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Synthesis of Disaccharidic Sub-Units of a New Series of Heparin Related Oligosaccharides.

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Abstract – The chemical synthesis of disaccharides **1** and **2**, useful building-blocks for the preparation of a new series of heparin related oligosaccharides containing the unusual sequence $(GlcN\text{-}IdoA)_n$, is described. In addition, the orthogonality of the protective groups would allow access to a wide array of differently sulfated oligosaccharides. As the simplest members of this new class of oligomer, the synthesis of sulfated disaccharides **3** and **4** fully deprotected is reported.

Key words: carbohydrates; glycosidation; biologically active compounds; sulfonation

INTRODUCTION

Heparin and heparan sulfate are complex sulfated polysaccharides composed of alternating units of hexuronic acid (D-glucuronic or L-iduronic acid) and D-glucosamine in (1→4) linkages. Most of the biological activities known to be associated with these glycosaminoglycans are due to interactions between their negatively charged chains and various proteins.[1] Such interactions range from highly specific, as described for the antithrombin-binding region in heparin,[2] to relatively nonspecific electrostatic associations. Among the proteins that heparin binds, a group of proteins which have attracted particular interest in recent years, namely fibroblast growth factors (FGFs).[3] The FGF family is comprised of seven members having a variety of growth and differentiation activities, of which the best characterised are the acidic (FGF-1) and basic (FGF-2).

To better understand the nature of the interaction between heparin and FGFs, a number of heparin related fragments, different in length, in composition of monosaccharide units and/or in sulfation pattern must be prepared and their binding properties tested. Conformational analysis as well as modelling of these fragments should be useful for obtaining information on structure-activity relationships.

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Heparin fragments are generally obtained from native polysaccharide through nitrous acid deamination at pH 3.9 (usually followed by reduction with NaB^3H_4) or digestion with heparin lyases (heparinase and heparanase):[4] both the procedures invariably lead to oligosaccharides having an uronic acid at the non-reducing end. Also, heparin fragments prepared by chemical synthesis usually have an uronic acid residue at the non-reducing end. On the other hand, fragments starting with a glucosamine at the non-reducing terminus should exhibit a sensibly altered electrostatic pattern as well as quite different conformational and binding properties.

To our knowledge, a systematic approach to the synthesis of a new kind of oligomer such as $(GlcN\text{-}HexA)_n$ has never been reported. Therefore, we started a programme aimed at the synthesis of heparin related oligosaccharide fragments which bear a glucosamine unit at the non-reducing end, focusing our attention on the significance of the 6-*O*-sulfation of the glucosamine units. As a matter of fact, it has been reported[5] that, whereas the idopyranosiduronyl 2-*O*-sulfates seem to be essential for the interaction with FGF-1 and FGF-2, the role played by the glucosamine 6-*O*-sulfates still remains unclear. To assess the influence of the 6-*O*-sulfation on conformation and binding to FGFs, we planned a synthetic protocol suitable for obtaining all possible oligosaccharides where the *O*-6 position of each glucosamine unit may, or may not, be sulfated. Our synthetic strategy is based on preparing and assembling disaccharide moieties having the glucosamine-uronic acid sequence with various patterns of orthogonal protective groups. This approach would allow access to a wide array of new differently sulfated oligosaccharides. In particular, our project is founded on the use of two strategically protected disaccharide building-blocks (**1** and **2**).

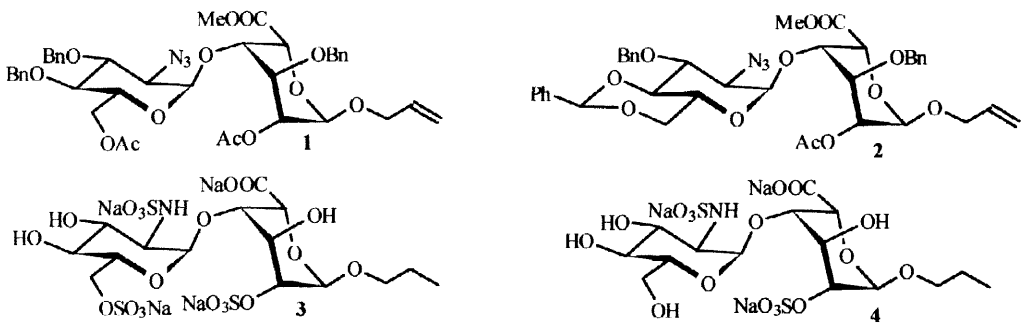


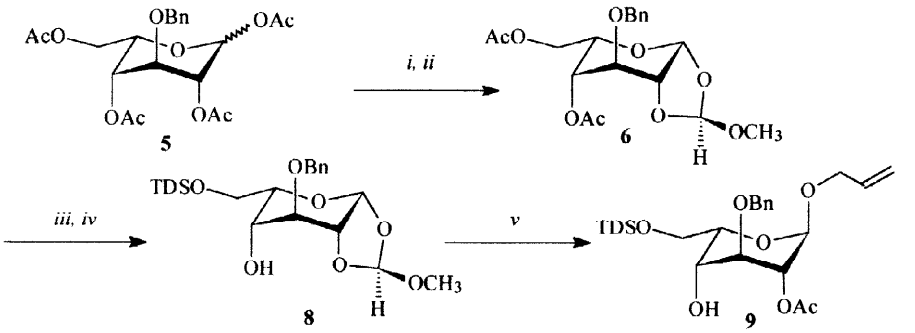
Figure 1

We describe here the synthesis of disaccharides **1** and **2** (Figure 1); in addition, we present the synthesis of the propyl glycosides of two partially sulfated disaccharides (**3** and **4**), obtained from building-block **1**, which could be helpful standards for conformational analysis.

RESULTS AND DISCUSSION

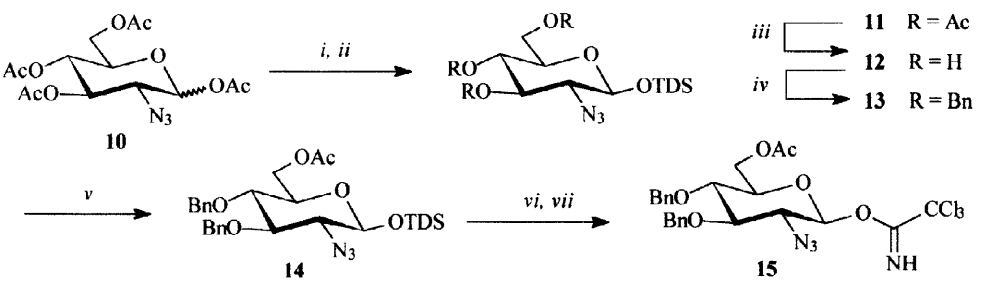
Our synthetic strategy was based on the use of benzyl ethers as permanent groups and acetates as temporary groups to protect those hydroxyls destinated to be *O*-sulfated. The synthesis of both compounds **1** and **2**

(Figure1) was achieved from a common idopyranosyl acceptor, obtained as follows. 1,2,4,6-Tetra-*O*-acetyl-3-*O*-benzyl-L-idopyranose **5**[6] was converted into methyl orthoester **6**. Deacetylation, followed by regioselective 6-*O*-silylation and opening of the orthoester with allyl alcohol in the presence of trimethylsilyl triflate gave alcohol **9** (Scheme 1).



Scheme 1. *(i)* $TiBr_4$, CH_2Cl_2:EtOAc 1:1, 0°C; *(ii)* MeOH, sym-collidine (47%); *(iii)* NaOMe, MeOH; *(iv)* Thexyldimethylsilyl chloride, TEA, DMAP, dry dichloromethane (63%); *(v)* Allyl alcohol, molecular sieves, *Tert*-butyldimethylsilyltriflate, dry dichloromethane (85%).

Known 1,3,4,6-tetra-*O*-acetyl-2-azido-2-deoxy-D-glucopyranose **10** was prepared by diazo-transfer from trifluoromethanesulfonyl azide (CAUTION: reported to be explosive when not in solution!)[7] to D-glucosamine

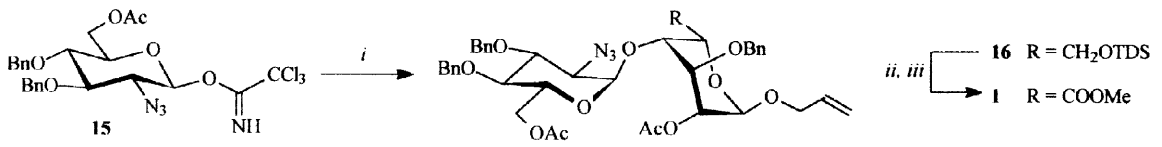


Scheme 2. *(i)* gaseous NH_3, THF:MeOH 7:3; *(ii)* Thexyldimethylsilyl chloride, Imidazole, dry DMF; *(iii)* MeONa, MeOH; *(iv)* NaH, BnBr, dry DMF; *(v)* Ac_2O:HOAc 2:1, $ZnCl_2$ (84% from **10**); *(vi)* TBAF, HOAc, dry THF; *(vii)* trichloroacetonitrile, K_2CO_3, dry dichloromethane (58% from **14**).

