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Synthesis of the disaccharides methyl 4-O-(2′/3′-O-sulfo-β-D-glucopyranosyluronic acid)-2-amino-2-deoxy-α-D-glucopyranoside disodium salts, related to heparin biosynthesis

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The synthesis of the disaccharides methyl 4-O-(2′/3′-O-sulfo-β-D-glucopyranosyluronic acid)-2-amino-2-deoxy-α-D-glucopyranoside **3** and **4** as disodium salts is described. Allyl 4,6-*O*-benzylidene-α-D-glucopyranoside **6** was converted to trichloroacetimidate **20**. Glycosylation of **20** with **5** promoted by $BF_3 \cdot OEt_2$ gave disaccharide **21**. Deacetylation of **21** followed by monoacetylation of the resultant diol **22** afforded the two monoacetylated disaccharides **23** and **24**. Sulfation and deprotection of each disaccharide gave the desired sulfated compounds **3** and **4**.

Keywords: heparin biosynthesis, carbohydrates, sulfated disaccharides, synthesis

Introduction

Heparin and heparan sulfate [1–4], two chemically closely related glycosaminoglycans, are present in many living organisms. Heparin has attracted considerable attention because of its anticoagulant properties, which are used at the clinic for prevention and treatment of venous thrombosis. Heparin inhibits blood coagulation through activation of antithrombin III (AT III), a physiological inhibitor of coagulation, accelerating the inactivation of the serine proteases involved in clotting [5–8].

The carbohydrate backbone of heparin and heparan sulfate is composed of hexuronic acid (D-GlcA and L-IdoA) and D-glucosamine (GlcN) units, arranged in alternating sequence. Sulfate substituents occur mainly at C-2 (*N*-sulfate groups) and C-6 of the GlcN and at C-2 of the IdoA units (*O*-sulfate groups). The possible sulfation sites are only partially occupied, and this variability along with that of the hexuronic acid sequence provides the basis for the extensive structural complexity and heterogeneity typical of these compounds.

Besides the major *O*-sulfation sites, two types of low abundance *O*-sulfate groups have been detected both in heparin and heparan sulfate. A 3-*O*-sulfate group in the GlcN is an essential substituent in the antithrombin binding pentasaccharide sequence [9]. Another rare *O*-sulfate group is present in GlcA residues, at the C-2 or C-3 position, both in heparin and heparan sulfate. The sulfated GlcA unit has been ascribed a potentially important role in control of cell proliferation [10, 11].

The occurrence of sulfated GlcA in heparin-related glycosaminoglycans was first demonstrated by Bienkowsky and Conrad, who analysed the labelled hexuronic acid-2,5-anhydro[1-^{3}H]mannitol (HexA-aMan$_R$) disaccharide fragments obtained by deaminative cleavage of heparin, followed by reduction of the products with NaB^3H_4 [12]. The biosynthesis of this component was demonstrated through enzymatic sulfation of a mouse mastocytoma microsomal polysaccharide; a metabolically labelled GlcA(O-SO_3)-aMan$_R$ component was isolated [13]. Furthermore, it was demonstrated that this unit arose

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through actual sulfation of GlcA and not by back-epimerization of sulfated IdoA units. While it was concluded that the sulfate group must be located either at C-2 or C-3, its precise location remained to be determined. Therefore, **1** and **2** represent the two putative structures of this fragment (Fig. 1).

We now report the synthesis of title disaccharides **3** and **4** which, after a deaminative cleavage/reduction procedure, were compared by anion-exchange HPLC with the natural fragment in order to confirm unambiguously its chemical structure.

Materials and methods

General

Reagents and dry solvents were added *via* oven-dried syringes through septa. Thin-layer chromatography (TLC): Merck silica gel *60 F_{254}* plates; detection by spraying with a 1:1 mixture of 20% H_2SO_4 solution and a solution of I_2 (10 g) and KI (100 g) in H_2O (500 ml) or with a solution containing H_2SO_4 (31 ml), ammonium molybdate (21 g), and $Ce(SO_4)_2$ (1 g) in water (500 ml) followed by heating. Flash column chromatography (FC): Merck silica gel 60 (230–400 mesh). M.p.: Buchi apparatus; uncorrected. Specific rotations ($[\alpha]_D$): Perkin-Elmer 241 polarimeter 20 °C. ^{1}H- and ^{13}C-NMR Spectra: Bruker-AC-300 or Bruker-AM-500 instrument. The chemical shifts for the spectra in D_2O (0.04 M solution at 303 K) are referenced to TSP.

Allyl 3-O-acetyl-4,6-O-benzylidene-β-D-glucopyranoside (7)

Five hundred mg (1.62 mmol) of **6** [14] in 20 ml of vinyl acetate were shaken 4 h at 45° with lipase P on Celite (300 mg). Filtration of the enzyme and evaporation of the solvent yielded 540 mg (95%) of **7** contaminated by traces of the regioisomer. An analytical sample was purified by FC (SiO_2, hexane/AcOEt 7:3). $[\alpha]_D = -48.4°$ ($c = 1$, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$): 7.45–7.30 (m, 5 H, Ph—*H*); 6.00–5.82 (m, 1 H, OCH_2CH=CH_2); 5.48 (s, PhC*H*); 5.21 (t, 1 H, $J = 9.7$, H—3); 5.39–5.20 (m, 2 H, OCH_2CH=CH_2); 4.50 (d, 1 H, $J = 7.7$, H—1); 4.40–4.30 (m, 2 H, H—6_{eq} and 1 H of OCH_2CH=CH_2); 4.15 (dd, $J = 12.7$, 6.3, 1 H of OCH_2CH=CH_2); 3.78 (t, 1 H, $J = 9.9$, H—6_{ax}); 3.7–3.55 (m, 2 H, H—2, H—4); 3.50 (dt, 1 H, $J = 9.9$, 4.9, H—5) 2.58 (d, 1 H, $J = 2.3$, OH); 2.09 (s, 3 H, Ac). Anal. Calc. for $C_{18}H_{22}O_7$: C 61.71, H 6.33; found: C 61.45, H 6.48.

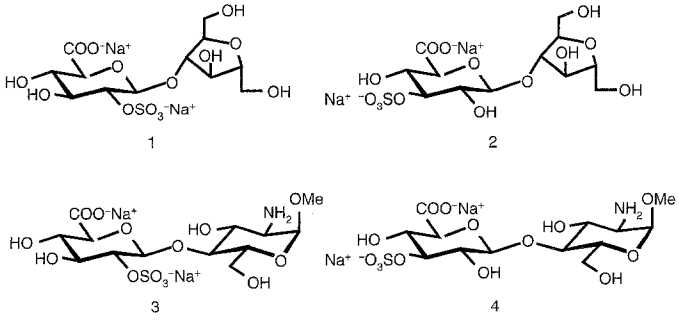

Figure 1.

