolved in acetonitrile (40 ml) and benzaldehyde dimethyl acetal (3.6 ml, 23.4 mmol) was added. The pH was adjusted to 4 using camphor-10-sulfonic acid. After 3 h, TLC analysis showed complete conversion of the starting material. The reaction was neutralized by addition of triethylamine and the mixture was evaporated *in vacuo*. The residue was dissolved in ethyl acetate (500 ml), and the solution was washed successively with saturated aq. $NaHCO_3$ (3 × 50 ml) and brine (1 × 50 ml), dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (eluent ethyl acetate–hexanes, 2 : 5 v/v) to give compound **3** (5.43 g, 88%) as a clear oil, R_f 0.38 (2% acetone in DCM); ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.47–7.24 (m, 10H, ArH), 5.91 (ddd, 0.5H, C*H*=CH_2, $^3J_{trans}$ = 17.1 Hz, $^3J_{cis}$ = 10.8 Hz, ^3J = 6.6 Hz), 5.72 (ddd, 0.5H, C*H*=CH_2, $^3J_{trans}$ = 17.7 Hz, $^3J_{cis}$ = 9.6 Hz, ^3J = 7.5 Hz), 5.55 (s, 1H, C*H*Ph), 5.27–5.08 (m, 2H, CH=CH_2), 5.00–4.77 (m, 2H, CH_2Ph), 4.46 (d, 1H, H-1, $^3J_{1,2}$ = 7.2 Hz), 4.35–4.29 (m, 1H, OCH), 3.83–3.55 (m, 5H, H-2, -3, -4, H_2-6), 3.46–3.36 (m, 1H, H-5), 2.40–2.38 (m, 1H, 2-OH), 1.32, 1.30 (2 d, CH_3CH, ^3J = 6.3 Hz, ^3J = 6.0 Hz); ^{13}C NMR (300 MHz; $CDCl_3$) δ_C 140.0, 139.2 (*C*H=CH_2), 138.5, 137.5 (Ar-C, quart), 129.2–126.2 (Ar-CH), 116.9, 115.5 (CH=*C*H_2), 101.5 (*C*HPh), 100.4, 99.3 (C-1), 81.8, 81.6, 80.6, 78.8, 78.7, 76.2, 74.3, 73.4, 73.2, 69.0, 66.5, 66.4 (CH_3*C*H, C-2, -3, -4, -5, Ph*C*H_2, C-6), 22.0, 21.1 (*C*H_3CH). (Calc. for $C_{24}H_{28}O_6$: C, 69.88; H, 6.84. Found: C, 69.81, H, 6.66%).

(*R*/*S*)-But-3-en-2-yl 2-*O*-benzoyl-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside 4

To a cooled (0 °C) solution of compound **3** (5.33 g, 12.9 mmol) in pyridine (14 ml) was added benzoyl chloride (2.2 ml, 19.4 mmol). The mixture was stirred at room temperature for 18 h. The reaction was quenched by addition of methanol (10 ml) and the mixture was evaporated *in vacuo*. After coevaporation with toluene, the residue was dissolved in ethyl acetate (500 ml) and washed successively with saturated aq. $NaHCO_3$ (2 × 50 ml) and brine (1 × 50 ml). The organic layer was dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (eluent 0→1% acetone in DCM) to give compound **4** (5.87 g, 88%) as a white solid, R_f 0.46 (2% acetone in DCM); ^{1}H NMR (300 MHz; $CDCl_3$) δ 8.00–7.95 (m, 2H, ArH, Bz), 7.52–7.00 (m, 13H, ArH), 5.82 (ddd, 0.5H, C*H*=CH_2, $^3J_{trans}$ = 16.8 Hz, $^3J_{cis}$ = 10.8 Hz, ^3J = 6.6 Hz), 5.57 (ddd, 0.5H, C*H*=CH_2, $^3J_{trans}$ = 17.1 Hz, $^3J_{cis}$ = 10.2 Hz, ^3J = 6.9 Hz), 5.50 (s, 1H, C*H*Ph), 5.29–4.96 (m, 2H, CH=CH_2), 4.84–4.60 (m, 2H, CH_2Ph), 4.47, 4.46 (2d, 1H, H-1, $^3J_{1a,2a}$ = 7.8 Hz, $^3J_{1b,2b}$ = 8.1 Hz), 4.35–4.10 (m, 1H, OCH), 3.84–3.63 (m, 5H, H-2, -3, -4, H_2-6), 3.46–3.31 (m, 1H, H-5), 1.21, 1.17 (2d, 3H, CH_3CH, ^3J = 6.3 Hz); ^{13}C NMR (300 MHz; $CDCl_3$) δ_C 169.5, 169.4 (C=O, Bz), 140.0, 139.9 (*C*H=CH_2), 139.2, 138.6, 138.3 (Ar-C, quart), 137.7, 137.6 (Ar-CH, Bz), 133.4–126.3 (Ar-CH), 116.9, 115.5 (CH=*C*H_2), 101.5 (*C*HPh) 100.4, 99.4 (C-1), 82.0, 81.9, 81.8, 78.9, 78.8, 78.4, 77.6, 77.4, 76.2, 74.3, 74.3, 74.2, 73.4, 73.1, (CH_3*C*H, C-2, -3, -4, -5, Ph*C*H_2, C-6), 21.1, 20.6 (*C*H_3CH). (Calc. for $C_{31}H_{32}O_7$: C, 72.08; H, 6.24. Found: C, 72.11; H, 6.11%).

(*R*/*S*)-But-3-en-2-yl 2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranoside 5

To a cooled (0 °C) solution of compound **4** (0.97 g, 1.88 mmol) and triethylsilane (1.5 ml, 9.4 mmol) in DCM (10 ml) was added TFA (730 µl, 9.4 mmol) dropwise. After 1 h, the reaction mixture was allowed to warm to room temperature and stirring was continued for 1 h. The reaction mixture was diluted with ethyl acetate (150 ml), washed successively with saturated aq. $NaHCO_3$ and brine, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (eluent 0→2% acetone in DCM) to give compound **5** (0.58 g, 60%) as a colorless oil, R_f 0.25 (2% acetone in DCM); ^{1}H NMR (300 MHz; $CDCl_3$) δ 8.04 (d, 2H, ArH, Bz), 7.60–7.18 (m, 13H, ArH), 5.85 (ddd, 0.5H, C*H*=CH_2, $^3J_{trans}$ = 16.8 Hz, $^3J_{cis}$ = 10.5 Hz, ^3J = 6.6 Hz), 5.50 (ddd, 0.5H, C*H*=CH_2, $^3J_{trans}$ = 17.7 Hz, $^3J_{cis}$ = 10.2 Hz, ^3J = 7.2 Hz), 5.28–5.00 (m, 3H, H-2, CH=CH_2), 4.75–4.57 (m, 5H, H-1, PhCH_2), 4.23–4.15 (m,

1H, CH_3CH), 3.82–3.62 (m, 4H, H_2-6, H-4, -3), 3.54–3.45 (m, 1H, H-5), 2.75 (d, 1H, 4-OH), 1.21, 1.11 (2d, 3H, CH_3CH, ^3J = 6.3 Hz); ^{13}C NMR (300 MHz) δ_C 165.3 (C=O, Bz), 140.1, 139.4 ($CH{=}CH_2$), 138.3, 138.0 133.2 (Ar-CH, Bz, Ar-C, quart), 130.3–127.9 (Ar-CH), 116.8, 115.2 ($CH{=}CH_2$), 100.0, 98.7 (C-1), 82.6, 82.5 (C-3), 76.7, 75.8 (CH_3CH), 74.5, 74.3, 74.2, 74.0, 73.9, 73.7, 72.7, 72.6 (C-2, -4, -5, $PhCH_2$), 70.9, 70.8 (C-6), 21.9, 20.5 ($CHCH_3$). (Calc. for $C_{31}H_{34}O_7$: C, 71.80; H, 6.61. Found: C, 71.98; H, 6.55%).