(70% yield after acetylation). Conversion of **10** into trichloroacetimidate **15** was achieved in 7 steps (Scheme 2). After selective 1-*O*-deacetylation and silylation of the anomeric position, compound **11** was fully deacetylated and benzylated (→**13**). Regioselective 6-*O*-debenzylation and acetylation was accomplished using zinc chloride in a 2:1 mixture of acetic anhydride:acetic acid,[8] giving compound **14** (81% yield from **10**).

Finally, removal of the thexyldimethylsilyl group and treatment with trichloroacetonitrile in dichloromethane in the presence of potassium carbonate afforded β-trichloroacetimidate **15** (58% from **14**). Condensation of the alcohol **9** with **15** in toluene in the presence of *tert*-butyldimethylsilyl triflate gave disaccharide **16** in 83% yield. The α configuration of the newly formed glycosidic linkage was ascertained through ^1H NMR spectroscopy: H-2' appeared as a doublet of doublets at δ 3.34 ($J_{2',1'}$ = 3.5 Hz, $J_{2',3'}$ = 10.2 Hz), as expected for a 1,2-*cis* linkage.

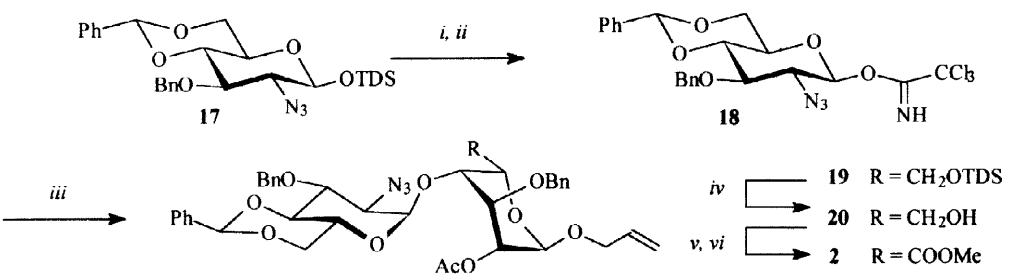
Jones oxidation, followed by the treatment with an ethereal solution of diazomethane, converted disaccharide **16** directly into methyl idopyranosiduronate **1** in 59% yield (Scheme 3).



Scheme 3. *(i)* Acceptor **9**, *Tert*-butyldimethylsilyl triflate, -20°C, dry toluene (83%); *(ii)* Jones' reagent, acetone; *(iii)* MeOH, diazomethane (59% from **16**).

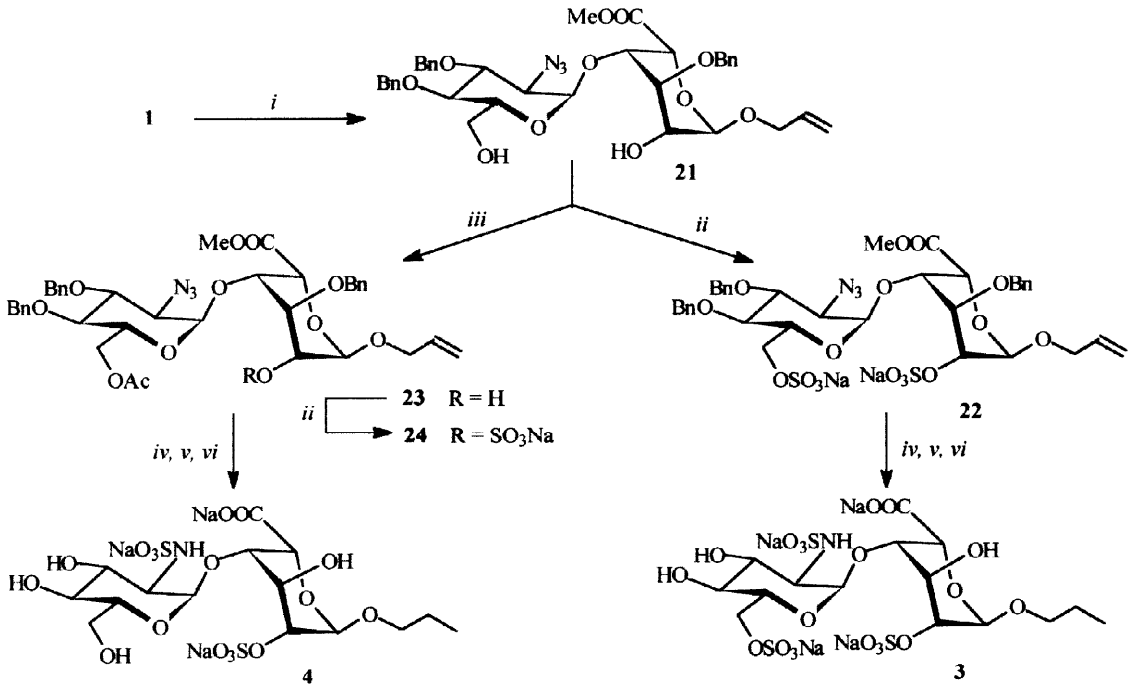
Compound **1** is a useful building-block for the synthesis of larger oligosaccharides, through the elongation of the saccharidic chain at the reducing terminus. On the other hand, disaccharide **2** gives access to more complex oligosaccharides allowing elongation both at the reducing and at the non-reducing end. The preparation of disaccharide **2** started from known intermediate **17**, easily obtained from **12**.[9] Desilylation of **17**, followed by treatment with trichloroacetonitrile and potassium carbonate in dichloromethane afforded β imidate **18** (71% yield from **17**) which was condensed with the previously described alcohol **9** in the presence of *tert*-butyldimethylsilyl triflate affording disaccharide **19** in 84% yield. The α configuration of the newly formed glycosidic linkage in disaccharide **19** was confirmed through NMR spectroscopy: in the proton spectrum H-1b showed a doublet at δ 4.83 ($J_{1,2}$ = 3.8 Hz) as expected for a 1,2-*cis* linkage.

After desilylation (88% yield), oxidation of the alcohol **20** was achieved using pyridinium dichromate and acetic anhydride in dichloromethane, followed by the treatment with an ethereal solution of diazomethane to give methyl idopyranosiduronate **2** (80% from **20**, Scheme 4).



Scheme 4. *(i)* TBAF, HOAc, dry THF; *(ii)* trichloroacetonitrile, K_2CO_3, dry dichloromethane (71% from **17**); *(iii)* acceptor **9**, *tert*-butyldimethylsilyl triflate, -20°C, dry toluene (84%); *(iv)* TBAF, dry THF (88%); *(v)* PDC, Ac_2O, dry dichloromethane; *(vi)* MeOH, diazomethane, (80% from **20**).

Conversion of the building-block **1** into the disaccharides **3** and **4** was accomplished as follows. Zemplèn deacetylation of **1** gave diol **21** in quantitative yield, which was then 2a,6b-di-*O*-sulfated using sulfur trioxide-trimethylamine complex in dry DMF (→**22**, 86%). Sequential saponification of the methyl ester, hydrogenolysis of benzyl ethers with contemporary reduction of the azido function, and N-sulfation amino group were performed without isolation of the intermediate products to give disaccharide **3** in 90% overall yield (Scheme 5).



Scheme 5. *(i)* MeOH, NaOMe (qu.); *(ii)* SO_3·NMe_3, DMF (86% from **21**, 78% from **23**); *(iii)* Ac_2O, Py (1 eq.), DMAP, -40°C (69%); *(iv)* aq. 2.5 M NaOH, MeOH; *(v)* H_2, $Pd(OH)_2$/C, MeOH:H_2O 1:1, HOAc; *(vi)* SO_3·NMe_3, satd aq. $NaHCO_3$ (90% from **22**, 86% from **24**).