Allyl 4,6-O-benzylidene-3-O-chloroacetyl-β-D-glucopyranoside (8)

Six hundred mg (1.95 mmol) of **6** and 3 ml of trifluroethyl chloroacetate in 15 ml of toluene/THF 4:1 were stirred 24 h at 45° with lipase P on Celite (300 mg). Filtration of the enzyme, evaporation of the solvent and FC (SiO_2, hexane/AcOEt 6.5:3.5) gave 557 mg (74%) of **8**. $[\alpha]_D = -49.2°$ ($c = 1$, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$): 7.45–7.30 (m, 5 H, Ph—*H*); 6.0–5.8 (m, 1 H, OCH_2CH=CH_2); 5.49 (s, 1 H, PhC*H*); 5.40–5.20 (m, 3 H, H—3, OCH_2CH=CH_2); 4.52 (d, 1 H, $J = 7.7$, H—1); 4.51–4.29 (m, 2 H, H—6_{eq} and 1 H of OCH_2CH=CH_2); 4.15 (dd, 1 H, $J = 12.6$, 6.1, 1 H of OCH_2CH=CH_2); 4.12 (s, 2 H, CH_2Cl); 3.79 (t, 1 H, $J = 9.9$, H—6_{ax}); 3.7–3.6 (m, 2 H, H—2, H—4); 3.50 (dt, 1 H, $J = 9.9$, 4.9, H—5); 2.58 (d, 1 H, $J = 2.3$, OH). Anal. calc. for $C_{18}H_{21}ClO_7$: C 56.18, H 5.50; found: C 55.98, H 5.71.

Allyl 3-O-acetyl-4,6-O-benzylidene-2-O-pivaloyl-β-D-glucopyranoside (9)

To a solution of 500 mg (1.45 mmol) of **7** in 12 ml of CH_2Cl_2, 260 µl (2.2 mmol) of PivCl and 400 µl of Et_3N (2.9 mmol) were added. The mixture was stirred overnight at room temperature then diluted with H_2O. The organic layer was washed sequentially with 5% HCl solution, satd. $NaHCO_3$ solution and H_2O, dried (Na_2SO_4) and evaporated. FC (SiO_2, hexane/AcOEt 9:1) gave 570 mg (92%) of **9**. $[\alpha]_D = -68.9°$ ($c = 1$, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$): 7.50–7.30 (m, 5 H, Ph—*H*); 5.90–5.70 (m, 1 H, OCH_2CH=CH_2); 5.48 (s, 1 H, PhC*H*); 5.36 (t, 1 H, $J = 9.7$, H—3); 5.30–5.15 (m, 2 H, OCH_2CH=CH_2); 5.03 (br. t, H—2); 4.62 (d, 1 H, $J = 7.8$, H—1); 4.40–4.31 (m, 2 H, H—6_{eq} and 1 H of OCH_2CH=CH_2); 4.11 (dd, 1 H, $J = 12.9$, 6.1, 1 H of OCH_2CH=CH_2); 3.85–3.70 (t, 1 H, $J = 9.7$, H—6_{ax}); 3.70 (t, 1 H, $J = 9.7$, H—4); 3.52 (dt, 1 H, $J = 9.7$, 4.8, H—5); 2.01 (s, 3 H, Ac); 1.17 {s, 9 H, $(CH_3)_3C$}. Anal. calc. for $C_{23}H_{30}O_8$: C 63.58, H 6.96; found: C 63.76, H 7.17.

Allyl 2-O-acetyl-4,6-O-benzylidene-3-O-chloroacetyl-β-D-glucopyranoside (10)

To a solution of 500 mg (1.29 mmol) of **8** in 12 ml of CH_2Cl_2 1 ml of pyridine and 620 µl of Ac_2O were added at 0°. The mixture was stirred 48 h at room temperature then diluted with H_2O. The organic layer was washed sequentially with 5% HCl solution, satd. $NaHCO_3$ solution and H_2O, dried (Na_2SO_4) and evaporated. FC (SiO_2,

hexane/AcOEt 8:2) gave 429 mg (78%) of **10**. $[\alpha]_D = -81.2°$ ($c = 1$, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$): 7.45–7.30 (m, 5 H, Ph—*H*); 5.92–5.70 (m, 1 H, OCH_2CH=CH_2); 5.50 (s, 1 H, PhC*H*); 5.35 (t, 1 H, $J = 9.6$, H—3); 5.31–5.16 (m, 2 H, OCH_2CH=CH_2); 5.06 (br. t, 1 H, H—2); 4.64 (d, 1 H, $J = 7.8$, H—1); 4.40–4.34 (m, 2 H, H—6_{eq} and 1 H of OCH_2CH=CH_2); 4.11 (m, 1 H of OCH_2CH=CH_2); 4.02 (s, 2 H, CH_2Cl); 3.85–3.70 (m, 2 H, H—6_{ax}, H—4); 3.52 (dt, 1 H, $J = 9.6$, 4.8, H—5); 2.07 (s, 3 H, Ac). Anal. calc. for $C_{20}H_{23}ClO_8$: C 56.28, H 5.43; found: C 56.12, H 5.61.

Allyl 4,6-O-benzylidene-2,3-di-O-pivaloyl-β-D-gluco-pyranoside (11)

To a solution of **6** (8.4 g, 27.24 mmol) in dry toluene under N_2, Et_3N (30.4 ml, 217.92 mmol), 4-dimethylamino-pyridine (332 mg, 2.72 mmol) and, after 10 min, PivCl (19.9 ml, 163.44 mmol) were added. The mixture was refluxed for 6 h, then cooled and diluted with CH_2Cl_2 and water. The organic layer was washed sequentially with 5% HCl solution, satd. $NaHCO_3$ solution and H_2O, dried (Na_2SO_4) and evaporated. FC (SiO_2, hexane/AcOEt 9.5:0.5 → 9:1) afforded 10.89 g of **11** (82%). $[\alpha]_D = -74.1°$ ($c = 1$, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$): 7.45–7.30 (m, 5 H, Ph—*H*); 5.87–5.76 (m, 1 H, OCH_2CH=CH_2); 5.50 (s, 1 H, PhC*H*); 5.34 (t, 1 H, $J = 9.7$, H—3); 5.28–5.16 (m, 2 H, OCH_2CH=CH_2); 5.07 (br. t, 1 H, H—2); 4.62 (d, 1 H, $J = 8.0$, H—1); 4.40–4.32 (m, 2 H, H—6_{eq} and 1 H of OCH_2CH=CH_2); 4.06 (dd, 1 H, $J = 12.9$, 6.3, 1 H of OCH_2CH=CH_2); 3.80 (t, 1 H, $J = 9.7$, H—6_{ax}); 3.71 (t, 1 H, $J = 9.7$, H—4); 3.51 (dt, 1 H, $J = 9.7$, 4.9, H—5); 1.16, 1.14 {2 s, 18 H, (CH_3)$_3$C}. Anal. calc. for $C_{26}H_{36}O_8$: C 65.53, H 7.61; found: C 65.24, H 7.47.