(*R*/*S*)-But-3-en-2-yl 2,4-di-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranoside 6

To a solution of **5** (600 mg, 1.2 mmol) in pyridine (5 ml) at 0 °C was added benzoyl chloride (243.9 mg, 1.7 mmol) and the mixture was stirred overnight at room temperature. The reaction mixture was quenched by addition of methanol (5 ml) and concentrated *in vacuo*. The residue was dissolved in ethyl acetate (100 ml) and washed successively with saturated aq. $NaHCO_3$ (2 × 30 ml) and brine (1 × 20 ml). The organic layer was dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified by silica gel chromatography (eluent 2% acetone in DCM) to give **6** (809.5 mg, 76%) as a white solid, R_f 0.50 (3% acetone in DCM); ^{1}H NMR (300 MHz; $CDCl_3$) δ 8.04–7.96 (m, 4H, ArH, Bz), 7.61–6.97 (m, 16H, ArCH), 5.90 (ddd, 0.5H, $CH{=}CH_2$, $^3J_{trans}$ = 16.8 Hz, $^3J_{cis}$ = 10.8 Hz, ^3J = 6.3 Hz), 5.53 (ddd, 0.5H, $CH{=}CH_2$, $^3J_{trans}$ = 17.4 Hz, $^3J_{cis}$ = 10.2 Hz, ^3J = 7.2 Hz), 5.42–5.03 (m, 3H, H-2, $CH{=}CH_2$), 4.73–4.50 (m, 5H, H-1, $PhCH_2$), 4.35–4.21 (m, 2H, CH_3CH, H-4), 4.06–3.98 (m, 3H, H_2-6, H-3), 3.51–3.45 (m, 1H, H-5), 1.24, 1.14 (2d, 3H, CH_3CH, ^3J = 6.6 Hz); ^{13}C NMR (300 MHz; $CDCl_3$) δ_C 165.5, 165.1 (CO, Bz), 140.0, 139.3 ($CH{=}CH_2$), 138.1, 137.6, 133.5, 133.3 (Ar-CH, Bz, Ar-C, quart), 130.0–127.7 (Ar-CH), 117.0, 115.3 ($CH{=}CH_2$), 99.9, 98.6 (C-1), 80.0, 79.9 (C-3), 76.8, 75.9 (CH_3CH), 74.3, 74.2, 74.0, 73.9, 73.8, 71.8, 70.1 (C-2, -4, -5, PhH_2, C-6), 21.9, 20.5 (CH_3CH, allyl). (Calc. for $C_{38}H_{38}O_8$: C, 73.29; H, 6.15. Found: C, 73.09; H, 5.91%).

(*E*/*Z*)-But-2-en-2-yl 2,4-di-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranoside 7

Wilkinson's catalyst (247 mg, 0.27 mmol) was dissolved in THF (1 ml) and *n*-BuLi (169 µl, 1.6 M) was added. The mixture was stirred for 5 minutes and transferred into a refluxing solution of **6** (555.7 mg, 0.89 mmol) in THF (2 ml). After 2 h, the mixture was evaporated *in vacuo* and the residue was purified by flash silica gel column chromatography (eluent acetone–triethylamine–DCM 3 : 5 : 92, v/v/v) to give **7** (386.5 mg, 69.9%) as a slightly yellow-colored oil, R_f 0.51 (3% acetone in DCM); ^{1}H NMR (300 MHz; $CDCl_3$) δ 8.07–7.99 (m, 4H, ArH, Bz), 7.62–7.00 (m, 16H, ArH), 5.57–4.47 (m, 7H, H-1, -2, $CHCH_3$, $PhCH_2$), 4.35–4.21 (m, 2H, CH_3CH, H-4), 4.12–3.69 (m, 4H, H_2-6, H-3, -5), 1.88, 1.68 (2s, 3H, CCH_3) 1.54–1.44 (m, 3H, CH_3CH); ^{13}C NMR (300 MHz; $CDCl_3$) δ_C 165.5, 165.2 (CO, Bz), 152.1, 149.5 (C=CH), 138.1, 137.6, 133.6, 133.5 (Ar-CH, Bz, Ar-C, quart), 130.1–127.7 (Ar-CH), 106.9 ($CHCH_3$), 99.1, 98.9 (C-1), 79.9, 79.8 (C-3), 74.4, 74.3, 74.0, 73.9, 73.8, 73.7, 73.5, 71.7, 71.6, 70.0 (C-2, -4, -5, PhC_2, C-6), 19.2, 15.6 (CCH_3), 12.1, 10.4 (CH_3CH); MALDI-TOF MS *m*/*z* 646 $[M + Na]^+$.

(*R*/*S*)-But-3-en-2-yl 2-azido-2-deoxy-4,6-di-*O*-isopropylidene-β-D-glucopyranoside 9

(*R*/*S*)-But-3-en-2-yl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-D-glucopyranoside[12f] **8** (5.5 g, 14.3 mmol) as a solution in methanol (50 ml) was treated with NaOMe (75 mg, 1.4 mmol). After 3 h, TLC analysis showed complete conversion of the starting material into one product. The reaction mixture was neutralized using Dowex-H^+, filtered, and evaporated *in vacuo*. The residue was co-concentrated from toluene (3 × 15 ml), dissolved in a mixture of acetone (50 ml) and DMP (15 ml), and the pH was adjusted to 4 using *p*-TsOH. After stirring of the mixture at 40 °C for 5 h, TLC analysis showed complete conversion of the starting material into one product. The mixture was neutralized by addition of triethylamine and was then evaporated *in vacuo*. The residue was dissolved in DCM, and the solution was washed successively with saturated aq. $NaHCO_3$ and brine, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (eluent 20% ethyl acetate in hexanes) to give compound **9** (3.6 g, 85%) as a clear oil, R_f 0.46 (20% ethyl acetate in hexanes); ^{1}H NMR (300 MHz; $CDCl_3$) δ 5.89 (ddd, 0.5H, $CH{=}CH_2$, $^3J_{trans}$ = 17.1 Hz, $^3J_{cis}$ = 10.5 Hz, ^3J = 6.6 Hz), 5.79 (ddd, 0.5H, $CH{=}CH_2$, $^3J_{trans}$ = 17.7 Hz, $^3J_{cis}$ = 10.2 Hz, ^3J = 8.1 Hz), 5.26–5.09 (m, 2H, $CH{=}CH_2$), 4.45 (d, 1H, H-1, $^3J_{trans}$ = 8.1 Hz), 4.35–4.26 (m, 1H, CH_3CH), 3.93–3.84, 3.81–3.73 (2 m, 2H, H_2-6), 3.59–3.43 (m, 2H, H-4, -3), 3.39–3.32 (m, 1H, H-2), 3.22–3.14 (m, 1H, H-5), 2.98 (br s, 1H, 3-OH), 1.50, 1.41 (2 s, 6H, CMe_2), 1.33, 1.31 (2 d, 3H, CH_3CH, ^3J = 6.3 Hz); ^{13}C NMR (300 MHz; $CDCl_3$) δ_C 140.0, 138.8 ($CH{=}CH_2$), 118.2, 115.8 ($CH{=}CH_2$), 101.3, 99.6 (C-1), 100.3 [$(CH_3)_2C_q$], 78.0, 76.5 (CH_3CH), 74.0, 73.9 (C-4), 72.6 (C-3), 67.5, 67.4 (C-5), 67.2, 66.9 (C-2), 62.2 (C-6), 29.3, 19.4 (CMe_2), 22.0, 20.6 (CH_3CH). (Calc. for $C_{13}H_{21}N_3O_5$: C, 52.16; H, 7.07; N, 14.04. Found: C, 51.98; H, 7.20; N, 14.12%).