Experimental

General methods.—^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AC 300, Bruker Am 500 and Varian Gemini 200 spectrometers for solutions in $CDCl_3$. In NMR spectra of disaccharidic compounds, the indexes a and b are referred to the reducing and the non-reducing terminus, respectively. Melting points were determined with a Büchi apparatus and are not corrected. Optical rotations were measured at room temperature with a Perkin-Elmer 241 polarimeter. IR spectra were recorded on Perkin Elmer 681 Infrared Spectrophotometer. TLC was carried out on Merck Silica-gel 60 F_{254} plates (0.25 mm thickness), and spots were visualized by spraying with a solution containing H_2SO_4 (31 mL), ammonium molybdate (21 g) and $Ce(SO_4)_2$ (1 g) in 500 mL water, followed by heating at 110 °C for 5 min. Column chromatography was performed by the flash procedure using Merck Silica-gel 60 (230-400 mesh). Elemental analyses were performed using the Carlo Erba elemental analyser 1108. In the description of the ^{13}C spectra signals corresponding to aromatic carbons were omitted.

4,6-Di-*O*-acetyl-3-*O*-benzyl-α-L-idopyranose-1,2-methyl orthoacetate (6). A solution of **5**[6] (2.0 g, 4.6 mmol) in dry dichloromethane and dry ethyl acetate (1/1 vol., 40 mL) was cooled at 0°C; $TiBr_4$ (2.5 g, 6.8 mmol) was added. The mixture was allowed to warm to room temperature; after 6 h the reaction was quenched with an excess of potassium acetate and stirred until decoloration. The solid was filtered through a celite pad and the solvent was evaporated to afford a brown oil which was dissolved in dry dichloromethane (6 mL) under N_2; sym-

collidine (4.0 mL, 32.2 mmol) and methanol (1 mL, 23 mmol) were added and the reaction was stirred at room temperature for 20 h. After washing the organic phase with satd aq. $NaHCO_3$ the solution was dried over $NaSO_4$ and filtered; the solvent was removed and the crude product purified by flash chromatography (8:2 hexane:ethyl acetate + 1% triethylamine) yielding **6** as a brown oil (870 mg, 47%): $[\alpha]_D^{23}$ –8.3 (c 1, chloroform); ν_{max}(liquid film) 1740, 1493, 1054 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 7.41-7.26 (m, 5H, H_{Ar}), 5.52 (d, 1H, $J_{1,2}$ = 2.3 Hz, H-1), 4.84-4.64 (m, 3H, H-4, C*H*$_2$Ph), 4.19-4.13 (m, 3H, H-5, H-6, H-6'), 4.10-4.05 (m, 2H, H-2, H-3), 3.25 (s, 3H, OCH_3), 2.07 (s, 6H, 2 OAc), 1.70 (s, 3H, CCH_3). Anal. Calcd for $C_{20}H_{26}O_9$ (410.4): C, 58.53; H, 6.39. Found: C, 58.72; H, 6.42.

3-*O*-Benzyl-6-*O*-thexyldimethylsilyl-α-L-idopyranose-1,2-methyl orthoacetate (8). Orthoester **6** (870 mg, 2.12 mmol) was dissolved in dry methanol (20 mL) under N_2 and cooled at 0°C; 0.9 M sodium methoxide in dry methanol (200 μL) was added and after 2 h the solution was neutralized with ion exchange resin (Amberlite IR-120, H^+ form). After filtration and evaporation of the solvent the product was dissolved in dry dichloromethane (10 mL); triethylamine (393 μL, 2.97 mmol), 2,4-dimethylaminopyridine (10 mg, 0.083 mmol) and thexyldimethylsilyl chloride (540 μL, 2.75 mmol) were added. After stirring 24 h at room temperature water was added and the reaction mixture extracted with dichloromethane (2 × 10 mL); the organic layers were dried ($NaSO_4$), filtered and evaporated. Flash chromatography (9:1 hexane:ethyl acetate + 1% triethylamine) gave **8** as a yellow oil (623 mg, 63%): $[\alpha]_D^{23}$ –4.0 (c 1, chloroform); ν_{max}(liquid film) 3700-3100 (br), 1260, 1105, 728 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 7.38-7.25 (m, 5H, H_{Ar}), 5.45 (d, 1H, $J_{1,2}$ = 2.3 Hz, H-1), 4.67 (d, 1H, J = 11.6 Hz, C*H*HPh), 4.59 (d, 1H, J = 11.6 Hz, CH*H*Ph), 4.14 (m, 1H, $J_{3,2}$ = 2.3 Hz, H-3), 4.04 (t, 1H, $J_{1,2}$ = $J_{2,3}$ = 2.3 Hz, H-2), 3.90-3.72 (m, 4H, H-4, H-5, H-6, H-6'), 3.29 (s, 3H, OCH_3), 2.68 (d, 1H, J = 10.1 Hz, OH), 1.71 (s, 3H, CCH_3), 1.62 (m, 1H, CH_{thexyl}), 0.91 (s, 3H, $CH_{3\ thexyl}$), 0.88 (s, 6H, $CH_{3\ thexyl}$), 0.86 (s, 3H, $CH_{3\ thexyl}$), 0.14 (s, 6H, CH_3 on Si). Anal. Calcd for $C_{24}H_{40}O_7Si$ (468.6): C, 61.51; H, 8.60. Found: C, 61.80; H, 8.35.

Allyl 2-*O*-acetyl-3-*O*-benzyl-6-*O*-thexyldimethylsilyl-α-L-idopyranoside (9). Compound **8** (140 mg, 0.30 mmol) was dissolved in allyl alcohol (10 mL) under inert atmosphere; 4 Å molecular sieves were added, then the mixture was cooled at 0°C and 0.1 M *tert*-butyldimethylsilyl triflate in dichloromethane (300 μL, 0.030 mmol) was dropped. After 10 min. the reaction was neutralized with triethylamine, filtered through a celite pad and evaporated. Flash chromatography (9:1 hexane:ethyl acetate) afforded compound **9** as a pale yellow oil (126 mg, 85%): $[\alpha]_D^{23}$ –53.8 (c 1, chloroform); ν_{max}(liquid film) 3402, 1745, 1261, 1231, 1080, 732 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.38-7.25 (m, 5H, H_{Ar}), 5.91 (m, 1H, CH_2CH=CH_2), 5.30 (m, 1H, CH_2CH=C*H*H), 5.18 (m, 1H, CH_2CH=C*H*H), 5.00 (bs, 1H, H-1), 4.82 (s, 1H, H-2), 4.77 (d, 1H, J = 12.1 Hz, C*H*HPh), 4.59 (d, 1H, J = 12.1 Hz, CH*H*Ph), 4.30-4.19 (m, 2H, C*H*HCH=CH_2, H-5), 4.02 (m, 1H, CH*H*CH=CH_2), 3.80 (s, 1H, H-6), 3.78 (s, 1H, H-6'), 3.72 (bd, 1H, $J_{4,3}$ = 3.0 Hz, $J_{4,OH}$ = 10.5 Hz, H-4), 3.64 (t, 1H, $J_{3,4}$ = $J_{3,2}$ = 3.0 Hz, H-3), 2.57 (d, 1H, J = 10.5 Hz, OH), 2.05 (s, 3H, OAc), 1.61 (m, 1H, CH_{thexyl}), 0.90 (s, 3H, $CH_{3\ thexyl}$), 0.87 (s, 6H, $CH_{3\ thexyl}$), 0.86

(s, 3H, $CH_{3\ thexyl}$), 0.15 (s, 6H, CH_3 on Si). Anal. Calcd for $C_{26}H_{42}O_7Si$ (494.7): C, 63.13; H, 8.56. Found: C, 63.42; H, 8.59.