Allyl 2,3-di-O-pivaloyl-6-O-trityl-β-D-glucopyranoside (13)

A solution of **11** (6.64 g, 13.9 mmol) in CH_2Cl_2 (250 ml) was cooled at 0 °C, then 70% trifluoroacetic acid (25 ml) was added dropwise. The mixture was stirred at 0 °C up to completion (2 h), then poured into water and neutralized with solid $NaHCO_3$. After filtration, the organic layer was washed with water, dried (Na_2SO_4) and evaporated. The crude allyl 2,3-di-*O*-pivaloyl-β-D-glucopyranoside (**12**) (5.3 g) was directly used in the next reaction. A solution of crude **12** (5.2 g, 13.38 mmol) and trityl chloride (8.58 g, 30.78 mmol) in dry pyridine (100 ml) was refluxed for 1.5 h, then cooled to room temperature and diluted with CH_2Cl_2 and water. The organic layer was sequentially washed with 5% HCl, satd. $NaHCO_3$ and water, dried (Na_2SO_4) and evaporated. FC (SiO_2, hexane/AcOEt 9:1), provided **13** (7.12 g, 84% from **11**). $[\alpha]_D = -18.3°$ ($c = 0.6$, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$): 7.50–7.21 (m, 15 H, Ph—*H*); 5.92–5.79 (m, 1 H, OCH_2CH=CH_2); 5.30–5.16 (m, 2 H, OCH_2CH=CH_2); 5.03–4.97 (m, 2 H, H—2, H—3); 4.51 (d, 1 H, $J = 7.3$, H—1); 4.37, 4.06 (2 br. dd, 2 H, OCH_2CH=CH_2); 3.66 (br. t, 1 H, H—4); 3.48–3.39 (m, 3 H, H—5, H—6); 2.48 (br. d, OH); 1.17 {s, 18 H, (CH_3)$_3$C}. Anal. calc. for $C_{38}H_{46}O_8$: C 72.36, H 7.35; found: C 72.54, H 7.47.

Allyl 4-O-benzyl-2,3-di-O-pivaloyl-6-O-trityl-β-D-gluco-pyranoside (14)

To a solution of **13** (7.1 g, 11.25 mmol) in freshly distilled N,N-dimethylformamide (200 ml) silver oxide (7.3 g, 31.5 mmol) was added; the suspension was stirred under nitrogen at room temperature for 15 min, then benzyl bromide (6 ml, 50.62 mmol) was added dropwise, keeping the mixture at room temperature in the dark. The reaction was monitored by TLC (hexane/AcOEt 9:1) and after completion (48 h) the mixture was filtered over a Celite pad and the filtrate diluted with CH_2Cl_2. The organic layer was washed with water, dried (Na_2SO_4) and evaporated. FC (SiO_2, toluene/CH_2Cl_2 9.5:0.5) afforded 5.69 g (70%) of **14**. $[\alpha]_D = -16.2°$ ($c = 1$, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$): 7.60–7.18 (m, 20 H, Ph—*H*); 5.98–5.85 (m, 1 H, OCH_2CH=CH_2); 5.30 (t, 1 H, $J = 9.5$, H—3); 5.28–5.10 (m, 2 H, OCH_2CH=CH_2); 5.11 (dd, 1 H, $J = 9.5$, 8.0, H—2); 4.56 (d, 1 H, $J = 8.0$, H—1); 4.44, 4.27 (ABq, 2 H, $J = 10.6$, OCH_2Ph); 4.42, 4.16 (2 dd, 2 H, $J = 12.8$, 4.9 and 12.8, 6.3, OCH_2CH=CH_2); 3.88 (t, 1 H, $J = 9.5$, H—4); 3.59–3.48 (m, 2 H, H—5, H—6_a); 3.20 (dd, 1 H, $J = 10.1$, 3.8, H—6_b); 1.16, 1.14 {2 s, 18 H, (CH_3)$_3$C}. Anal. calc. for $C_{45}H_{52}O_8$: C 74.97, H 7.27; found: C 75.36, H 7.13.

Allyl 4-O-benzyl-6-O-trityl-β-D-glucopyranoside (15)

To a solution of **14** (5.63 g, 7.81 mmol) in CH_2Cl_2 (150 ml), cooled to −78 °C, diisobutylaluminium hydride solution (1.2 M in toluene, 32.5 ml, 39.05 mmol) was added and the mixture stirred at −78 °C for 2 h. The reaction was quenched with MeOH and the mixture was poured into a satured solution of Rochelle salt (sodium and potassium tartrate) and diluted with AcOEt (500 ml). The organic layer was dried (Na_2SO_4) and evaporated. FC (SiO_2, hexane/AcOEt 1:1) afforded the diol **15** (3.07 g, 71%). $[\alpha]_d = -7.7°$ ($c = 0.35$, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$): 7.57–7.20 (m, 20 H, Ph—*H*); 6.10–5.96 (m, 1 H, OCH_2CH=CH_2); 5.40–5.24 (m, 2 H, OCH_2CH= CH_2); 4.61, 4.38 (ABq, 2 H, $J = 11.0$, OCH_2Ph); 4.46, 4.22 (2 dd, 2 H, $J = 12.5$, 5.2 and 12.5, 6.2, OCH_2CH=CH_2); 4.36 (d, 1 H, $J = 8.1$, H—1); 3.71–3.48 (m, 4 H, H—2, H—3, H—4, H—6_a); 3.44–3.40 (m, 1 H, H—5); 3.22 (dd, 1 H, $J = 10.0$, 4.0, H—6_b); 2.65–2.35 (br. s, 2 H, OH). Anal. calc. for $C_{35}H_{36}O_6$: C 76.06, H 6.57; found: C 76.27, H 6.39.

Allyl 2,3-di-O-acetyl-4-O-benzyl-6-O-trityl-β-D-gluco-pyranoside (16)

To a solution of diol **15** (3.05 g, 5.51 mmol) in dry CH_2Cl_2 (100 ml), pyridine (3.55 ml, 44.08 mmol) and 4-

dimethylaminopyridine (cat.) were added under nitrogen. The mixture was cooled to 0 °C and Ac_2O (2.08 ml, 22.04 mmol) was added, then the reaction was kept at room temperature up to completion (1.5 h). The mixture was poured in ice-cold water and the organic layer was sequentially washed with 5% HCl, satd. $NaHCO_3$ and water, dried (Na_2SO_4) and evaporated. FC (SiO_2, hexane/AcOEt 8:2) gave diacetate **16** (3.33 g, 95%). $[\alpha]_D = -39.0°$ ($c = 1$, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$): 7.59–7.19 (m, 20 H, Ph—*H*); 6.01–5.88 (m, 1 H, OCH_2CH=CH_2); 5.34–5.20 (m, 2 H, OCH_2CH=CH_2); 5.17 (t, 1 H, $J = 8.8$, H—3); 5.04 (br. t, 1 H, $J = 8.8$, H—2); 4.56 (d, 1 H, $J = 8.8$, H—1); 4.42 (dd, 1 H, OCH_2CH=CH_2); 4.37, 4.28 (ABq, 2 H, $J = 10.9$, OCH_2Ph); 4.19 (dd, 1 H, $J = 6.0$, OCH_2CH=CH_2); 3.93 (t, 1 H, $J = 8.8$, H—4); 3.60 (br. d, 1 H, $J = 10.2$, H—6_a); 3.47–3.43 (br. d, 1 H, H—5); 3.18 (dd, 1 H, $J = 10.2$, 3.4, H—6_b); 2.08, 1.93 (2 s, 6 H, 2 Ac). Anal. calc. for $C_{39}H_{40}O_8$: C 73.57, H 6.33; found: C 73.76, H 6.51.