(*R*/*S*)-But-3-en-2-yl 2-azido-3-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-2-azido-β-D-glucopyranoside 12

To a solution of compound **9** (2.58 g, 7.4 mmol) in DMF (20 ml) was added NaH (460 mg, 11.1 mmol; 60% NaH). The mixture was cooled to 0 °C, benzyl bromide (1.3 ml, 11.1 mmol) was added dropwise, and the mixture was stirred at room temperature for 2 h. The reaction was quenched by addition of methanol (10 ml) and the mixture was evaporated *in vacuo*. The residue was dissolved in aq. acetic acid (80%, 50 ml) and the solution was heated to 50 °C for 8 h. The solvents were evaporated off *in vacuo* and the residue was co-concentrated from toluene (3 × 50 ml). The crude product and imidazole (1.1 g, 16.5 mmol) were dissolved in DMF (5 ml) and *tert*-butylchlorodiphenylsilane (TBDPSCl) (2.4 ml, 9.3 mmol) was added dropwise. The mixture was stirred for 18 h at room temperature and then poured into ice–water (500 ml) and extracted with DCM (3 × 100 ml). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (eluent 20% ethyl acetate in hexanes) to give compound **12** (3.8 g, 88%) as a clear oil, R_f 0.30 (20% ethyl acetate in hexanes); ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.72–7.66 (m, 5H, Ph, Bn) 7.43–7.30 (m, 10H, $SiPh_2$), 5.91 (ddd, 0.5H, $CH{=}CH_2$, $^3J_{trans}$ = 17.1 Hz, $^3J_{cis}$ = 10.8 Hz, ^3J = 6.6 Hz), 5.75 (ddd, 0.5 H, $CH{=}CH_2$, $^3J_{trans}$ = 17.7 Hz, $^3J_{cis}$ = 9.9 Hz, ^3J = 8.1 Hz), 5.28–5.03 (m, 2H, $CH{=}CH_2$), 4.94–4.75 (m, 2H, $PhCH_2$,), 4.38–4.26 (m, 2H, H-1, CH_3CH, $^3J_{1,2}$ = 8.1 Hz), 3.93–3.84 (m, 2H, H_2-6), 3.70–3.64 (m, 1H, H-4), 3.43–3.21 (m, 3H, H-2, -5, -3), 2.64 (d, 1H, 4-OH), 1.32 (d, 3H, CH_3CH, ^3J = 6.3 Hz), 1.07, 1.06, 1.05 [3s, 9H, $C(CH_3)_3$]; ^{13}C NMR (300 MHz; $CDCl_3$) δ_C 140.2, 139.0 ($CH{=}CH_2$), 138.5–127.9 (Ar-C_q, Ar-CH) 117.8, 115.4 ($CH{=}CH_2$), 100.6, 99.0 (C-1), 83.1, 83.0 (C-3), 76.9, 75.7, 75.4, 75.3, 75.2, 75.1, 72.1, 66.1, 65.9, 64.7, 64.6 ($PhCH_2$, CH_3CH, C-2, -4, -5, -6), 27.1, 26.9 (CMe_3, *t*-butyl), 21.9, 20.4 (CH_3CH), 19.5, 19.3 (CMe). (Calc. for $C_{33}H_{41}N_3O_5Si$: C, 67.43; H, 7.03; N, 7.15. Found: C, 67.46; H, 7.14; N, 7.03%).

(*R*/*S*)-But-3-en-2-yl 2-azido-3-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-4-*O*-*p*-methoxybenzyl-β-D-glucopyranoside 13

Compound **12** (3.9 g, 6.6 mmol) was dissolved in DMF (5 ml). Under stirring, NaH (405 mg, 9.9 mmol; 60% NaH in mineral oil) was added over a period of 5 minutes. The mixture was cooled (0 °C), *p*-methoxybenzyl chloride (1.4 ml, 9.9 mmol) was added dropwise, and the mixture was stirred at room temperature for 5 h before being poured into ice–water (600 ml), and the aqueous layer was extracted with ethyl acetate (3 × 100 ml).

The combined organic layers were successively washed with saturated aq. $NaHCO_3$ (2 × 20 ml) and brine (1 × 20 ml), dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (eluent 0→5% ethyl acetate in hexanes) to give compound **13** (3.2 g, 70.2%) as a clear oil, R_f 0.40 (20% ethyl acetate in hexanes); ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.79–7.71 (m, 5H, ArH, benzyl), 7.47–7.33 (m, 10H, Ph_2Si), 7.14–7.08 (m, 2H, *o*-ArH, PMB, $^3J_{H,H}$ = 8.4 Hz), 6.84–6.80 (m, 2H, *m*-ArH, PMB), 6.04 (ddd, 0.5H, C*H*=CH_2, $^3J_{trans}$ = 17.1 Hz, $^3J_{cis}$ = 10.8 Hz, ^3J = 6.6 Hz), 5.80 (ddd, 0.5 H, C*H*=CH_2, $^3J_{trans}$ = 17.7 Hz, $^3J_{cis}$ = 9.9 Hz, ^3J = 8.1 Hz), 5.31–5.21 (m, 2H, CH=CH_2), 5.14–4.80 (m, 4H, ArCH_2,), 4.45–4.33 (m, 2H, H-1, CH_3C*H*), 3.95–3.72 (m, 6H, H_2-6, H-4, OCH_3), 3.57–3.31 (3H, H-2, -5, -3), 1.33 (d, 3H, CH_3CH, ^3J = 6.3 Hz), 1.06 (s, 9H, C(Me_3); ^{13}C NMR (300 MHz; $CDCl_3$) δ_C 159.8 (Ar-*C*, PMB), 140.4, 139.2 (*C*H=CH_2), 136.3, 135.9, 135.2 (Ar-C_q) 130.1–127.9 (Ar-CH), 117.8, 115.2 (CH=*C*H_2), 100.9, 99.2 (C-1), 83.8, 83.6 (C-3), 79.9, 79.0, 78.1, 77.8, 77.4, 75.8, 75.6, 75.4, 72.1, 71.0, 67.0, 66.8 (CH_3*C*H, C-2, -4, -5), 76.1, 76.0, 75.1, 70.2, 66.5, 63.2, 63.1 (Ar*C*H_2, C-6) 55.6 (*C*H_3, OMe), 27.8, 26.9 (C(Me_3), 22.1, 21.9 (*C*H_3CH), 19.5 (*C*Me_3). Calc. for $C_{41}H_{49}N_3O_6Si$: C, 69.56; H, 6.98; N, 5.94. Found: C, 69.37; H, 7.11; N, 6.25%).

(*E*/*Z*)-But-2-en-2-yl 2-azido-3-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-4-*O*-*p*-methoxybenzyl-β-D-glucopyranoside 14

Wilkinson's catalyst (64.8 mg, 0.07 mmol) was dissolved in THF (1 ml) and *n*-BuLi (52 µl, 0.08 mmol) was added. The mixture was stirred for 5 minutes and then transferred into a refluxing solution of compound **13** (548.6 mg, 0.77 mmol) in THF (2 ml). After 1 h, the mixture was evaporated *in vacuo* and the residue was purified by flash silica gel column chromatography (eluent ethyl acetate–triethylamine–hexanes, 20 : 5 : 75 v/v/v) to give compound **14** (423.3 mg, 78%) as a clear oil, R_f 0.40 (20% ethyl acetate in hexanes); ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.75–7.29 (m, 15 H, Ph), 7.12–7.08 (m, 2H, *o*-ArH, PMB), 6.83–6.79 (m, 2H, *m*-ArH, PMB), 5.05–4.43 (m, 5H, C*H*CH_3, ArCH_2), 4.18–3.34 (m, 10H, H-1, -2, -3, -4, -5, -6a,b, OMe), 1.69, 1.61 (2d, 6H, 2 × CH_3, $^3J_{cis/trans}$ = 6.6 Hz), 1.10 (s, 9H, CMe_3); ^{13}C NMR (300 MHz; $CDCl_3$) δ_C 159.6 (Ar-C_q, PMB), 151.7, 149.1 (*C*=CH), 138.3–135.0 (Ar-C), 129.9–127.8 (Ar-CH), 106.0 (*C*HCH_3), 99.5, 99.2 (C-1), 83.6, 83.4, 77.6, 77.5, 66.4 (C-3, -4, -5), 75.9, 75.1, 62.9, 62.7 (Ar*C*H_2, C-2, -6), 55.5 (OMe), 27.0, 26.8 (C(Me_3), 15.7 (C*C*H_3), 12.2, 10.4 (CH*C*H_3); MALDI-TOF *m*/*z* 730 $[M + Na]^+$.