Thexyldimethylsilyl 6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (14). Compound **10**[7] (33 g, 88.47 mmol) was dissolved in a 7:3 THF:MeOH mixture (600 mL) and cooled to 0°C. Gaseous ammonia was bubbled until the starting material disappeared, then nitrogen was bubbled until ammonia was removed and the solvent was evaporated under reduced pressure. The residue was dissolved in DMF (10 mL) and thexyldimethylsilyl chloride (20.8 mL, 106 mmol) and imidazole (12 g, 177 mmol) were added. The mixture was stirred for 20 h, then the solvent was evaporated. The residue was dissolved in dichloromethane (200 mL) and washed with 5% ammonium chloride (2 × 200 mL); the organic layer was dried and evaporated, obtaining crude **11**. Compound **11** was dissolved in MeOH (20 mL) and a catalytic amount of sodium methoxide (1M solution in methanol, 3 mL) was added. After 3 h the mixture was neutralized with acidic resin (Amberlite IR-120, H^+ form), filtered and evaporated giving compound **12**. Crude **12** was dissolved in dry DMF (40 mL), then benzyl bromide (47 mL, 397 mmol) was added dropwise and NaH (8.5 g, 354 mmol) was added portionwise; the mixture was stirred overnight, then the reaction was quenched with methanol, and diluted with diethyl ether (300 mL). The mixture was washed with water and the aqueous phase was extracted with diethyl ether (2 × 200 mL), the organic layers were dried ($NaSO_4$), filtered and evaporated, giving crude **13**. To a solution of **13** in a 2:1 mixture of acetic anhydride:acetic acid (210 mL) a solution of freshly fused zinc chloride (100 g, 735 mmol) in acetic anhydride:acetic acid (2:1, 210 mL) was added. The reaction was stirred for 4 h at room temperature, then was quenched with water and extracted with ethyl acetate. The organic layers were collected and washed with satd aq. $NaHCO_3$ (2×200 mL) and water, dried (Na_2SO_4), filtered and concentrated. Flash chromatography (hexane:ethyl acetate 9:1) afforded compound **14** as a yellow oil (41 g, 81% overall yield); $[\alpha]_D^{23}$ –7.0 (c 1.3, chloroform); ν_{max}(liquid film) 2101, 1739, 1251, 1038 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.40-7.24 (m, 10H, H_{Ar}), 4.93 (d, 1H, J = 10.8 Hz, C*H*HPh), 4.86 (d, 1H, J = 11.0 Hz, C*H*HPh), 4.79 (d, 1H, J = 10.8 Hz, CH*H*Ph), 4.58 (d, 1H, J = 11.0 Hz, CH*H*Ph), 4.51 (d, 1H, J = 7.3 Hz, H-1), 4.34 (bd, 1H, $J_{6,5}$ = 1.6 Hz, $J_{6,6'}$ = 11.0 Hz, H-6), 4.13 (ddd, 1H, $J_{5,6}$ = 1.6 Hz, $J_{5,6'}$ = 5.4 Hz, $J_{5,4}$ = 1.4 Hz, H-5), 3.49-3.34 (m, 4H, H-3, H-4, H-2, H-6'), 2.03 (s, 3H, OAc), 1.65 (m, 1H, CH_{thexyl}), 0.93 (s, 6H, $CH_{3\ thexyl}$), 0.89 (s, 6H, $CH_{3\ thexyl}$), 0.20 (s, 3H, CH_3 on Si), 0.19 (s, 3H, CH_3 on Si). Anal. Calcd for $C_{30}H_{43}O_6N_3Si$ (569.7): C, 63.24; H, 7.61; N, 7.37. Found: C, 63.42; H, 7.32; N, 7.15.

6-*O*-Acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl trichloroacetimidate (15). Compound **14** (446 mg, 0.785 mmol) was dissolved in dry THF (5 mL) and cooled to -40°C. HOAc (53 μL, 0.942 mmol) and 1M tetrabutylammonium fluoride in THF (942 μL, 0.942 mmol) were added. After 25 h the reaction was quenched with brine, the organic layer was washed with brine (2×20 mL) and the aqueous phases were unified and extracted with ethyl acetate (3×20 mL). The mixture was dried over Na_2SO_4, filtered and the solvent evaporated. The crude product was dissolved in dry dichloromethane (8 mL) containing 4 Å molecular sieves.

Dry potassium carbonate (142 mg, 1.03 mmol) and trichloroacetonitrile (387 μL, 3.86 mmol) were added and the mixture was stirred for 20 h. After filtration through celite and evaporation of the solvent the crude product was purified by flash chromatography (8:2 hexane:ethyl acetate + 1% triethylamine) yielding 45 mg of α anomer (10%). Further elution gave **15** as a white solid (262 mg, 58%): mp 85-86°; $[\alpha]_D^{23}$ +13.0 (c 1.1, chloroform); $\nu_{max}(CHCl_3)$ 3315, 2100, 1658 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.73 (bs, 1H, NH), 7.46-7.20 (m, 10H, H_{Ar}), 5.62 (d, 1H, J = 7.9 Hz, H-1), 4.93 (d, 1H, J = 10.7 Hz, C*H*HPh), 4.85 (d, 2H, CH_2Ph), 4.59 (d, 1H, J = 10.7 Hz, CH*H*Ph), 4.33 (dd, 1H, $J_{6,6'}$ = 12.7 Hz, $J_{6,5}$ = 1.5 Hz, H-6), 4.22 (ddd, 1H, $J_{5,6}$ = 1.5 Hz, $J_{5,6'}$ = 3.5 Hz, $J_{5,4}$ = 11.8 Hz, H-5), 3.71-3.56 (m, 4H, H-2, H-3, H-4, H-6'), 2.01 (s, 3H, OAc). Anal. Calcd for $C_{24}H_{25}O_6N_4Cl_3$ (571.8): C, 50.41; H, 4.41; N, 9.80. Found: C, 50.18; H, 4.35; N, 9.93.

Allyl (6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-2-*O*-acetyl-3-*O*-benzyl-6-*O*-thexyldimethylsilyl-α-L-idopyranoside (16). Imidate **15** (1.9 g, 3.4 mmol) and alcohol **9** (937 mg, 2.0 mmol) were dissolved in dry toluene (5 mL) under argon atmosphere and the solution was cooled to -20°C. A 1M solution of *tert*-butyldimethylsilyl triflate in dry dichloromethane (600 μL, 0.6 mmol) was added dropwise and after 30 min. the reaction was neutralized with triethylamine and the solvent evaporated. Flash chromatography (85:15 hexane:ethyl acetate) afforded disaccharide **16** as a pale yellow oil (1.5 g, 83%): $[\alpha]_D^{23}$ +8.0 (c 1, chloroform); ν_{max}(liquid film) 3950, 2100, 1739, 1231, 1098, 1022, 829 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.47-7.27 (m, 15H, H_{Ar}), 5.90 (m, 1H, CH_2CH=CH_2), 5.29 (m, 1H, C*H*HCH=CH_2), 5.17 (m, 1H, CH*H*CH=CH_2), 4.96 (t, 1H, $J_{2,3}$ = $J_{2,1}$ = 2.6 Hz, H-2a), 4.93-4.82 (m, 5H, H-1a, H-1b, CH_2Ph, C*H*HPh), 4.77 (d, 1H, J = 11.8 Hz, C*H*HPh), 4.68 (d, 1H, J = 11.8 Hz, CH*H*Ph), 4.57 (d, 1H, J = 11.2 Hz, CH*H*Ph), 4.27-4.16 (m, 4H, H-5a, H-6a, H-6'a, C*H*HCH=CH_2), 4.04 (m, 1H, CH*H*CH=CH_2), 3.98-3.89 (m, 4H, H-4a, H-3b, H-5b, H-3a), 3.84-3.81 (m, 2H, H-6b, H-6'b), 3.51 (t, 1H, $J_{4,5}$ = $J_{4,3}$ = 9.3 Hz, H-4b), 3.34 (dd, 1H, $J_{2,3}$ = 10.2 Hz, $J_{2,1}$ = 3.5 Hz, H-2b), 2.10 (s, 3H, OAc), 1.99 (s, 3H, OAc), 1.61 (m, 1H, CH_{thexyl}), 0.90 (s, 3H, $CH_{3\ thexyl}$), 0.87 (s, 3H, $CH_{3\ thexyl}$), 0.84 (s, 6H, $CH_{3\ thexyl}$), 0.12 (s, 3H, CH_3 on Si), 0.11 (s, 3H, CH_3 on Si); ^{13}C NMR (75.46 MHz, $CDCl_3$) δ 170.48 (s, C=O), 170.01 (s, C=O), 133.94 (d, CH_2=*C*HCH_2), 117.16 (t, *C*H_2=CHCH_2), 97.10, 96.01 (2d, C-1a, C-1b), 80.40, 77.99, 72.53, 70.93, 69.67, 69.26, 68.59, 63.64, (8 d, C-2a, C-3a, C-4a, C-5a, C-2b, C-3b, C-4b, C-5b), 75.50, 74.93, 72.42, 68.18, 62.76, 62.16 (6 t, 3 *C*H_2Ph, CH_2=CHCH_2, C-6a, C-6b), 34.03 (d, CH_{thexyl}), 29.68 (s, $C_{q\ thexyl}$), 25.92, 20.79, 20.30, 18.54 (4 q, 6C, 2 *C*H_3C=O, 4 *C*$H_{3\ thexyl}$), -3.25, -3.42 (2q, CH_3 on Si). Anal. Calcd for $C_{48}H_{65}O_{12}N_3Si$ (904.0): C, 63.77; H, 7.25; N, 4.65. Found: C, 64.01; H, 7.37; N, 4.91.