Allyl 2,3-di-O-acetyl-4-O-benzyl-β-D-glucopyranoside (17)

To a suspension of **16** (3.26 g, 5.12 mmol) in MeOH (140 ml), catalytic *p*-toluenesulfonic acid was added. After completion (1.5 h, clear solution) the mixture was neutralized with solid $NaHCO_3$, filtered by suction and the solvent evaporated under reduced pressure. Chromatographic purification (SiO_2, hexane/AcOEt 7:3 → 6.5:3.5) of the crude residue afforded **17** (1.51 g, 75%). $[\alpha]_D = -52.0°$ ($c = 1$, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$): 7.39–7.23 (m, 5 H, Ph—*H*); 5.90–5.77 (m, 1 H, OCH_2CH=CH_2); 5.28–5.16 (m, 2 H, OCH_2CH=CH_2); 5.23 (t, 1 H, $J = 9.6$, H—3); 4.89 (dd, 1 H, $J = 9.6$, 7.9, H—2); 4.64, 4.59 (ABq, 2 H, $J = 11.2$, OCH_2Ph); 4.55 (d, 1 H, $J = 7.9$, H—1); 4.30, 4.09 (2 dd, 2 H, $J = 13.5$, 5.0 and 13.5, 5.9, OCH_2CH=CH_2); 3.89 (ddd, 1 H, $J = 9.7$, 4.7, 2.8, H—6_a); 3.80–3.72 (m, 2 H, H—6_b, H—4); 3.42 (br. dt, 1 H, $J = 9.7$, 3.4, H—5); 2.02, 1.96 (2 s, 6 H, 2 Ac); 1.92 (br. d, 1 H, OH). Anal. calc. for $C_{20}H_{26}O_8$: C 60.90, H 6.64; found: C 61.02, H 6.81.

Methyl (allyl 2,3-di-O-acetyl-4-O-benzyl-β-D-glucopyranosid) uronate (18)

A solution of **17** (877 mg, 2.22 mmol) in acetone (10 ml) was cooled to −5 °C and a solution of chromium trioxide in 3.5 M sulfuric acid (3 ml, 3.33 mmol; 1.15 g of CrO_3 in 10 ml of 3.5 M H_2SO_4) was added dropwise. The mixture was stirred at room temperature up to completion (6 h), then was poured in ice-cold water and diluted with $CHCl_3$. The organic layer was dried over Na_2SO_4, filtered and the solvent evaporated under reduced pressure. The crude residue (462 mg, colourless oil) was dissolved in MeOH at room temperature, then ethereal diazomethane was added until the solution showed a pale yellow colour. Evaporation of the solvent and FC (SiO_2, hexane/AcOEt 8:2 → 6:4) gave the methyl uronate **18** (640 mg, 70%). White powder, mp 114–116 °C, $[\alpha]_D = -59.2°$ ($c = 1$, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$): 7.39–7.18 (m, 5 H, Ph—*H*); 5.87–5.75 (m, 1 H, OCH_2CH=CH_2); 5.28–5.16 (m, 3 H, H—3, OCH_2CH=CH_2); 4.94 (dd, 1 H, $J = 9.2$, 7.6, H—2); 4.60, 4.53 (ABq, 2 H, $J = 11.2$, OCH_2Ph); 4.58 (d, 1 H, $J = 7.6$, H—1); 4.34–4.03 (m, 2 H, OCH_2CH=CH_2); 3.99–3.92 (m, 2 H, H—4, H—5); 3.74 (s, 3 H, CO_2CH_3); 2.04, 1.93 (2 s, 6 H, 2 Ac). Anal. calc. for $C_{21}H_{26}O_9$: C 59.71, H 6.20; found: C 59.93, H 6.10.

Methyl 2,3-di-O-acetyl-4-O-benzyl-D-glucopyranosyluronate (19)

To a solution of **18** (600 mg, 1.42 mmol) in dry tetrahydrofuran (20 ml) 1,5-cyclooctadiene-bis[methyldiphenylphosphine]-iridium hexafluorophosphate (0.01 eq.) was added. The stirred solution was degassed, placed under dry and oxygen-free nitrogen, and degassed once more. The catalyst was activated by hydrogen during which operation the slightly red suspension became yellow. To affect isomerization, the solution was degassed once more after 5 min and left at room temperature for 2 h under an atmosphere of dry and oxygen-free nitrogen. TLC analysis showed complete conversion of the allyl ether into the vinyl ether. The solvent was evaporated and the residual oil was dissolved in CH_2Cl_2 and washed with satd. $NaHCO_3$ and water, dried (Na_2SO_4) and evaporated. FC on a short column (hexane/AcOEt 7:3) afforded the vinyl ether (quant).

To a solution of 2-propenyl glycoside (603 mg, 1.42 mmol) in acetone/water 9:1 (20 ml) mercuric chloride (808 mg, 2.98 mmol) and mercuric oxide (738 mg, 3.41 mmol) were added. The suspension was stirred at room temperature for 24 h, then filtered over a Celite pad and the liquor evaporated. The residue was dissolved in CH_2Cl_2 and washed with satd. $NaHCO_3$, water, 30% potassium iodide and water, dried (Na_2SO_4) and evaporated. FC (SiO_2, hexane/AcOEt 7:3 → 1:1) afforded **19** (445 mg, 82%). White crystals, mp 152–154 °C, $[\alpha]_D = +32.7°$ ($c = 1$, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$, major anomer): 7.35–7.20 (m, 5 H, Ph—*H*); 5.57 (t, 1 H, $J = 9.7$, H—3); 5.45 (br. t, 1 H, H—1); 4.85 (dd, 1 H, $J = 9.7$, 3.7, H—2); 4.62, 4.54 (ABq, 2 H, $J = 11.2$, OCH_2Ph); 4.05 (d, 1 H, $J = 9.7$, H—5); 3.88 (t, 1 H, $J = 9.7$, H—4); 3.74 (s, 3 H, CO_2CH_3); 3.21 (br. d, 1 H, OH); 2.06, 1.95 (2 s, 6 H, 2 Ac). Anal. calc. for $C_{18}H_{22}O_9$: C 56.54, H 5.80; found: C 56.83, H 5.91.

Methyl (2,3-di-O-acetyl-4-O-benzyl-α-D-glucopyranosyl tricholoro-acetimidate) uronate (20)

Product **19** (320 mg, 0.837 mmol) dissolved in dry CH_2Cl_2 (15 ml) was added through a double tipped needle into a two-necked flask sealed with rubber septa and cooled to 0 °C. Trichloroacetonitrile (843 μl, 8.37 mmol)

and DBU (20 μl) were added to the mixture. The reaction was stirred at 0 °C and monitored by TLC. After 2 h the solvent was removed and the residue was filtered quickly through a short pad of SiO_2 (hexane/AcOEt 7:3 → 1:1 containing 0.5% of triethylamine) affording α-trichloroacetimidate **20** as colourless oil (390 mg, 88%). ^{1}H-NMR (300 MHz, $CDCl_3$): 8.67 (s, 1 H, N*H*); 7.35–7.21 (m, 5 H, Ph—*H*); 6.54 (d, 1 H, $J = 3.7$, H—1); 5.62 (t, 1 H, $J = 9.9$, H—3); 5.07 (dd, 1 H, $J = 9.9$, 3.7, H—2); 4.61, 4.53 (ABq, 2 H, $J = 11.3$, OCH_2Ph); 4.48 (d, 1 H, $J = 9.9$, H—5); 3.96 (t, 1 H, $J = 9.9$, H—4); 3.74 (s, 3 H, CO_2CH_3); 2.00 and 1.95 (2 s, 6 H, 2 Ac). Anal. calc. for $C_{20}H_{22}Cl_3NO_9$: C 45.60, H 4.21, N 2.66; found: C 45.97, H 4.29, N 2.81.