2-Azido-3-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-4-*O*-*p*-methoxybenzyl-α,β-D-glucopyranosyl trichloroacetimidate 16

Compound **14** (102.0 mg, 0.14 mmol) was dissolved in acetone–water (10 ml; 9/1 v/v) and red HgO (15.2 mg, 0.07 mmol) and $HgBr_2$ (100.9 mg, 0.28 mmol) were added. After stirring of the mixture for 18 h, TLC analysis showed complete conversion. The reaction mixture was concentrated *in vacuo* and the residue was purified by silica gel column chromatography (eluent 20% ethyl acetate in hexanes). The obtained product was dissolved in DCM (2 ml) and trichloroacetonitrile (110 µl, 1.1 mmol) was added. The mixture was cooled (−10 °C) and DBU (8.6 µl, 0.06 mmol) was added dropwise. After stirring of the mxiture for 1 h, TLC analysis showed complete conversion of the starting material. The solvent was evaporated off *in vacuo* and the residue was purified by flash silica gel column chromatography (eluent 20% ethyl acetate in hexanes) to give compound **16** (72.0 mg, 78.2%) as a clear oil, R_f 0.50 (α) and 0.33 (β) (20% ethyl acetate in hexanes); ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.66–7.23 (m, 20H, ArH, NH), 6.47 (d, 0.5H, H-1α, ^3J = 3.3 Hz), 5.62 (d, 0.5H, H-1β, ^3J = 8.4 Hz), 4.98–4.60 (m, 4H, ArCH_2), 4.06–3.41 (m, 9H, H-2, -3, -4, -5, H_2-6, OCH_3), 1.02 (s, 9H, CMe_3); ^{13}C NMR (300 MHz; $CDCl_3$) δ_C 132.2–123.4 (Ar-C), 92.9 (C-1β), 91.1 (C-1α), 79.3, 73.7, 72.1, 71.9, 71.3, 71.2, 70.9, 62.2, 59.7, 58.4, 58.2 (C-2, -3, -4, -5, C_2-6), 51.6, 51.5 (OCH_3), 23.2, 23.1 (C(Me_3), 15.6 (*C*(Me_3); MALDI-TOF MS *m*/*z* 821 $[M + Na]^+$.

2-Azido-3-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-4-*O*-*p*-methoxybenzyl-α,β-D-glucopyranosyl fluoride 17

To a cooled (−30 °C) solution of **15** (131.2 mg, 0.19 mmol) in DCM (1 ml) was added DAST (28 µl, 0.23 mmol). The cooling bath was removed and after 20 min, TLC analysis indicated complete conversion of the starting material. The mixture was cooled (−30 °C) and methanol (200 µl) was added. The solvent was concentrated *in vacuo* and the residue dissolved in DCM (100 ml). The solution was washed successively with saturated aqueous $NaHCO_3$ (20 ml) and brine (20 ml), dried ($MgSO_4$), filtered and the filtrate concentrated *in vacuo*. The residue was purified by silica gel column chromatography (eluent 20% ethyl acetate in hexanes) to give compound **17** (99.7 mg, 80%) as a clear oil, R_f = 0.52 (20% ethyl acetate in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.67–7.31 (m, 15H, Ar-C*H*), 7.09–7.07 (m, 2H, *o*-Ar-*CH*, *p*-methoxyphenyl), 6.81–6.79 (m, 2H, *m*-Ar-*CH*, *p*-methoxyphenyl), 5.65 (d, 1H, $J_{1,F}$ = 53.1 Hz), 4.92–4.65 (m, 4H, Ar-CH_2), 4.00–3.79 (m, 8H, H-3, -4, -5, -6a,b, OCH_3), 3.52–3.48, 3.43–3.40 (2m, 1H, H-2), 1.06 (s, 9H, C(CH_3)$_3$); ^{13}C NMR (300 MHz, $CDCl_3$) δ 159.0 (Ar-C_q, *p*-methoxybenzyl), 136.5–133.8 (Ar-C), 130.4–126.7 (Ar-CH), 107.7, 104.4 (C-1), 80.1, 79.8, 76.7, 74.8, 74.6, 73.5 (C-3, -4, -5), 62.8, 62.5 (C-2), 61.5 (C-6), 55.6 (C-2), 26.7 (C(*C*H_3)$_3$), 19.9 (*C*(CH_3)$_3$). MALDI-TOF *m*/*z* 678 $[M + Na]^+$ (Calc. for $C_{37}H_{42}FN_3O_5Si$: C, 67.76; H, 6.45; N, 6.41. Found: C, 68.03; H, 6.43; N, 6.48%).

(*R*/*S*)-But-3-en-2-yl 4-*O*-(2-azido-3-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-4-*O*-*p*-methoxybenzyl-α-D-glucopyranosyl)-2-*O*-benzoyl-3,6-di-*O*-benzoyl-β-D-glucopyranoside 18

Method A. A suspension of **14** (355.7 mg, 0.5 mmol), **5** (365.1 mg, 0.7 mmol) and activated 4 Å molecular sieves (300 mg) in a mixture of diethyl ether (15 ml) – DCM (3 ml) was stirred for 2 h at room temperature. The reaction mixture was cooled (−20 °C), TMSOTf (9 µl, 0.05 mmol) was added, and the mixture was stirred for 30 minutes, when TLC analysis showed no further reaction occurring. The reaction mixture was quenched by the addition of triethylamine and concentrated *in vacuo*. The crude product was dissolved in DCM (100 ml), and the solution was washed with saturated aq. $NaHCO_3$ (2 × 20 ml) and brine (1 × 20 ml) successively dried ($MgSO_4$), filtered, and the solvent was removed *in vacuo*. The residue was purified by silica gel column chromatography (eluent 2.5% ethyl acetate in toluene) to give disaccharide **18** (151.7 mg, 30%) and the corresponding β-anomer (50.6 mg, 10%).

Method B. A suspension of **17** (23.6 mg, 0.04 mmol), **5** (25.9 mg, 0.05 mmol) and activated 4 Å molecular sieves (100 mg) in DCM (1 ml) was stirred at room temperature for 1 h. The mixture was cooled (0 °C) and $ZrCp_2Cl_2$ (44.2 mg, 0.11 mmol) and AgOTf (18.5 mg, 0.07 mmol) were added. The suspension was allowed to warm to room temperature and after stirring for 18 h, TLC analysis showed complete conversion of the donor. The mixture was filtered and the residue washed with DCM (200 ml). The combined organic layers were subsequently washed with saturated aqueous $NaHCO_3$ (2 × 20 ml) and brine (20 ml), dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. Purification of the residue by silica gel column chromatography (eluent: 20% ethyl acetate in hexanes) gave disaccharide **18** (20.6 mg, 49.6%).

Method C. A suspension of **16** (177.9 mg, 0.23 mmol), **5** (311 mg, 0.60 mmol) and activated 4 Å molecular sieves (200 mg) in diethyl ether (3 ml) and DCM (0.6 ml) was stirred at room temperature for 1.5 h. The mixture was cooled (0 °C)