Allyl (6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-methyl-2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosiduronate (1). A solution of disaccharide **16** (800 mg, 0.885 mmol) in acetone (10 mL) was cooled at 0°C; Jones' reagent (2.5 g of CrO_3 in 10 mL of 3.5M H_2SO_4) was added (750 μL, 2.2 eq). The reaction was stirred at the same temperature and after 10 h another portion of Jones' reagent (same amount) was added. After 5 h the reaction was poured into ice-cold water and extracted with chloroform (3×100 mL). The organic layers were washed with water until the pH was neutral, dried over Na_2SO_4 and evaporated. The crude

product was dissolved in methanol (10 mL) and an ethereal solution of diazomethane was added until the starting material disappeared. After evaporation of the solvent flash chromatography (7:3 hexane:ethyl acetate) afforded methyl ester **1** a white foam (410 mg, 59%): $[\alpha]_D^{23}$ +11.3 (c 1, chloroform); $\nu_{max}(CHCl_3)$ 2104, 1758, 1740, 1550, 881 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.41-7.30 (m, 5H, H_{Ar}), 5.90 (m, 1H, CH_2CH=CH_2), 5.28 (m, 1H, CH_2CH=C*H*H), 5.17 (m, 1H CH_2CH=CH*H*), 5.05 (bs, 1H, H-1a), 4.96 (bs, 1H, H-2a), 4.90 (d, 1H, $J_{1,2}$ = 3.4 Hz, H-1b), 4.82 (bs, 3H, CH_2Ph, H-5a), 4.81 (d, 1H, J = 11.2 Hz, C*H*HPh), 4.80 (d, 1H, J = 11.2 Hz, C*H*HPh), 4.68 (d, 1H, J = 11.2 Hz, CH*H*Ph), 4.58 (d, 1H, J = 11.2 Hz, CH*H*Ph), 4.34 (bd, 1H, $J_{6,6'}$ = 12.3 Hz, H-6b), 4.25 (m, 1H, C*H*HCH=CH_2), 4.17 (dd, 1H, $J_{6,6'}$ = 12.3 Hz, $J_{5,6'}$ = 3.9 Hz, H-6'b), 4.12-4.04 (m, 2H, CH*H*CH=CH_2, H-4a), 3.95-3.84 (m, 3H, H-3a, H-3b, H-5b,), 3.78 (s, 3H, COOCH_3), 3.51 (t, 1H, $J_{4,3}$ = $J_{4,5}$ = 9.4 Hz, H-4b), 3.27 (dd, 1H, $J_{1,2}$ = 3.4 Hz, $J_{2,3}$ = 10.2 Hz, H-2b), 2.10 (s, 3H, OAc), 2.05 (s, 3H, OAc); ^{13}C NMR (75.46 MHz, $CDCl_3$) δ 170.45, 169.96, 162.52 (3s, C=O), 133.37 (d, CH_2*C*H=CH_2), 117.43 (t, CH_2CH=*C*H_2), 97.67, 97.20, (2d, C-1a, C-1b), 79.99, 77.56, 72.93, 72.19, 69.81, 67.63, 63.37 (7d, 8C, C-2a, C-3a, C-4a, C-5a, C-2b, C-3b, C-4b, C-5b), 75.321, 74.74, 68.83, 62.37 (4t, 3 *C*H_2Ph, C-6b), 72.20 (t, *C*H_2CH=CH_2), 52.22 (q, COO*C*H3), 20.78 (q, 2 *C*H_3C=O). Anal. Calcd for $C_{41}H_{47}O_{13}N_3$ (789.8): C, 62.35; H, 6.00; N, 5.32. Found: C,62.59; H, 6.28; N, 5.19.

2-Azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl trichloroacetimidate (18). Compound **17** (7.0 g, 13.4 mmol) was dissolved in dry THF (20 mL) and the solution was cooled to –40°C. HOAc (0.961 mL, 16.8 mmol) and 1M tetrabutylammonium fluoride in THF (16.8 mL, 16.8 mmol) were added. After 4 h the reaction was diluted with ethyl acetate and washed with brine (2×200 mL). The aqueous phase was extracted with ethyl acetate (3×100 mL), then the organic layers were dried (Na_2SO_4), filtered and evaporated. The crude product was dissolved in dry dichloromethane (30 mL), dry potassium carbonate (2.96 g, 21.44 mmol) and trichloroacetonitrile (13,8 mL, 138 mmol) were added. After 5 h the reaction was filtered through celite and the solvent evaporated. Crystallization (8:2 hexane:ethyl acetate) afforded β-trichloroacetimidate **17** (5.0 g, 71%) as white needles: mp 102-103°C; $[\alpha]_D^{23}$ –55 (c 1, chloroform); $\nu_{max}(CHCl_3)$ 3320, 2100, 1671 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.76 (bs, 1H, NH), 7.55-7.23 (m, 10H, H_{Ar}), 5.70 (d, 1H, J = 8.3 Hz, H-1), 5.58 (s, 1H, C*H*Ph), 4.95 (d, 1H, J = 11.5 Hz, C*H*HPh), 4.82 (d, 1H, CH*H*Ph), 4.40 (dd, 1H, $J_{6,5}$ = 4.9 Hz, $J_{6,6'}$ = 10.1 Hz, H-6), 3.81 (t, 1H, $J_{6',5}$ = $J_{6,6'}$ = 10.1 Hz, H-6'), 3.79 (t, 1H, $J_{4,3}$ = $J_{4,5}$ = 10.1 Hz, H-4), 3.74-3.65 (m, 2H, H-2, H-3), 3.57 (dt, 1H, $J_{5,6}$ = 4.9 Hz, $J_{5,6'}$ = $J_{5,4}$ = 10.1 Hz, H-5). Anal. Calcd for $C_{22}H_{21}O_5N_4C_{13}$ (527): C, 50.07; H, 4.01; N, 10.62. Found: C, 50.31; H, 4.13; N, 10.37.

Allyl (2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl)-(1→4)-2-*O*-acetyl-3-*O*-benzyl-6-*O*-thexyldimethylsilyl-α-L-idopyranoside (19). Acceptor **9** (1.49 g, 3.0 mmol) and donor **18** (2.3 g, 4.36 mmol) were dissolved in dry toluene (20 mL) under argon. The mixture was cooled to -20°C and *tert*-butyldimethylsilyl triflate (1M solution in dry dichloromethane, 429 μL, 0.429 mmol) was added. After 1,5 h the reaction mixture was neutralized with triethylamine, and the solvent was evaporated under reduced pressure. The

crude product was purified by flash chromatography (9:1 hexane:ethyl acetate) affording **19** as a colourless oil (2.1 g, 84%): $[\alpha]_D^{23}$ –32.2 (c 1.2, chloroform); ν_{max}(liquid film) 2100, 1738, 1450, 1236, 830 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.53-7.22 (m, 15H, H_{Ar}), 5.90 (m, 1H, CH_2CH=CH_2), 5.55 (s, 1H, C*H*Ph), 5.33-5.17 (m, 2H, CH_2CH=CH_2), 4.97-4.91 (m, 3H, H-1a, H-2a, C*H*HPh), 4.83 (d, 1H, $J_{1,2}$ = 3.8 Hz, H-1b), 4.80 (d, 1H, J = 12.0 Hz, C*H*HPh), 4.75 (d, 1H, J = 12.4 Hz, CH*H*Ph), 4.70 (d, 1H, J = 12.0 Hz, CH*H*Ph), 4.26 (m, 1H, C*H*HCH=CH_2), 4.22-4.13 (m, 2H, H-5a, H-6b), 4.04 (m, 1H, CH*H*CH=CH_2), 4.0 (t, 1H, $J_{3,4}$ = $J_{3,2}$ = 9.4 Hz, H-3b), 3.94 (t, 1H, $J_{3,4}$ =$J_{3,2}$ = 3.9 Hz, H-3a), 3.91-3.78 (m, 4H, H-4a, H-6a, H-6'a, H-5b), 3.70 (t, 1H, $J_{6,6'}$ = $J_{6',5}$ = 9.8 Hz, H-6'b), 3.67 (t, 1H, $J_{4,5}$ = $J_{4,3}$ = 9.4 Hz, H-4b), 3.38 (dd, 1H, $J_{2,1}$ = 3.8 Hz, $J_{2,3}$ = 9.4 Hz, H-2b), 2.08 (s, 3H, OAc), 1.65 (m, 1H, CH_{thexyl}), 0.90 (s, 6H, 2 $CH_{3\ thexyl}$), 0.67 (s, 6H, 2 $CH_{3\ thexyl}$), 0.16 (s, 3H, CH_3 on Si), 0.14 (s, 1H, CH_3 on Si); ^{13}C NMR (75.46 MHz, $CDCl_3$) δ 170.07 (s, C=O), 133.92 (d, CH_2CH=CH_2), 117.26 (t, CH_2CH=CH_2), 101.52 (d, *C*HPh), 97.45, 97.13 (2 d, C-1a, C-1b), 82.72, 76.38, 73.00, 72.43, 69.37, 63.26 (8 d, C-2a, C-2b, C-3a, C-3b, C-4a, C-4b, C-5a, C-5b), 74.91, 72.57, 68.73, 62.30 (4 t, 5C, C-6a, C-6b, 2 *C*H_2Ph *C*H_2CH=CH_2), 34.06 (d, CH_{thexyl}), 25.19 (s, $C_{q\ thexyl}$), 20.84, 20.33, 18.55 (3 q, 5C, 4 $CH_{3\ thexyl}$, *C*H_3C=O), -3.3 (2 q, 2 CH_3 on Si). Anal. Calcd for $C_{46}H_{61}O_{11}N_3Si$ (860.1): C, 64.24; H, 7.15; N, 4.89. Found: C, 64.41; H, 7.12; N, 5.03.