Methyl 4-O-(methyl 2,3-di-O-acetyl-4-O-benzyl-β-D-glucopyranosyluronate)-3,6-di-O-benzyl-2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranoside (21)

Trichloroacetimidate **20** (176 mg, 0.33 mmol) and acceptor **5** [15] (142 mg, 0.28 mmol) were dissolved in dry CH_2Cl_2 (10 ml) and added through a double tipped needle into a two-necked flask containing activated powdered 4 Å molecular sieves. The mixture was cooled to 0 °C and stirred under nitrogen atmosphere, then freshly distilled boron trifluoride etherate (7 μl, 0.05 mmol) was added. The reaction was completed in 20 min and the mixture was neutralized with satd. $NaHCO_3$ and diluted with CH_2Cl_2. The mixture was filtered and the filtrate was washed with satd. $NaHCO_3$ and water, dried (Na_2SO_4) and evaporated. FC (SiO_2, hexane/AcOEt 8:2 → 7:3) gave disaccharide **21**. White crystals (95 mg, 47%), mp 159–161 °C. $[\alpha]_D$+36.6° ($c = 1$, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$): 7.44–7.21 (m, 20 H, Ph—*H*); 5.03 (t, 1 H, $J = 9.2$, H—3′); 5.02 (br. s, 2 H, NHCO_2CH_2Ph); 4.86 (t, 1 H, $J = 9.2$, H—2′); 4.91–4.42 (m, 8 H, 3 OCH_2Ph, H—1, H—1′); 3.99 (br. t, 1 H, H—4); 3.90 (br t, 1 H, H—2); 3.88 (t, 1 H, $J = 9.2$, H—4′); 3.77 (dd, 1 H, $J = 3.1$, 10.8, H—6_a); 3.72 (d, 1 H, $J = 9.2$, H—5′); 3.62–3.57 (m, 2 H, H—6_b, H—5); 3.54 (s, 3 H, CO_2CH_3); 3.47 (br. t, 1 H, H—3); 3.29 (s, 3 H, OCH_3); 1.98, 1.90 (2 s, 6 H, 2 Ac). ^{13}C-NMR (75.46 MHz, $CDCl_3$): 170.44 (s, CO); 169.97 (s, CO); 168.64 (s, CO); 156.47 (s, CO); 139.49 (s); 138.24 (s); 137.03 (s); 129.32–127.83 (m, CH arom.); 100.87 (d, C—1′); 99.51 (d, C—1); 78.77 (d); 78.20 (2 d); 75.24 (t); 75.15 (t); 75.00 (d); 74.73 (d); 74.33 (t); 72.93 (d); 71.08 (d); 68.11 (t); 67.45 (t); 55.86 (d); 54.94 (q); 53.05 (q); 21.28 (q, 2C, 2CH_3CO). Anal. calc. for $C_{47}H_{53}NO_{15}$: C 64.74, H 6.13, N 1.61; found: C 64.98, H 5.94, N 1.81.

Methyl 4-O-(methyl 4-O-benzyl-β-D-glucopyranosyluronate)-3,6-di-O-benzyl-2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranoside (22)

0.5 M sodium methoxide (61 μl) in dry MeOH was added dropwise to a solution of **21** (266 mg, 0.3 mmol) in MeOH-CH_2Cl_2 2:1 (21 ml). After stirring at room temperature (4 h) the solution was neutralized with Amberlite IR 120 resin (H^+), then filtered by suction. The solvent was evaporated and the residue purified by flash chromatography (SiO_2, hexane/AcOEt 1:1 → 4:6) affording diol **22** (205 mg, 85%). Glass, $[\alpha]_D = +31.0°$ ($c = 1$, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$): 7.45–7.18 (m, 20 H, Ph—*H*); 5.02 (br. s, 2 H, NHCO_2CH_2Ph); 4.89–4.52 (m, 8 H, 3 OCH_2Ph, H—1, H—1′); 4.04–3.97 (m, 2 H, H—2, H—4); 3.74–3.59 (m, 6 H, H—2′, H—3′, H—4′, H—5, H—6); 3.58 (s, 3 H, CO_2CH_3); 3.49 (br. t, 1 H, H—3); 3.36 (d, 1 H, $J = 8.1$, H—5′); 3.29 (s, 3 H, OCH_3); 2.60–2.45 (br. s, 2 H, OH). Anal. calc. for $C_{43}H_{49}NO_{13}$: C 65.55, H 6.27, N 1.78; found: C 65.83, H 6.11, N 1.92.

Methyl 4-O-(methyl 2-O-acetyl-4-O-benzyl-β-D-glucopyranosyluronate)-3,6-di-O-benzyl-2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranoside (24) and Methyl 4-O-(methyl 3-O-acetyl-4-O-benzyl-β-D-glucopyranosyluronate)-3,6-di-O-benzyl-2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranoside (23)

Under N_2 dry pyridine (38 μl) was added to a solution of disaccharide **22** (186 mg, 0.236 mmol) in dry CH_2Cl_2 (6 ml). Ac_2O (22 μl, 0.236 mmol) was added at 0 °C, then the solution was warmed to room temperature and monitored by TLC (benzene/AcOEt 9:5). After 20 h, the mixture was poured into ice-cold water and extracted with CH_2Cl_2. The organic phase was washed sequentially with 5% HCl, satd. $NaHCO_3$ and water, dried (Na_2SO_4) and evaporated. FC (SiO_2, benzene/AcOEt 9:2 → 9:2.5) afforded **21** (23 mg), **24** (white powder, 53 mg, 27%) and **23** (white powder, 25 mg, 13%), along with 44 mg of mixed fractions; further elution provided unreacted **22** (32 mg). Acetate **21** was deacetylated once more; the resulting diol (18 mg, 87%) combined with the aforementioned unreacted **22** was submitted again to the monoacetylation procedure. After usual workup, chromatographic purification of crude product including mixed fractions gave further 40 mg of **24** and 20 mg of **23**. Product **24**: $[\alpha]_D = +43.8°$ ($c = 1$, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$): 7.40–7.16 (m, 20 H, Ph—*H*); 5.02 (br. s, 2 H, NHCO_2CH_2Ph); 4.94–4.43 (m, 7 H, 3 OCH_2Ph, H—1′); 4.77 (t, 1 H, $J = 8.7$, H—2′); 4.65 (d, 1 H, $J = 4.7$, H—1); 4.03–3.86 (m, 2 H, H—2, H—4); 3.80 (dd, 1 H, $J = 10.9$, 3.0 H—6_a); 3.72–3.45 (m, 6 H, H—3, H—3′, H—4, H—5′, H—5, H—6_b); 3.55 (s, 3 H, CO_2CH_3); 3.29 (s, 3 H, OCH_3); 2.36 (d, 1 H, $J = 4.9$, OH—3); 2.02 (s, 3 H, Ac). Anal. calc. for $C_{45}H_{51}NO_{14}$: C 65.13, H 6.19, N 1.69; found: C 65.27, H 6.38, N 1.52.