and trimethylsilyl trifluoromethanesulfonate (2.1 μl, 0.01 mmol) was added. After 15 min, TLC analysis showed complete consumption of the donor. The reaction was quenched by addition of triethylamine. After filtration, the residue was washed with DCM (50 ml) and the combined organic phases were concentrated *in vacuo*. The residue was dissolved in DCM (100 ml) and the solution was washed subsequently with saturated aqueous $NaHCO_3$ (2 × 20 ml) and brine (20 ml), dried ($MgSO_4$), filtered and the filtrate concentrated *in vacuo*. Purification of the residue by silica gel column chromatography (eluent: 2.5% ethyl acetate in toluene) gave disaccharide **18** (165.2 mg, 60.2%) and the corresponding β-anomer (51.7 mg, 18.1%), $R_f = 0.39$ (20% ethyl acetate in hexanes); ^{1}H NMR (500 MHz; $CDCl_3$) δ 8.13 (m, 2H, *o*-ArH, Bz), 7.71–7.14 (m, 30H, ArH), 6.88, 6.87 (2s, 2H, *o*-ArH, PMB), 5.91 (ddd, 0.5H, C*H*=CH$_2$, $^3J_{trans} = 16.7$ Hz, $^3J_{cis} = 10.8$ Hz, $^3J = 5.9$ Hz), 5.57 (ddd, 0.5H, C*H*=CH$_2$, $^3J_{trans} = 17.3$ Hz, $^3J_{cis} = 9.7$ Hz, $^3J = 7.0$ Hz), 5.65, 5.64 (2d, 1H, H-1′, $^3J_{1',2'} = 5.5$ Hz), 5.45–5.42 (m, 1H, H-2), 5.19, 5.08 (2dd, 2H, CH=C*H*$_2$, $^3J_{trans} = 17.3$ Hz, $^3J_{cis} = 10.3$ Hz), 4.98–4.78 (m, 8H, ArC*H*$_2$), 4.70, 4.69 (2d, 1H, β-H-1, $^3J_{1,2} = 8.1$Hz), 4.36–4.22 (m, 1H, OC*H*CH$_3$), 4.09–4.02 (m, 1H, H-3), 3.97–3.92 (m, 1H, H-3′), 3.87–3.64 (m, 11H, H-4′, -4, -5′, -5, H$_2$-6, -6′, ArOC*H*$_3$), 3.32 (dd, 1H, H-2′, $^3J_{1',2'} = 3.8$ Hz), 1.27, 1.18 (2d, 3H, C*H*$_3$CH, $^3J = 6.5$ Hz), 1.09 (s, 9H, C(Me)$_3$); ^{13}C NMR (500 MHz; $CDCl_3$) δ_C 170.3 (*C*O), 135.3, 134.1 (=*C*H), 129.2–121.6 (Ar-CH), 108.7 (Ar-CH, PMB), 110.2, 109.1 (=CH$_2$), 93.0, 91.8 (C-1), 90.9 (α-C-1′), 70.6, 69.1 (CH$_3$*C*H), 75.2, 66.2 (C-3), 73.3 (C-3′), 71.2, 68.8, 65.5, 62.7, 55.8, 55.7, 48.9 (C-4, -5, -4′, -5′, Ar*C*H$_2$, O*C*H$_3$), 69.5, 69.0, 67.8 (C-6, -6′) 68.9 (C-2), 57.0 (C-2′), 27.0, 26.9 (C(*Me*)$_3$), 20.0 (*C*(Me)$_3$), 14.8, 13.9 (CH*C*H$_3$) (Calc. for $C_{68}H_{75}N_3O_{12}Si$: C, 70.75; H, 6.55; N, 3.64. Found: C, 71.02; H, 6.43; N, 3.48%).

(*R*/*S*)-But-3-en-2-yl 4-*O*-(2-azido-3-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-α-D-glucopyranosyl)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranoside 19

Compound **18** (98.8 mg, 0.086 mmol) was dissolved in DCM (25 ml) and TFA (500 μl) and water (10 μl) were added dropwise. After stirring of the mixture for 1.5 h, TLC analysis showed complete consumption of the starting material. The reaction mixture was diluted with toluene (50 ml) and concentrated *in vacuo*. The residue was purified by flash silica gel column chromatography (eluent 20% ethyl acetate in hexanes) to give compound **19** (85.6 mg, 99.5%) as a clear oil, R_f 0.26 (20% ethyl acetate in hexanes); ^{1}H NMR (500 MHz; $CDCl_3$) δ 8.04–8.02 (m, 2H, *o*-ArH, Bz), 7.61–7.14 (m, 28H, ArH), 5.85 (ddd, 0.5H, C*H*=CH$_2$, $^3J_{trans} = 16.5$ Hz, $^3J_{cis} = 11$ Hz, $^3J = 6.5$ Hz), 5.48 (ddd, 0.5H, C*H*=CH$_2$, $^3J_{trans} = 17.5$ Hz, $^3J_{cis} = 10.5$ Hz, $^3J = 7.0$ Hz), 5.55, 5.54 (2d, 1H, H-1′, $^3J_{1',2'} = 5$ Hz), 5.32–5.28 (m, 1H, H-2), 5.15–4.97 (m, 2H, CH=C*H*$_2$), 4.83–4.82 (m, 1H, H-1), 4.74–4.43 (m, 6H, ArC*H*$_2$), 4.25–4.14 (m, 1H, OCH), 3.98–3.93 (m, 1H, H-3), 3.79–3.57 (m, 9H, H-3′, -4′, -4, -5′, -5, H$_2$-6, -6′), 3.15–3.12 (m, 1H, H-2′), 2.42, 2.22 (2d, 1H, 4′-OH, $^3J = 4.5$ Hz, $^3J = 4.0$ Hz), 1.19, 1.10 (2d, 3H, C*H*$_3$CH, $^3J = 6.5$ Hz), 1.00 (s, 9H, CMe$_3$); ^{13}C NMR (300 MHz: $CDCl_3$) δ_C 165.3 (CO, benzoyl), 140.2, 139.3 (=CH), 138.4–133.0 (Ar-C), 130.1–127.7 (Ar-CH), 116.8, 115.1 (=*C*H$_2$), 99.8, 98.4 (C-1β), 97.5 (C-1′α), 83.9, 79.8, 75.7, 75.4, 75.1, 74.9, 74.5, 74.2, 74.0, 73.6, 73.2, 73.1, 72.5, 71.9, 69.6, 69.5, 64.0, 62.9 (C-2, -3, -4, -5, -6, -2′, -3′, -4′, -5′, -6′, CH$_3$-*C*H, Ar*C*H$_2$) 27.2 (C*Me*$_3$), 21.9, 20.5 (CH*C*H$_3$), 19.5 (*C*Me$_3$) (Calc. for $C_{60}H_{67}N_3O_{11}Si$: C, 69.68; H, 6.53; N, 4.06. Found: C, 69.78; H, 6.79; N, 3.74%).

(*R*/*S*)-But-3-en-2-yl 4-*O*-[2-azido-3-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-4-*O*-(2,4-di-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranosyl)-α-D-glucopyranosyl]-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranoside 20

Compound **19** (188.2 mg, 0.18 mmol) was dissolved in DCM (1 ml) and the solution was stirred at room temperature over molecular sieves (300 mg, 4Å) for 1 h. In a second flask donor **7** (283.5 mg, 0.42 mmol) was dissolved in DCM (2 ml) and the solution was stirred at room temperature over molecular sieves (500 mg, 4Å) for 1 h. Both solutions were cooled to −20 °C and TMSOTf (3.2 μl, 0.018 mmol) was added to the acceptor solution. Subsequently, the donor solution was added to the acceptor in portions (100 μl) over a period of 3 h. When addition was complete, the reaction mixture was stirred for an additional 30 minutes. The mixture was neutralized using triethylamine, filtered, and the filtrate was concentrated *in vacuo*. The residue was dissolved in DCM (100 ml), washed successively with saturated aq. $NaHCO_3$ (2 × 20 ml) and brine (1 × 20 ml), dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified by flash silica gel chromatography (eluent 20% ethyl acetate in hexanes), followed by Sephadex LH-20 size-exclusion column chromatography (50% methanol in DCM) to give trisaccharide **20** (215 mg, 75%), R_f 0.33 (20% ethyl acetate in hexanes); ^{1}H NMR (500 MHz; $CDCl_3$) δ 8.03–7.70 (2m, 6H, *o*-ArH, Bz), 7.59–6.86 (m, 44H, ArH), 5.76 (ddd, 0.5H, C*H*=CH$_2$, $^3J_{trans} = 17.0$ Hz, $^3J_{cis} = 10.5$ Hz, $^3J = 6.0$ Hz), 5.49–5.36 (m, 4.5H, C*H*=CH$_2$, CH=C*H*$_2$, H-1′, -2″), 5.28–5.24 (m, 1H, H-2), 5.28–4.33 (m, 12H, ArC*H*$_2$, H-1″β, -1β), 4.19–4.07 (m, 2H, OC*H*, H-4″), 3.92–3.30 (m, 14H, H-3, -4, -5, H$_2$-6, H-3′, -4′, H-5′, H$_2$-6′, H-3″, -5″, H$_2$-6), 3.08 (dd, 1H, H-2′, $^3J_{1,2} = 4$ Hz), 1.13, 1.05 (2d, 3H, C*H*$_3$CH, $^3J = 6.5$ Hz), 1.01 (s, 9H, CMe$_3$); ^{13}C NMR (300 MHz; $CDCl_3$) δ_C 165.6, 165.5, 165.0 (*C*=O), 140.3, 139.3 (*C*H=CH$_2$), 138.8–132.8 (Ar-C), 130.3–127.5 (Ar-CH), 116.9, 115.0 (CH=*C*H$_2$), 100.4, 99.9, 98.6, 97.4 (C-1, -1′, -1″), 84.2, 80.6, 76.2, 75.7, 74.8, 74.7, 74.5, 74.4, 74.2, 74.0, 72.6, 72.5, 72.4 (C-2, -3, -4, -5, -2′, -3′, -4′, -5′, -2″, -3″, -4″, -5″, OCH), 75.9, 74.4, 73.1, 70.1, 68.8, 68.7, 61.3 (Ar*C*H$_2$, C-6, -6″, -6″), 27.4 (C*Me*$_3$), 22.0, 20.6 (CH*C*H$_3$), 19.8 (*C*Me$_3$) (Calc. for $C_{94}H_{97}N_3O_{18}Si$: C, 71.24; H, 6.17; N, 2.65. Found: C, 71.09; H, 6.37; N, 2.41%).