Allyl (2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl)-(1→4)-2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranoside (20). 1M tetrabutylammonium fluoride in dry THF (2.39 mL) was added to a solution of **19** (1.63 g, 1.90 mmol) in dry THF (10 mL) at 0°C. The mixture was stirred at 0°C and after 3.5 h water was added and after dilution with ethyl acetate the organic layer was washed with brine (2×40 mL). The collected aqueous layers were extracted with ethyl acetate (3×20 mL); the organic layers were dried (Na_2SO_4), filtered and evaporated under reduced pressure. Flash chromatography (6:4 hexane:ethyl acetate) gave **20** as a white solid (1.21 g, 88%): mp 41-43°C; $[\alpha]_D^{23}$ –66.0 (c 1.18, chloroform); ν_{max}(liquid film) 3350-3100 (b), 2100, 1741, 1440 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.50-7.20 (m, 15H, H_{Ar}), 4.92 (m, 1H, CH_2CH=CH_2), 5.55 (s, 1H, C*H*Ph), 5.31 (m, 1H, CH_2CH=C*H*H), 5.20 (m, 1H, CH_2CH=CH*H*), 4.99 (t, 1H, $J_{2,3}$ = $J_{2,1}$ = 2.3 Hz, H-2a), 4.95 (d, 1H, J = 11.0 Hz, C*H*HPh), 4.90 (bs, 1H, H-1a), 4.82 (d, 1H, J = 11.8 Hz, C*H*HPh), 4.76 (d, 1H, $J_{1,2}$ = 3.9 Hz, H-1b), 4.75 (d, 1H, J = 11.0 Hz, CH*H*Ph), 4.64 (d, 1H, J = 11.8 Hz, CH*H*Ph), 4.32-4.22 (m, 3H, C*H*HCH=CH_2, H-6b, H-5a), 4.04 (m, 1H, CH*H*CH=CH_2), 4.00 (t, 1 H, $J_{3,2}$ = $J_{3,4}$ = 9.8 Hz, H-3b), 3.95-3.83 (m, 3H, H-5b, H-6a, H-3a), 3.76-3.64 (m, 4H, H-4a, H-4b, H-6'b, H-6'a), 3.39 (dd, 1H, $J_{2,1}$ = 3.9 Hz, $J_{2,3}$ = 9.8 Hz, H-2b), 2.10 (s, 3H, OAc); ^{13}C NMR (75.46 MHz, $CDCl_3$) δ 170.17 (s, C=O), 134.43 (d, CH_2CH=CH_2), 117.50 (t, CH_2CH=CH_2), 101.43 (d, *C*HPh), 97.62, 97.22 (2d, C-1a, C-1b), 82.54, 76.52, 72.72, 71.28, 68.56, 67.66, 63.48, 63.09 (8d, C-2a, C-2b, C-3a, C-3b, C-4a, C-4b, C-5a, C-5b), 74.98, 72.20, 68.44, 62.16, 61.87 (5t, *C*H_2CH=CH_2, 2 *C*H_2Ph, C-6a, C-6b), 20.8 (q, *C*H_3CO). Anal. Calcd for $C_{38}H_{43}O_{11}N_3$ (717.7): C, 63.59; H, 6.04; N, 5.85. Found: C, 63.21; H, 6.30; N, 6.02.

Allyl (2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl)-(1→4)-methyl-2-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosiduronate (2). In a round bottomed flask containing freshly activated 4 Å molecular sieves (200 mg) and a solution of disaccharide **20** (204 mg, 0.285 mmol) and acetic anhydride (28 μL, 0.285 mmol) in dry dichloromethane (5 mL) a solution of PDC (536 mg, 1.42 mmol) in dry dichloromethane (5 mL) was dropped. After stirring at room temperature for 20 h the mixture was filtered over silica gel, then concentrated and dissolved in methanol (5 mL). An ethereal solution of diazomethane was added until carboxylic acid disappeared. The solution was concentrated and the crude product was purified by flash chromatography (85:15 hexane:ethyl acetate) affording **2** (170 mg, 80%) as a white solid: mp 40-42°C; $[\alpha]_D^{23}$ –29.0 (c 1.04, chloroform); $\nu_{max}(CHCl_3)$ 2100, 1760, 1732, 1548, 885 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.60-7.30 (m, 15H, H_{Ar}), 5.89 (m, 1H, CH_2CH=CH_2), 5.53 (s, 1H, CHPh), 5.33-5.17 (m, 2H, CH_2CH=CH_2), 5.06 (bs, 1H, H-1a), 4.95 (bs, 1H, H-2a), 4.91 (d, 1H, J = 11.2 Hz, CHHPh), 4.83 (d, 1H, J = 11.7 Hz, CHHPh), 4.84 (d, 1H, $J_{5,4}$ = 2.8 Hz, H-5a), 4.79 (d, 1H, $J_{1,2}$ = 3.8 Hz, H-1b), 4.72 (d, 1H, J = 11.2 Hz, CHHPh), 4.63 (d, 1H, J = 11.7 Hz, CHHPh), 4.29 (dd, 1H, $J_{6,6'}$ = 10.0 Hz, $J_{6,5}$ = 4.7 Hz, H-6b), 4.20-4.04 (m, 3H, CH_2CH=CH_2, H-4a), 3.94 (t, 1H, $J_{3,2}$ = $J_{3,4}$ = 9.4 Hz, H-3b), 3.89 (t, 1H, $J_{3,2}$ = $J_{3,4}$ = 3.0 Hz, H-3a), 3.86-3.80 (m, 1H, H-5b), 3.78 (s, 3H, $COOCH_3$), 3.60 (dd, 1H, $J_{6',6}$ = $J_{6',5}$ = 10.0 Hz, H-6'b), 3.64 (t, 1H, $J_{4,3}$ = $J_{4,5}$ = 9.4 Hz, H-4b), 3.33 (dd, 1H, $J_{2,1}$ = 3.8 Hz, $J_{2,3}$ = 9.4 Hz, H-2b), 2.08 (s, 3H, OAc); ^{13}C NMR (75.46 MHz, $CDCl_3$) δ 170.07 (s, C=O), 169.49 (s, C=O), 133.44 (d, CH_2CH=CH_2), 117.54 (t, CH_2CH=CH_2), 101.55 (d, CHPh), 98.14, 97.83 (2d, C-1a, C-1b), 82.55, 76.02, 73.69, 72.46, 67.53, 63.20, 63.04 (7d, 8C, C-2a, C-2b, C-3a, C-3b, C-4a, C-4b, C-5a, C-5b), 74.81, 72.31, 68.87, 68.56 (4t, 2 CH_2Ph, CH_2CH=CH_2, C-6b), 52.24 (q, $COOCH_3$), 20.79 (q, CH_3C=O). Anal. Calcd for $C_{39}H_{43}O_{12}N_3$ (745.8): C, 62.81; H, 5.81; N, 5.63. Found: C, 62.80; H, 5.76; N, 5.41.

Allyl *O*-(2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-methyl-3-*O*-benzyl-α-L-idopyranosiduronate (21). Compound **1** (105 mg, 0.133 mmol) was dissolved in dry methanol (5 mL) and a 0.9M solution of sodium methoxide (15 μL, 0.013 mmol) was added. After 5 h the reaction was neutralized with IR-120 (H^+ form), the resin was removed by filtration and the solvent evaporated. The crude product was used in the next steps without further purification.