Product **23**: $[\alpha]_D = +61.3°$ ($c = 1$, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): 7.41–7.20 (m, 20 H, Ph—*H*); 5.02 (br. s, 2 H, NHCO_2CH_2Ph); 4.91 (t, 1 H, $J = 9.3$, H—3′); 4.88–4.43 (m, 8 H, 3 OCH_2Ph, H—1; H—1′); 4.07–3.92 (m, 2 H, H—2, H—4); 3.78 (t, 1 H, $J = 9.3$, H—4′); 3.72–3.36 (m, 6 H, H—5′, H—6, H—5, H—3, H—

2′); 3.56 (s, 3 H, CO_2CH_3); 3.29 (s, 3 H, OCH_3); 3.18 (d, 1 H, $J = 3.6$, OH—2); 2.01 (s, 3 H, Ac). Anal. calc. for $C_{45}H_{51}NO_{14}$: C 65.13, H 6.19, N 1.69; found: C 65.32, H 6.03, N 1.45.

*Methyl 4-O-(methyl 2-O-acetyl-4-O-benzyl-3-O-sulfo-β-D-glucopyranosyluronate)-3,6-di-O-benzyl-2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranoside, sodium salt (**26**)*

A solution of **24** (75 mg, 0.09 mmol) in dry N,N-dimethylformamide (8 ml) was stirred for 20 h at 60 °C in the presence of freshly purified sulfur trioxide-trimethylamine complex (94 mg, 0.68 mmol) [16]. The mixture was cooled to room temperature and the solvent concentrated to minimal volume. The residue was purified on a short column (SiO_2, $CHCl_3$/MeOH 9:1). The pure fractions were concentrated, dissolved in MeOH and eluted from a column of Amberlyst 15 (Na^+) resin affording disaccharide **26** (65 mg, 78%) as a yellow glass, $[\alpha]_D = +50.3°$ ($c = 1$, $CHCl_3$). ^{1}H-NMR (500 MHz, CD_3OD): 7.42–7.15 (m, 20 H, Ph—*H*); 5.06, 4.51 (ABq, 2 H, $J = 10.4$, OCH_2Ph); 5.04, 4.97 (ABq, 2 H, $J = 12.2$, $NHCO_2CH_2Ph$); 4.94, 4.48 (ABq, 2 H, $J = 10.6$, OCH_2Ph); 4.91 (t, 1 H, $J = 8.6$, H—2′); 4.68, 4.53 (ABq, 2 H, $J = 11.4$, OCH_2Ph); 4.62 (br. d, 1 H, H—1); 4.61 (d, 1 H, $J = 8.6$, H—1′); 4.57 (t, 1 H, $J = 8.6$, H—3′); 3.84 (br. dd, 1 H, H—6_a); 3.81 (br. t, 1 H, H—4); 3.78 (t, 1 H, $J = 8.6$, H—4′); 3.76–3.69 (m, 3 H, H—2, H—5′, H—6_b); 3.63–3.58 (m, 2 H, H—5, H—3); 3.42 (s, 3 H, CO_2CH_3); 3.35 (s, 3 H, OCH_3); 2.04 (s, 3 H, Ac). Anal. calc. for $C_{45}H_{50}NO_{17}SNa$: C 58.00, H 5.41, N 1.50; found: C 58.23, H 5.13, N 1.68.

*Methyl 4-O-(methyl 3-O-acetyl-4-O-benzyl-2-O-sulfo-β-D-glucopyranosyluronate)-3,6-di-O-benzyl-2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranoside, sodium salt (**25**)*

A solution of **23** (34 mg, 0.041 mmol) in dry N,N,-dimethylformamide (4 ml) was stirred for 30 h at 60 °C in the presence of sulfur trioxide-trimethylamine complex (58 mg, 0.4 mmol). The mixture was processed as described for the preparation of **26**, affording **25** (38 mg, 99%) as colourless glass, $[\alpha]_D = +35.6°$ ($c = 1$, MeOH); ^{1}H-NMR (500 MHz, CD_3OD): 7.45–7.15 (m, 20 H, Ph—*H*); 5.14 (t, 1 H, $J = 8.5$, H—3′); 5.02 (br. s, 2 H, $NHCO_2CH_2Ph$); 5.05, 4.98 (ABq, 2 H, $J = 12.5$, OCH_2Ph); 4.68–4.48 (2ABq, 4 H, $J = 11.1$, $J = 11.8$, 2 OCH_2Ph); 4.62 (d, 1 H, $J = 3.3$, H—1); 4.57 (d, 1 H, $J = 8.5$, H—1′); 4.26 (t, 1 H, $J = 8.5$, H—2′); 4.24 (br. dd, 1 H, H—6_a); 3.90–3.85 (m, 1 H, H—5); 3.83 (t, 1 H, $J = 8.5$, H—4′); 3.76 (dd, 1 H, $J = 3.3$, H—2); 3.74–3.70 (m, 2 H, H—3, H—4); 3.69 (br. dd, 1 H, H—6_b); 3.64 (d, 1 H, $J = 8.5$, H—5′); 3.47 (s, 3 H, CO_2CH_3); 3.33 (s, 3 H, OCH_3); 2.00 (s, 3 H, Ac). Anal. calc. for $C_{45}H_{50}NO_{17}SNa$: C 58.00, H 5.41, N 1.50; found: C 58.16, H 5.39, N 1.56.

*Methyl 4-O-(4-O-benzyl-3-O-sulfo-β-D-glucopyranosyluronic acid)-3,6-di-O-benzyl-2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranoside, disodium salt (**28**)*

To a solution of **26** (10 mg, 0.01 mmol) in MeOH (0.6 ml) at 0 °C 2.5 M sodium hydroxide (150 μl) was added. After stirring at room temperature (2 h) the mixture was diluted with MeOH, then eluted from a column of Dowex 50 W-X8 resin (20–50 mesh, H^+). The fractions containing the product were concentrated, dissolved in MeOH and finally eluted from the same resin in Na^+ form. FC (SiO_2, $CHCl_3$/MeOH 8:2) afforded **28** (8 mg, 83%). Structure of **28** was confirmed by ^{1}H-NMR (disappearance of $-CO_2CH_3$ and $-COCH_3$ signals) and it was directly used for the hydrogenation without any further characterization because of its relative instability. ^{1}H-NMR (300 MHz, CD_3OD): 7.45–7.15 (m, 20 H, Ph—*H*); 5.10–4.50 (m, 10 H, 4 OCH_2Ph, H—1, H—1′); 4.45 (t, 1 H, $J = 8.6$, H—3′); 4.09 (dd, 1 H, $J = 10.8$, 3.2, H—2); 4.00–3.50 (m, 8 H); 3.32 (s, 3 H, OCH_3).

*Methyl 4-O-(4-O-benzyl-2-O-sulfo-β-D-glucopyranosyluronic acid)-3,6-di-O-benzyl-2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranoside, disodium salt (**27**)*

Hydrolysis of **25** (11 mg, 0.012 mmol) with 2.5 M sodium hydroxide (150 μl) in MeOH (0.6 ml) was performed as described for the preparation of **28**, affording **27** (9 mg, 85%) the structure of which was confirmed by ^{1}H-NMR (see **28**). **27** was immediately submitted to hydrogenation. ^{1}H-NMR (300 MHz, CD_3OD): 7.45–7.15 (m, 20 H, Ph—*H*); 5.12–4.50 (m, 10 H, 4 OCH_2Ph, H—1, H—1′); 4.28–4.18 (m, 1 H, H—2′); 4.00–3.60 (m, 8 H); 3.31 (s, 3 H, OCH_3).