(*R*/*S*)-Isobutyl 4-*O*-[2-acetamido-2-deoxy-6-*O*-*tert*-butyldiphenylsilyl-4-*O*-(β-D-glucopyranosyl)-α-D-glucopyranosyl]-β-D-glucopyranoside 21

Compound **20** (200 mg, 0.13 mmol) was dissolved in methanol (10 ml) saturated with K_2CO_3. After stirring of the mixture for 48 h, TLC analysis showed complete consumption of the starting material. The reaction mixture was filtered, neutralized with Dowex H^+, filtered, and concentrated *in vacuo*. The residue was dissolved in pyridine (2 ml) and cooled (0 °C). Thioacetic acid (2 ml) was added dropwise and the mixture was stirred at 0 ° C for 18 h before being evaporated *in vacuo*, and the residue was purified by silica gel chromatography (eluent 0→1% methanol in DCM) to give the reduced trisaccharide (99.2 mg, 59%). The trisaccharide was subjected to catalytic hydrogenation in ethanol (25 ml) with $Pd(OAc)_2$ (5 mg) as catalyst. After 48 h the reaction mixture was filtered and evaporated to give pure **21** (49.3 mg, 76.3%), ^{1}H NMR (500 MHz; CD_3OD) δ 7.73–7.36 (m, 10H, ArH), 5.32–5.29 (2d, 1H, H-1′α, $^3J = 3.5$ Hz), 4.71–4.65, 4.34–4.16 (2m, 2H, H-1β, -1″β), 4.14–3.18 (m, 19H, H-2, -3, -4, -5, H$_2$-6, H-2′, -3′, -4′, -5′, H$_2$-6, H-2″, H-3″, H-4″, H-5″, H$_2$-6″, OCH), 1.84–0.94 (m, 20H, CMe$_3$, Ac, CHC*H*$_3$, CH$_2$C*H*$_3$, C*H*$_2$CH$_3$); ^{13}C NMR (600 MHz; MeOH) δ_C 168.5 (CO), 129.1–120.4 (Ar-C), 96.4, 96.1 (C-1, -1″), 92.5 (C-1′), 71.2, 70.7, 70.6, 70.4, 70.2, 70.0, 69.5, 69.6, 69.0, 68.8, 68.6, 67.4, 67.0, 65.5, 63.3, 62.7 (C-2, -3, -4, -5, -6, -2′, -3′, -4′, -5′, -6′, -2″, -3″, -4″, -5″, -6″), 20.4 (CMe$_3$), 20.2, 19.5 (CH*C*H$_3$), 18.5 (*C*Me$_3$), 14.5 (CO*C*H$_3$); FAB MS *m*/*z* 862.39 [M + Na]$^+$.

(*R*/*S*)-Isobutyl 4-*O*-[2-acetamido-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-4-*O*-(β-D-glucopyranosyluronic acid)-α-D-glucopyranosyl]-β-D-glucuronic acid disodium salt 22

Compound **21** (24.0 mg, 0.03 mmol), TEMPO (0.4 mg, 0.003

mmol) and NaBr (2 mg, 0.02 mmol) were dissolved in water (450 μl). The pH was adjusted to 10 using 1 M aq. NaOH, and aq. 13% NaOCl adjusted to pH 10 using 4 M HCl was added dropwise. The pH dropped sharply due to the formation of uronic acid and the pH was maintained at 10 ± 0.5 by addition of 1 M NaOH. Addition of NaOCl was continued until the pH remained stable for a prolonged period of time (5 min). The reaction was quenched by addition of methanol (1 ml) and the reaction mixture was lyophilized. Purification of the crude product by reversed phase chromatography (eluent 0→50% methanol in water) gave trisaccharide **22** (23.1 mg, 89%) as a white solid, ^{1}H NMR (500 MHz; CD_3OD) δ 7.82–7.36 (m, 10H, ArH), 5.37–5.34 (m, 1H, H-1′α), 4.79–4.78, 4.52–4.37 (2m, 2H, H-1, -1″), 4.17–3.21 (m, 13H, H-2, -3, -4, -5, -2′, -3′, -4′, -5′, -2″, -3″, -4″, -5″, OCH), 1.86–0.90 (m, 20H, CMe_3, NHAc, $CHCH_3$, CH_2CH_3, CH_2CH_3); ^{13}C NMR (300 MHz; MeOH) δ_C 174.3, 172.7 (COONa), 137.2–136.1 (Ar-C) 130.9–128.7 (Ar-CH), 104.2, 102.7(C-1, -1″), 99.8 (C-1′), 79.5, 78.9, 78.3, 78.1, 77.9, 76.5, 75.6, 75.4, 74.8, 73.5, 73.3, 71.4 (C-2, -3, -4, -5, -3′, -4′, -5′, C-2″, -3″, -4″, -5″) 55.9, 55.7 (C-2′), 28.4, 28.3 (CMe_3), 21.2, 20.9 (CH_2CH_3), 19.7 (CMe_3). 10.5, 10.2 ($COCH_3$); MALDI-TOF *m*/*z* 934 $[M + Na]^+$.

(*R*/*S*)-Isobutyl 4-*O*-[2-acetamido-2-deoxy-4-*O*-(β-D-gluco-pyranosyluronic acid)-α-D-glucopyranosyl]-β-D-glucuronic acid disodium salt 23

Compound **22** (13.0 mg, 0.014 mmol) was dissolved in pyridine (1 ml), the solution was cooled (0 °C) and HF·pyridine (10 μl) was added. The reaction mixture was stirred for 18 h at room temperature. Solid $NaHCO_3$ (20 mg) was added and the solvent was evaporated off. The residue was purified by Sephadex G-25 (eluent water) to give trisaccharide **23** (6.8 mg, 74%), ^{1}H NMR (500 MHz; D_2O) δ 5.41 (d, 1H, H-1′α, ^3J = 3.0 Hz), 4.55–4.52 (m, 2H, H-1, -1″), 3.96–3.52 (m, 11H, H-3, -4, -5, -2′, -3′, -4′, -5′, -3″, -4″, -5″, OCH), 3.41–3.73, 3.30–3.26 (2m, 2H, H-2, -2″), 1.66–1.59, 1.55–1.48 (2m, 2H, CH_2CH_3) 1.23, 1.19 (2d, 3H, $CHCH_3$, ^3J = 6.0 Hz, ^3J = 6.5 Hz), 0.93–0.88 (m, 6H, CH_2CH_3, $COCH_3$); ^{13}C NMR (500 MHz; D_2O) δ_C 180.1, 172.3 ($COCH_3$), 101.3, 100.6 (C-1, -1″), 100.1 (C-1′), 78.7, 78.6, 78.3, 78.0, 77.9, 76.5, 75.6, 75.4, 74.8, 73.5, 73.3, 71.4 (C-2, -3, -4, -5, -3′, -4′, -5′, -2″, -3″, -4″, -5″), 62.8 (C-6′), 55.9, 55.7 (C-2′), 10.5, 10.2 ($COCH_3$); MALDI-TOF *m*/*z* 696 $[M + Na]^+$.