Allyl (2-azido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-sulfo-α-D-glucopyranosyl)-(1→4)-methyl-3-*O*-benzyl-2-*O*-sulfo-α-L-idopyranosiduronate disodium salt (22). Compound **21** (96 mg, 0.136 mmol) was dissolved in dry DMF (2 mL) and sulfur trioxide trimethylamine complex (190 mg, 1.36 mmol) was added. The reaction was warmed to 45°C and stirred for 18 h at this temperature; after addition of methanol (1 mL) the solvent was evaporated. The crude product was dissolved in ethanol and first eluted from a column of Amberlyst 15 (Na^+ form) resin (eluent: ethanol) and successively on silica gel 230-400 mesh (6:4 chloroform:ethanol + 1% triethylamine), obtaining compound **22** as a white glass (110 mg, 86%): $[\alpha]_D^{23}$ +2.5 (c 1, chloroform); ν_{max}(liquid film) 2100, 1754, 1642, 1476, 1247, 1205, 1008, 911 cm^{-1}; δ_H (300 MHz, D_2O) 7.69-7.51 (m, 5H, H_{Ar}), 6.13 (m, 1H, CH_2CH=CH_2), 5.56 (m, 1H, CH_2CH=CHH), 5.43 (m, 1H, CH_2CH=CHH), 5.34 (s, 1H, H-1a), 5.14 (d,

1H, $J_{1,2}$ = 3.4 Hz, H-1b), 5.03 (s, 1H, H-5a), 5.01-4.81 (m, 6H, 3 CH_2Ph), 4.53 (bs, 1H, H-2a), 4.44-4.27 (m, 5H, H-3a, CH_2CH=CH_2, H-6b, H-6'b), 4.21 (bs, 1H, H-4a), 3.98 (t, 1H, $J_{3,2}$ = $J_{3,4}$ = 8.6 Hz, H-3b), 3.92 (s, 3H, COOCH_3), 3.86-3.74 (m, 2H, H-4b, H-5b), 3.47 (dd, $J_{2,1}$ = 3.4 Hz, $J_{2,3}$ = 8.6 Hz, H-2b); ^{13}C NMR (125.76 MHz, 1:1 D_2O:CD_3OD, T = 303K) δ 171.91 (s, C=O), 137.75 (d, CH_2CH=CH_2), 117.82 (t, CH_2CH=CH_2), 99.37 (d, C-1a), 97.05 (d, C-1b), 80.32 (d, C-3b), 78.23 (d, C-4b), 72.03 (d, C-3a), 71.91 (2 d, C-4a, C-2a), 71.09 (d, C-5b), 69.58 (t, CH_2CH=CH_2), 67.70 (d, C-5a), 66.48 (d, C-6b), 63.93 (d, C-2b), 53.81 (q, COOCH_3). Anal. Calcd for $C_{39}H_{41}O_{17}N_3S_2Na_2$ (933.2): C, 48.84; H, 4.43; N, 4.50. Found: C, 49.09; H, 4.21; N, 4.14.

Propyl *O*-(2-deoxy-2-sulfamino-6-*O*-sulfo-α-D-glucopyranosyl)-(1→4)-2-*O*-sulfo-α-L-idopyranosiduronate tetrasodium salt (3).

(i) Saponification. Aq 2.5M NaOH (1.32 mL, 3.3 mmol) was dropped into a solution of sodium salt **22** (150 mg, 0.165 mmol) in methanol (3 mL) at 0°C; after 6 h the solution was diluted with methanol (1 mL), then was eluted (eluent: methanol) from a column of Dowex-50 W X8, (20-50 mesh, H^+ form). The fractions containing the product were concentrated to ca 3 mL and finally eluted from the same resin in the Na^+ form yielding a compound used for the next step without characterization.

(ii) Hydrogenation. The compound obtained from the previous reaction was dissolved in 1:1 methanol:water (4 mL), then $Pd(OH)_2$/C (30 mg) and a few drops of acetic acid were added and the reaction mixture was stirred in hydrogen atmosphere. After 36 h the mixture was filtered through a celite pad, concentrated and lyophilized obtaining 103 mg of the crude propyl glycoside.

(iii) N-sulfation. The hydrogenated product was dissolved in satd. aq $NaHCO_3$ (20 mL) and sulfur trioxide trimethylamine complex (114 mg, 0.825 mmol) was added. After stirring for 24 h another portion of sulfur trioxide trimethylamine complex (23 mg, 0.165 mmol) was added, maintaining the pH at ca 9.0 by addition of solid $NaHCO_3$. After 24 h more sulfur trioxide trimethylamine complex was added (20 mg, 0.140 mmol), always maintaining the pH at ca 9.0 by addition of solid $NaHCO_3$. Finally the solid was filtered off and the solvent evaporated. The crude product was eluted through a column of Sephadex G 10 using 10:90 water:ethanol (1×80 cm, V_o = 22 mL, V_t = 30 mL) affording **3** (108 mg, 90% from **21**) as a white glass. $[\alpha]_D^{23}$ +10.2 (c 0.9, water); ν_{max}(KBr) 3567, 3306, 2964, 2880, 1610, 1142 cm^{-1}; δ_H (500 MHz, D_2O) 5.35 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1b), 5.16 (d, 1H, $J_{1,2}$ = 2.5 Hz, H-1a), 4.52 (d, 1H, $J_{5,4}$ = 2.7 Hz, H-5a), 4.37 (dd, 1H, $J_{6,6'}$ = 11.4 Hz, $J_{6,5}$ = 3.0 Hz, H-6b), 4.26 (dd, 1H, $J_{2,1}$ = 2.5 Hz, $J_{2,3}$ = 5.1 Hz, H-2a), 4.23 (dd, 1H, $J_{2,3}$ = 5.1 Hz, $J_{3,4}$ = 3.9 Hz, H-3a), 4.21 (dd, 1H, $J_{6',5}$ = 2.3 Hz, $J_{6,6'}$ = 11.4 Hz, H-6'b), 4.07 (dd, $J_{3,4}$ = 3.9 Hz, $J_{4,5}$ = 2.7 Hz, 1H, H-4a), 3.99 (ddd, 1H, $J_{5,4}$ = 10.4 Hz, $J_{5,6}$ = 3.0 Hz, $J_{5,6'}$ = 2.3 Hz, H-5b), 3.68 (dd, 1H, $J_{3,2}$ = 10.4 Hz, $J_{3,4}$ = 9.5 Hz, H-3b), 3.68 (m, 1H, CHHCH$_2$CH$_3$), 3.58 (dd, $J_{4,3}$ = 9.5 Hz, $J_{4,5}$ = 10.4 Hz, 1H, H-4b), 3.58 (m, 1H, CHHCH$_2$CH$_3$), 3.26 (dd, 1H, $J_{2,1}$ = 3.6 Hz, $J_{2,3}$ = 10.4 Hz, H-2b), 1.61 (m, 2H, CH$_2$CH_2CH$_3$), 0.89 (m, 3H, CH$_2$CH$_2$CH_3); ^{13}C (125.76 MHz, D_2O) δ 170.5 (s, C=O), 101.2 (d, C-1a), 99.9 (d, C-1b), 78.5 (d, C-2a), 78.4 (d, C-4a), 73.7 (d, C-3b), 73.1 (d, C-5b), 72.9 (t, $CH_2CH_2CH_3$), 72.6 (d, C-4b), 71.0 (d, C-3a), 70.9 (d, C-5a), 69.1 (t, C-6b), 60.6 (d, C-2b), 24.6

(t, $CH_2CH_2CH_3$), 12.5 (q, $CH_2CH_2CH_3$). Anal. Calcd for $C_{15}H_{23}O_{20}NS_3Na_4$ (725.5): C, 24.83; H, 3.20; N, 1.93. Found: C, 24.81; H, 3.46; N, 2.12.