*Methyl 4-O-(3-O-sulfo-β-D-glucopyranosyluronic acid)-2-amino-2-deoxy-α-D-glucopyranoside, disodium salt (**4**)*

A solution of **28** (19 mg, 0.021 mmol) in MeOH/water 1:1 (3 ml) was hydrogenated in the presence of 10% Pd/C (20 mg) for 36 h, then filtered over a Celite pad, concentrated and finally lyophilized yielding **4** (13 mg, 99%). White solid, $[\alpha]_D = +35.6°$ ($c = 1$, water); ^{1}H-NMR (500 MHz, D_2O): 4.96 (d, 1 H, $J = 3.7$, H—1); 4.63 (d, 1 H, $J = 8.7$, H—1′); 4.34 (t, 1 H, $J = 8.7$, H—3′); 3.94 (dd, 1 H, $J = 12.3$, 2.6, H—6_a); 3.90 (dd, 1 H, $J = 12.3$, 4.5, H—6_b); 3.83 (d, 1 H, $J = 9.9$, H—5′); 3.82 (m, 1 H, H—5); 3.79 (t, 1 H, $J = 9.5$, H—3); 3.72–3.69 (m, 2 H, H—4, H—4′); 3.54 (t, 1 H, $J = 8.7$, H—2′); 3.41 (s, 3 H, OCH_3); 3.21 (dd, 1 H, $J = 9.5$, 3.7, H—2). ^{13}C-NMR (125.76 MHz, D_2O): 177.50 (s, CO); 104.79 (d, C—1′); 101.50 (d, C—1); 86.44 (d, C—3′); 81.90 (d, C—4); 78.48 (d, C—5′); 74.71 (d, C—2′); 74.34 (d, C—3); 73.30 (d, C—5); 73.18 (d, C—4′); 62.70 (t, C—6); 58.00 (q, OCH_3); 57.18 (d, C—2). Anal. calc. for $C_{13}H_{21}NO_{14}SNa_2$: C 31.65, H 4.29, N 2.84; found: C 31.78, H 4.12, N 2.56.

Methyl 4-O-(2-O-sulfo-β-D-glucopyranosyluronic acid)-2-amino-2-deoxy-α-D-glucopyranoside, disodium salt (3)

Disaccharide **27** (25 mg, 0.028 mmol) was hydrogenated as described for the preparation of **4** from **28** affording **3** (14 mg, 99%). White solid. $[\alpha]_D = +36.1°$ ($c = 1$, water). ^{1}H-NMR (500 MHz, D_2O): 4.96 (d, 1 H, $J = 3.7$, H—1); 4.69 (d, 1 H, $J = 8.5$, H—1′); 4.11 (t, 1 H, $J = 8.5$, H—2′); 3.97 (dd, 1 H, $J = 11.0$, 4.2, H—6_a); 3.94 (dd, 1 H, $J = 11.0$, 2.5, H—6_b); 3.95 (t, 1 H, $J = 9.6$, H—3); 3.84 (m, 1 H, H—5); 3.80 (d, 1 H, $J = 9.9$, H—5′); 3.73 (t, 1 H, $J = 8.5$, H—3′); 3.67–3.60 (m, 2 H, H—4, H—4′); 3.41 (s, 3 H, OCH_3); 3.23 (dd, 1 H, $J = 9.6$, 3.7 H—2). ^{13}C-NMR (125.76 MHz, D_2O): 177.80 (s, CO); 103.21 (d, C—1′); 99.38 (d, C—1); 82.92 (d, C—2′); 81.85 (d, C—4); 78.21 (d, C—5′); 77.17 (d, C—3′); 74.25 (d, C—4′); 73.21 (d, C—5); 72.10 (d, C—3); 62.26 (t, C—6); 58.10 (q, OCH_3); 56.68, (d, C—2). Anal. calc. for $C_{13}H_{21}NO_{14}SNa_2$. C 31.65, H 4.29, N 2.84; found: C 31.36, H 4.47, N 2.78.

Results

Whereas the glucosaminyl acceptor **5** was easily obtained according to literature procedure [15], the synthesis of the glycosyl donor was more troublesome. Because of the structural complexity of the disaccharides three major aspects were considered in the choice of the appropriate protective groups: (a) the presence of an *O*-sulfate group either in position 2 or 3; (b) the presence of a carboxylate group at C—6; (c) the β configuration of the glycosidic linkage which requires the presence of a participating group at *C*—2 of the glucuronic acid unit.

In the light of these considerations a suitable glycosyl donor should, e.g., contain two selectively removable *O*-acyl groups at C—2 and C—3 respectively to provide the access to both disaccharides.

In preliminary experiments, the easily available allyl 4,6-*O*-benzylidene-β-D-glucopyranoside **6** [14] was selectively acylated at C—3 with lipase P from *Pseudomonas cepacia* to give **7** or **8** depending on the activated ester (Fig. 2) [17]. A second chemical acylation gave **9** from **7** and **10** from **8**. However, attempts to effect selective deprotections on **9** and **10** gave migration of esters leading to mixture of products.

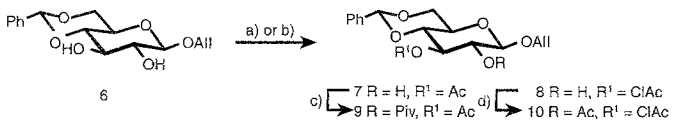

Figure 2. (a) vinyl acetate, lipase P, 45 °C, 95% of **7**; (b) trifluoroethyl chloroacetate, lipase P, toluene-THF 4:1, 74% of **8**; (c) PivCl, DMAP, Et_3N, CH_2Cl_2, rt, 92%; (d) Ac_2O, Py, CH_2Cl_2, rt, 78%.

We were forced by these shortcomings to change our synthetic approach: we envisaged the acetate **21** as suitable common precursor for both disaccharides **3** and **4**.

For the synthesis of disaccharide **21**, diol **6** was fully pivaloylated to give **11** (the acetyl groups showed to be too labile and to migrate easily) (Fig. 3).

Hydrolysis of the benzylidene acetal **11** with 70% trifluoroacetic acid afforded diol **12** (no migration products were observed); *O*-tritylation and *O*-benzylation with benzyl bromide and silver oxide [18] gave **14** in 30% overall yield from **6**. Finally, the pivaloyl groups were replaced with acetyl groups to ensure milder conditions in the final crucial steps of the synthesis; **14** was treated with a solution of diisobutylaluminium hydride (1.2 M in toluene), then acetylated to yield the fully protected **16** (68% from **14**).

Detritylation of **16** at room temperature with *p*-toluensulfonic acid in methanol (**17**, 75%), followed by Jones oxidation and esterification with diazomethane provided methyl glucuronate **18** in 70% yield. Deprotection of the anomeric position was performed as reported by van Boom *et al.* [19]: isomerization of the allyl ether to the 1-propenyl ether with 1,5-cyclooctadiene-bis (methyldiphenylphosphine)-iridium hexafluoro phosphate, followed by hydrolysis with mercuric chloride and mercuric oxide afforded hemiacetal **19** in 82% yield (Fig. 4). Compound **19** was converted into the α-trichloroacetimidate **20** by treatment with trichloroacetonitrile and DBU in CH_2Cl_2 [20] (88%). Condensation of **20** with **5** was promoted by boron trifluoride etherate in CH_2Cl_2 at 0 °C in the presence of 4 Å activated powdered molecular sieves and gave 47% of the β-disaccharide **21**.