(*R*/*S*)-But-3-en-2-yl 4-*O*-(2-acetamido-3-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-4-*O*-*p*-methoxybenzyl-α-D-gluco-pyranosyl)-3,6-di-*O*-benzyl-2-*O*-sulfo-β-D-glucopyranoside sodium salt 25

Compound **18** (41.8 mg, 36 μmol) was dissolved in methanol (10 ml) saturated with K_2CO_3. After being stirred for 18 h at room temperature, the reaction mixture was stirred with ion-exchange resin Dowex H^+ until neutral. The resulting suspension was filtered, and concentrated *in vacuo*. The residue was dissolved in pyridine (2.5 ml), the solution was cooled (0 °C), and thioacetic acid (2.5 ml) was added dropwise. The reaction mixture was allowed to warm to room temperature and was stirred for 18 h. The solvents were evaporated off *in vacuo* and the residue was purified by silica gel column chromatography (eluent 0→1% methanol in toluene) to give **24**. Compound **24** was dissolved in DMF (1 ml), SO_3·NEt_3 (56 mg, 0.3 mmol) was added, and the mixture was stirred at 50 °C for 18 h. The reaction mixture was allowed to cool to room temperature and saturated aq. $NaHCO_3$ (0.25 ml) was added. After being stirred for 1 h, the reaction mixture was lyophilized and the residue purified by LH-20 size-exclusion chromatography to yield **25** (28.0 mg, 67%) as a colorless glass, ^{1}H NMR (500 MHz; $CDCl_3$) δ 7.67–7.13 (m, 25H, ArH), 7.02, 7.01 (2s, 2H, *m*-ArH, PMB), 6.76, 6.74 (2s, 2H, *o*-ArH, PMB) 6.64–6.59 (m, 1H, NHAc), 5.82 (ddd, 0.5H, CH=CH_2, $^3J_{trans}$ = 17.0 Hz, $^3J_{cis}$ = 10.5 Hz, ^3J = 6.5 Hz), 5.69 (ddd, 0.5H, CH=CH_2, $^3J_{trans}$ = 17.5 Hz, $^3J_{cis}$ = 10.0 Hz, ^3J = 7.0 Hz), 5.25–4.71 (m, 11H, H-1′, CH=CH_2, $ArCH_2$), 4.59–4.27 (m, 2H, H-1, OCH), 4.19–3.20 (m, 15H, H-2, -3, -4, -5, H_2-6, -2′, -3′, -4′, -5′, H_2-6′, OMe), 1.75 (s, 3H, Ac), 1.21, 1.14 (2d, 3H, CH_3CH, ^3J = 6.0 Hz), 1.00 (s, 9H, CMe_3); ^{13}C NMR (300 MHz; $CDCl_3$) δ_C 174.9 (CO), 140.2, 139.1 (=*C*H), 138.4–137.6 (Ar-C), 133.6–127.7 (Ar-CH), 117.0, 114.9 (CH=CH_2), 110.0 (*p*-Ar-*C*, PMB), 98.6, 97.2 (C-1, -1′), 81.2, 76.9, 76.4, 75.3, 75.1, 74.8, 73.6, 73.3, 73.0, 69.6, 62.6, (C-2, -3, -4, -5, -6, -2′, -3′, -4′, -5′, -6′, $ArCH_2$, OCH_3, CH_3CH), 29.7, 26.8 (CMe_3), 22.3, 21.3, 19.8, 19.3 (CMe_3, $CHCH_3$) (Calc. for $C_{63}H_{74}NNaO_{15}SSi$: C, 64.76; H, 6.38; N, 1.20. Found: C, 64.87; H, 6.31; N, 1.09%). FAB MS *m*/*z* 1168.3 $[M + H]^+$.

(*R*/*S*)-Isobutyl 4-*O*-(2-acetamido-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-α-D-glucopyranosyl)-2-*O*-sulfo-β-D-glucopyranoside sodium salt 26

Compound **25** (24.7 mg, 0.021 mmol) was dissolved in ethanol (15 ml) and $Pd(OAc)_2$ (3 mg) was added. After stirring of the reaction mixture under an atmosphere of H_2 for 48 h, it was filtered, and concentrated *in vacuo* to give **26** (12.1 mg, 73.8%) as a colorless glass, ^{1}H NMR (500 MHz; CD_3OD) δ 7.68–7.65, 7.38–7.37 (m, 10H, ArH), 5.27–5.26 (m, 1H, H-1′), 4.56–4.53 (m, 1H, H-1), 3.98–3.53 (m, 12H, H-2′, -3, -3′, -4, -4′, -5, -5′, H_2-6, -6′, OCH), 1.75–0.92 (m, 20H, $C(O)CH_3$, Ac, $C(CH_3)_3$, CMe_3, CH_2CH_3); ^{13}C NMR (500 MHz; CD_3OD) δ_C 174.7 (CO), 135.7–126.5 (Ar-C_q, Ar-CH), 100.1 (C-1′), 101.2 (C-1), 80.5, 77.9, 76.4, 75.0, 74.1, 73.8, 73.6, 72.3, 71.0, 64.9, 63.5, 56.8 (C-2, -3, -4, -5, -6, -2′, -3′, -4′, -5′, -6′, OCH), 27.5, 26.3 (CMe_3), 21.3, 20.3, 19.8, 16.6 (CMe_3, $CHCH_3$, CH_2CH_3); MALDI-TOF *m*/*z* 803 $[M + Na]^+$.

(*R*/*S*)-Isobutyl 4-*O*-(2-acetamido-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-α-D-glucopyranosyl)-2-*O*-sulfo-β-D-glucuronic acid disodium salt 27

Aq. 13% NaOCl was adjusted to pH 8.5 by using 4 M aq. HCl. This mixture was added dropwise to a solution of compound **26** (13.9 mg, 17.8 μmol), TEMPO (0.28 mg, 2 μmol) and NaBr (0.71 mg, 7 μmol) in water (450 μl) at 0 °C. The pH of the resulting solution was carefully maintained at 8.5 ± 0.5 by addition of 1 M NaOH. Addition of the NaOCl solution and NaOH was continued until TLC analysis showed complete conversion of the starting material. The reaction mixture was directly purified by reversed phase chromatography (eluent 0→90% methanol in water) to give disaccharide **27** (9 mg, 62%) as a clear solid, ^{1}H NMR (500 MHz; CD_3OD) δ 7.68–7.65, 7.38–7.37 (m, 10H, ArH), 5.27–5.26 (m, 1H, H-1′), 4.94–4.90 (m, 1H, H-1), 4.59–4.50 (m, 1H, H-2), 3.98–3.53 (m, 10H, H-3, -4, -5, -2′, -3′, -4′, -5′, H_2-6, OCH), 2.02, 1.89 (2s, 3H, Ac), 1.67–0.85 (m, 17H, CH_3CH, CMe_3, CH_2CH_3); ^{13}C NMR (500 MHz; CD_3OD) δ_C 135.8–127.2 (Ar-C, Ar-CH), 100.2 (C-1′), 99.8 (C-1), 78.8 (C-2), 81.9, 78.6, 77.1, 73.6, 72.4, 70.5, 63.4, 61.8 (C-3, -4, -5, -3′, -4′, -5′, -6′, OCH), 54.4 (C-2′), 26.6, 26.2 (CMe_3), 21.8, 21.7 ($COCH_3$), 19.2, 17.9 (CH_2CH_3, $CHCH_3$), 14.8, 14.7 (CH_2CH_3); MALDI-TOF *m*/*z* 838 $[M + Na]^+$.