Allyl (6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-methyl-3-*O*-benzyl-α-L-idopyranosiduronate (23). Compound **21** (300 mg, 0.425 mmol) was dissolved in dichloromethane (5 mL) under inert atmosphere; the solution was cooled at –40°C, then 1M acetic anhydride in dichloromethane (1.08 mL, 1.08 mmol), 1M pyridine in dichloromethane (2.15 mL, 2.15 mmol) and a catalytic amount of 4-(N,N'-dimethyl)-aminopyridine were added. After 6 h the reaction was quenched with methanol and the solvent evaporated. The crude product was purified by flash chromatography (75:25 hexane:ethyl acetate) yielding **23** as a white solid (220 mg, 69%): mp 85-87°C; $[\alpha]_D^{23}$ +0.4 (c 1, chloroform); ν_{max}($CHCl_3$) 3650-3120 (br), 2102, 1760, 1738, 1541 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.39-7.25 (m, 5H, H_{Ar}), 5.93 (m, 1H, CH_2CH=CH_2), 5.32 (m, 1H, CH_2CH=C*H*H), 5.24 (m, 1H, CH_2CH=CH*H*), 5.12 (bs, 1H, H-1a), 5.00 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1b), 4.92 (d, 1H, $J_{5,4}$ = 1.7 Hz, H-5a), 4.83 (d, 1H, J = 11.5 Hz, C*H*HPh), 4.84 (bs, 2H, CH_2Ph), 4.77 (d, 1H, J = 11.5 Hz, C*H*HPh), 4.58 (d, 2H, 2 CH*H*Ph), 4.32-4.05 (m, 5H, H-4a, H-6b, H-6'b, CH_2CH=CH_2), 3.90-3.80 (m, 3H, H-3a, H-3b, H-5b), 3.79 (s, 3H, $COOCH_3$), 3.67-3.47 (m, 3H, H-2a, H-2b, H-4b), 2.07 (s, 3H, OAc); ^{13}C (300 MHz, $CDCl_3$) δ 170.47, 169.95 (2s, C=O), 133.67 (d, CH_2CH=CH_2), 117.38 (t, CH_2CH=CH_2), 100.80, 94.79 (2d, C-1a, C-1b), 81.09, 77.17, 71.74, 71.63, 70.01, 67.00, 66.22, 63.75, 62.20 (7d, 8C, C-2a, C-2b, C-3a, C-3b, C-4a, C-4b, C-5a, C-5b), 75.90, 74.75, 71.92, 68.97, 62.20 (5t, C-6b, 2 CH_2Ph, CH_2CH=CH_2), 52.47, ($COOCH_3$), 20.75 (CH_3C=O). Anal. Calcd for $C_{39}H_{45}O_{12}N_3$ (747.8): C, 62.64; H, 6.07; N, 5.62. Found: C, 62.88; H, 6.21; N, 5.50.

Allyl (6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-methyl-3-*O*-benzyl-2-*O*-sulfo-α-L-idopyranosiduronate sodium salt (24). Compound **23** (64 mg, 0.085 mmol) was dissolved in dry DMF (4 mL) and sulfur trioxide trimethylamine complex (59 mg, 0.43 mmol) was added. The mixture was warmed at 45°C with stirring for 3.5 h, then methanol (2 mL) was added and the solvent evaporated. The crude product was purified as described for compound **22** (eluent for flash chromatography: 10:0.5 chloroform:ethanol + 1% triethylamine), obtaining disaccharide **24** as a clear oil (56 mg, 78%): $[\alpha]_D^{23}$ +9.4 (c 1, chloroform); ν_{max}(liquid film) 2103, 1739, 1730, 1450, 1301, 1225 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 7.89-7.28 (m, 5H, H_{Ar}), 5.86 (m, 1H, CH_2CH=CH_2), 5.37 (bs, 1H, H-1a), 5.30-5.25 (m, 2H, H-1b, CH_2CH=C*H*H), 5.11 (m, 1H, CH_2CH=CH*H*), 4.93 (d, 1H, J = 10.7 Hz, C*H*HPh), 4.91 (bs, 1H, H-5a), 4.81 (d, 1H, J = 11.3 Hz, C*H*HPh), 4.79 (d, 1H, J = 11.5 Hz, C*H*HPh), 4.78 (d, 1H, J = 10.7 Hz, CH*H*Ph), 4.63 (bs, 1H, H-2a) , 4.54 (d, 1H, J = 11.3 Hz, CH*H*Ph), 4.53 (d, 1H, J = 11.5 Hz, CH*H*Ph), 4.34 (bs, 1H, H-3a), 4.27-4.18 (m, 2H, H-6b, C*H*HCH=CH_2), 4.11-4.03 (m, 3H, H-4a, H-6'b, CH*H*CH=CH_2), 3.76 (t, 1H, $J_{3,4}$ = $J_{3,2}$ = 9.4 Hz, H-3b), 3.72 (s, 3H, OAc), 3.56 (dd, 1H, $J_{5,6'}$ = $J_{5,4}$ =9.4 Hz, $J_{5,6}$ = 3.7 Hz, H-5b), 3.42 (t, 1H, $J_{4,5}$ = $J_{4,3}$ = 9.4 Hz, H-4b), 3.40 (dd, 1H, $J_{2,1}$ = 3.9 Hz, $J_{2,3}$ = 9.4 Hz, H-2b); ^{13}C NMR (125.76 MHz, $CDCl_3$, T = 303 K) δ 170.56 (s, C=O), 170.17 (s, C=O), 133.61 (d, CH_2CH=CH_2), 117.00 (t, CH_2CH=CH_2), 98.56 (d, C-1a), 93.71 (d, C-1b), 80.39 (d, C-3b), 77.25 (d, C-4b), 75.56, 74.80, 71.97 (3t, CH_2Ph), 69.81 (d, C-5b), 69.66 (d, C-2a), 69.41 (d, C-4a), 69.28 (t, CH_2CH=CH_2),

69.11 (d, C-3a), 67.04 (d, C-5a), 63.85 (d, C-2b), 62.62 (t, C-6b). Anal. Calcd for $C_{39}H_{44}O_{15}N_3SNa$ (849.84): C, 55.12; H, 5.22; N, 4.94. Found: C, 55.34; H, 4.98; N, 5.02.

Propyl *O*-(2-deoxy-2-sulfamino-α-D-glucopyranosyl)-(1→4)-2-*O*-sulfo-α-L-idopyranosiduronate trisodic salt (4). Compound **24** (53 mg, 0.063 mmol) was submitted to the same deprotection procedure described for the preparation of compound **3**, giving disaccharide **4** as a white glassy solid (33 mg, 86%): $[\alpha]_D^{23}$ +14.3 (c 0.9, water); ν_{max}(KBr) 3566, 3301, 2964, 2881, 1610, 1140 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 5.32 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1b), 5.12 (d, 1H, $J_{1,2}$ = 2.5 Hz, H-1a), 4.49 (d, 1H, $J_{4,5}$ = 2.6 Hz, H-5a), 4.23 (dd, 1H, $J_{2,1}$ =2.5 Hz, $J_{2,3}$ = 4.8 Hz, H-2a), 4.21 (dd, 1H, $J_{3,2}$ = 4.8 Hz, $J_{3,4}$ = 3.5 Hz, H-3a), 4.04 (dd, 1H, $J_{4,3}$ = 3.5 Hz, $J_{4,5}$ = 2.6 Hz, H-4a), 3.83 (dd, 1H, $J_{6,6'}$ = 12.8 Hz, $J_{6,5}$ = 5.9 Hz, H-6b), 3.82 (m, 1H, H-5b), 3.76 (dd, 1H, $J_{6',5}$ = 2.3 Hz, $J_{6',6}$ = 12.8 Hz, H-6'b), 3.67 (m, 1H, C*H*HCH$_2$CH$_3$), 3.65 (dd, 1H, $J_{3,2}$ = 10.4 Hz, $J_{3,4}$ = 8.5 Hz, H-3b), 3.56 (m, 1H, CH*H*CH$_2$CH$_3$), 3.43 (dd, 1H, $J_{4,3}$ = 8.5 Hz, $J_{4,5}$ = 10.5 Hz, H-4b), 3.20 (dd, 1H, $J_{2,1}$ = 3.5 Hz, $J_{2,3}$ = 10.4 Hz, H-2b), 1.59 (m, 2H, CH$_2$C*H*$_2$CH$_3$), 0.89 (m, 3H, CH$_2$CH$_2$C*H*$_3$); ^{13}C NMR (125.76 MHz, D_2O) δ 171.01 (s, C=O), 101.4 (d, C-1a), 100.2 (d, C-1b), 78.8 (d, C-4a), 78.7 (d, C-2a), 74.7 (d, C-5b), 74.0 (d, C-3b), 73.4 (t, *C*H$_2$CH$_2$CH$_3$), 73.1 (d, C-4b), 71.6 (d, C-3a), 71.1 (d, C-5a), 63.4 (t, C-6b), 61.2 (d, C-2b), 24.6 (t, CH$_2$*C*H$_2$CH$_3$), 12.5 (q, CH$_2$CH$_2$*C*H$_3$). Anal. Calcd for $C_{15}H_{24}O_{17}NS_2Na_3$ (623.44): C, 28.90; H, 3.88; N, 2.25. Found: C, 28.63; H, 3.76; N, 2.00.

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