Treatment of **21** with sodium methoxide in CH_2Cl_2-MeOH afforded diol **22** in 85% yield, which was *O*-acetylated with 1 equivalent of Ac_2O in CH_2Cl_2/pyridine (Fig. 5): the reaction was carefully monitored by TLC (eluent benzene/AcOEt 9:5) and stopped as soon as the formation of traces of the diacetylated disaccharide **21**

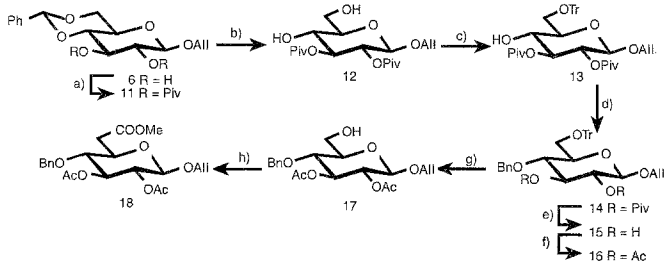

Figure 3. (a) PivCl, DMAP, Et_3N, CH_2Cl_2, reflux, 82%; (b) 70% TFA, CH_2Cl_2, quant.; (c) TrCl, Py, reflux, 84%; (d) BnBr, Ag_2O, DMF, 70%; (e) DIBAH, CH_2Cl_2, −78 °C, 71%; (f) Ac_2O, Py, DMAP, CH_2Cl_2, rt, 95%; (g) PTSA, MeOH, 75%; (h) CrO_3, H_2SO_4, acetone, rt then CH_2N_2, MeOH, rt, 70%.

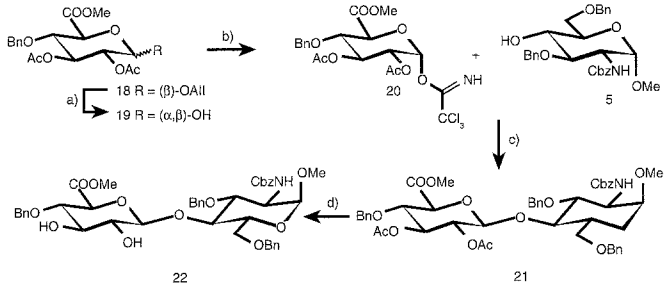

Figure 4. (a) {Ir(COD)[PMePh$_2$]$_2$}PF$_6$ cat., H_2, THF, rt, 2 h, then $HgCl_2$, HgO, acetone/H_2O 9:1, rt, 82%; (b) CCl_3CN, DBU, CH_2Cl_2, 0 °C, 88%; (c) BF_3 OEt_2, CH_2Cl_2, 0 °C, 47%; (d) MeONa in MeOH, 85%.

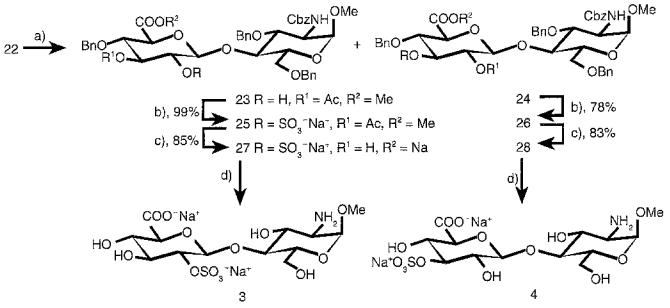

Figure 5. (a) 1 eq. of Ac_2O, CH_2Cl_2-Py, rt; (b) SO_3·NMe_3, DMF, 50 °C; (c) 2.5 M NaOH, MeOH; (d) H_2, Pd/C, MeOH/H_2O 1:1, quant.

was observed. The unreacted **22** was recovered and recycled, thus obtaining disaccharides 2′-*O*-acetylated **24** (70%) and 3′-*O*-acetylated **23** (20%). Regiochemistry of the acetylation was deduced from ^{1}H-NMR by comparison of the spectrum of **23** and **24** with that of **22** in the same solvent ($CDCl_3$). In compound **22** the signals associated to H—2′ and H—3′ resonates between 3.74 and 3.59 ppm. Compound **23** showed a downfielded triplet at 4.91 ppm (J = 9.2 Hz) assigned to H—3′; in fact, in a COSY experiment it gave two cross peaks with a signal at 3.78 ppm attributable to H—4′ and with a signal at 3.39 ppm attributable to H—2′, which, in turn, is coupled with a signal at 4.52 ppm due to H—1′. The downfield shift of the signal associated to H—3′ clearly shows the position of the acetylation.

On the other hand, compound **24** showed a triplet at 4.77 ppm (J = 8.7 Hz) with a cross peak with a signal at 3.52 ppm attributable to H—3′. The cross peak with H—1′ (resonating at 4.53 ppm) was not directly visible being covered by the cross peaks of the benzylic protons. However, a selective decoupling by irradiation at 4.77 showed the correlation with H—1′ and, because of the downfield shift with respect to **22**, confirmed that the compound **24** is acetylated in position 2.

O-Sulfation of **24** was achieved with sulfur trioxide-trimethylamine complex in N,N-dimethylformamide at 50 °C (Fig. 5); the trimethylammonium salt was purified by filtration through a short column of silica gel, then converted into the sodium salt using Amberlyst 15 resin (Na^+) to give disaccharide **26** in 78% yield. Sulfation of HO—3′ of **24** caused a downfield shift of the signal of H—3′ by ~0.80 ppm in $CDCl_3$ as solvent; the ^{1}H NMR spectrum showed a triplet for H—3′ at δ 4.57 (J = 8.6 Hz). Hydrolysis of the methyl ester and the acetyl group of **26** was performed with 2.5 M sodium hydroxide in methanol; conversion into the disodium salt was obtained through a first elution of the product on Dowex 50W in H^+ form, followed by a second elution on the same resin in Na^+ form [21]. Final flash chromatography provided **28** in 83% yield, which was hydrogenolysed in methanol-water in the presence of 10% Pd/C affording the 3′-*O*-sulfated disaccharide **4** in almost quantitative yield.

The analogous sequence was applied to the 3′-*O*-acetyl derivative **23**: once more *O*-sulfation was confirmed by the downfield shift of the signal of H—2′ by ~0.60 ppm. Saponification and hydrogenolysis provided disaccharide **3** in 83% overall yield.

The ^{1}H- and ^{13}C-NMR spectra (500 MHz) of **3** and **4** are in excellent agreement with the assigned structures; all the signals of both compounds were completely assigned through two-dimension homo and heterocorrelation experiments. In particular, in a solution of deuterium oxide, H—2′ of **3** was shown as a triplet at δ 4.11 (J = 9.2 Hz), whereas H—3′ of **4** was shown as a triplet at δ 4.34 (J = 8.9 Hz): these chemical shifts are characteristic of the corresponding *O*-sulfated positions [22].

The details of the deaminative cleavage, reduction and HPLC analysis have been published elsewhere [23].

Thereby it was demonstrated that the sulfated D-glucuronic acid unit occurring in heparan sulfate, and in heparin [6], carries its sulfate substituent at C-2 position.

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