(*R*/*S*)-Isobutyl 4-*O*-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-2-*O*-sulfo-β-D-glucuronic acid disodium salt 28

To a cooled (0 °C) solution of **27** (5 mg, 6 μmol) in pyridine (500 μl) was added HF·pyridine (5 μl; 70% HF in pyridine). The reaction mixture was allowed to warm to room temperature and was stirred for 18 h. The solution was neutralized with solid $NaHCO_3$ (10 mg) and the solvent was removed *in vacuo*. The residue was purified by reversed phase column chromatography (eluent 0→20% methanol in water) to give **28** (2.1 mg, 63%) as a white solid, ^{1}H NMR (500 MHz; D_2O) δ 5.42 (br s, 1H, H-1′), 4.92–4.87 (m, 1H, H-1), 4.38–4.35 (m, 1H, H-2), 4.09–3.51 (m, 10H, H-3, -4, -5, -2′, -3′, -4′, -5′, H_2-6, OCH), 2.13, 2.01 (2s, 3H, Ac), 1.76–1.56 (m, 2H, CH_2CH_3), 1.32–1.27, 1.02–0.96

(2m, CH_2CH_3, $CHCH_3$); ^{13}C NMR (500 MHz; D_2O) δ_C 99.8 (C-1), 97.0 (C-1′), 82.6, 81.4, 73.2, 71.5, 70.0, 61.2, 60.6, 60.9 (C-3, -4, -5, -3′, -4′, -5′, -6′, OCH), 79.7 (C-2), 53.7 (C-2′), 22.2, 22.1 ($COCH_3$), 20.0, 17.8 (CH_2CH_3, $CHCH_3$), 14.6 (CH_2CH_3); MALDI-TOF *m/z* 601 $[M + Na]^+$.

References

1 U. Lindahl, K. Lidholt, D. Spillman and L. Kjellén, *Thromb. Res.*, 1994, **75**, 1.
2 A. Yayon, M. Klagsbrun, J. D. Esko, P. Leder and D. M. Ornitz, *Cell*, 1991, **64**, 841.
3 S. Prestrelski, G. M. Fox and T. Arakawa, *Arch. Biochem. Biophys.*, 1992, **293**, 314.
4 I. Bjork and U. Lindahl, *Mol. Cell. Biochem.*, 1982, **48**, 161.
5 (*a*) U. Lindahl, *Heparin. Chemical and Biological Properties*, CRC Press, Boca Raton, FL, 1989; (*b*) U. Lindahl, M. Kusche-Gullberg and L. Kjellén, *J. Biol. Chem.*, 1998, **273**, 24979; (*c*) D. Spillmann and U. Lindahl, *Curr. Opin. Struct. Biol.*, 1994, **4**, 677.
6 M. Salmivirta, K. Lidholt and U. Lindahl, *FASEB J.*, 1996, **10**, 1270.
7 (*a*) M. Petitou, P. Duchaussoy, I. Lederman, J. Choay, P. Sinaÿ, J. C. Jacquinet and G. Torri, *Carbohydr. Res.*, 1986, **147**, 221; (*b*) C. M. Dreef-Tromp, J. E. M. Basten, M. A. Broekhoven, T. G. van Dinther, M. Petitou and C. A. A. van Boeckel, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 2081.
8 (*a*) C. A. A. van Boeckel, T. Beetz, J. N. Vos, A. J. M. de Jong, S. F. van Aelst, R. H. van der Bosch, J. M. R. Mertens and F. A. van der Vlugt, *J. Carbohydr. Chem.*, 1985, **4**, 293; (*b*) P. Westerduin, J. E. M. Basten, M. A. Broekhoven, V. de Kimpe, W. H. A. Kuijpers and C. A. A. van Boeckel, *Angew. Chem., Int. Ed. Engl.* 1996, **35**, 331; (*c*) M. Petitou, P. Duchaussoy, P.-A. Driguez, G. Jaurand, J.-P. Hérault, J.-C. Lormeau, C. A. A. van Boeckel and J.-M. Herbert, *Angew. Chem., Int. Ed. Engl.*, 1998, **37**, 3009.
9 (*a*) J. Kovensky, P. Duchaussoy, M. Petitou and P. Sinaÿ, *Tetrahedron*: *Asymmetry*, 1996, **7**, 3119; (*b*) J. Kovensky, P. Duchaussoy, F. Bono, M. Salmivirta, P. Sizun, J.-M. Herbert, M. Petitou and P. Sinaÿ, *Bioorg. Med. Chem.*, 1999, **7**, 1567; (*c*) P. Duchaussoy, G. Jaurand, P.-A. Diguez, I. Lederman, M.-L. Ceccato, F. Gourvenec, J.-M. Strassel, P. Sizun, M. Petitou and J.-M. Herbert, *Carbohydr. Res.*, 1999, **317**, 85; (*d*) M. Petitou, J.-P. Hérault, A. Bernat, P.-A. Driguez, P. Duchaussoy, J.-C. Lormeau and J.-M. Herbert, *Nature*, 1999, **398**, 417.
10 C. A. A. van Boeckel and M. Petitou, *Angew. Chem., Int. Ed. Engl.*, 1993, **32**, 1671.
11 (*a*) N. J. Davis and S. L. Flitsch, *Tetrahedron Lett.*, 1993, **34**, 1181; (*b*) A. E. J. de Nooy, A. C. Besemer and H. van Bekkum, *Carbohydr. Res.*, 1995, **269**, 89; (*c*) A. E. J. de Nooy, A. C. Besemer and H. van Bekkum, *Recl. Trav. Chim. Pays-Bas*, 1994, **113**, 165.
12 (*a*) G.-J. Boons and S. Isles, *Tetrahedron Lett.*, 1994, **35**, 3593; (*b*) G.-J. Boons and S. Isles, *J. Org. Chem.*, 1996, **61**, 4262; (*c*) G.-J. Boons, R. Gibson and R. Dickinson, *J. Chem. Soc., Perkin Trans. I*, 1997, 3357; (*d*) G.-J. Boons, B. Heskamp and F. Hout, *Angew. Chem., Int. Ed. Engl.* 1996, **35**, 2845; (*e*) M. Johnson, C. Arles and G.-J. Boons, *Tetrahedron Lett.*, 1998, **39**, 9801; (*f*) Y. Bai, G.-J. Boons, A. Burton, M. Johnson and M. Haller, *J. Carbohydr. Chem.*, 2000, **19**, 939; (*g*) G.-J. Boons, A. Burton and S. Isles, *Chem. Commun.*, 1996, 141.
13 J. A. L. M. van Dorst, A. F. Voskamp, J. P. Kamerling and J. F. G. Vliegenthart, *Liebigs Ann.*, 1997, 1227.
14 M. P. DeNinno, J. B. Etienne and K. C. Duplantier, *Tetrahedron Lett.*, 1995, **36**, 669.
15 A. Demchenko, T. Stauch and G.-J. Boons, *Synlett*, 1997, 818.
16 M. Masato, Y. Ito and T. Ogawa, *Carbohydr. Res.*, 1990, **195**, 199.
17 (*a*) G. H. Posner and S. R. Haines, *Tetrahedron Lett.*, 1985, **26**, 1823; (*b*) W. Rosenbrook, D. A. Riley and P. A. Lartey, *Tetrahedron Lett.*, 1985, **26**, 3.
18 H. Kunz, *Angew. Chem.*, 1987, **99**, 297.
19 K. Suzuki, H. Maeta, T. Suzuki and T. Matsumoto, *Tetrahedron Lett.*, 1989, **30**, 6879.
20 H. Paulsen, A. Wulff and M. Brenken, *Liebigs Ann. Chem.*, 1991, 1127.
21 S. D. Kuduk, J. B. Schwarz, X.-T. Chen, P. W. Glunz, D. Sames, G. Ragupathi, P. O. Livingston and S. J. Danishefsky, *J. Am. Chem. Soc.*, 1998, **120**, 12474.
22 T. Boecker, T. K. Lindhorst, J. Thiem and V. Vill, *Carbohydr. Res.*, 1992, **230**, 245.