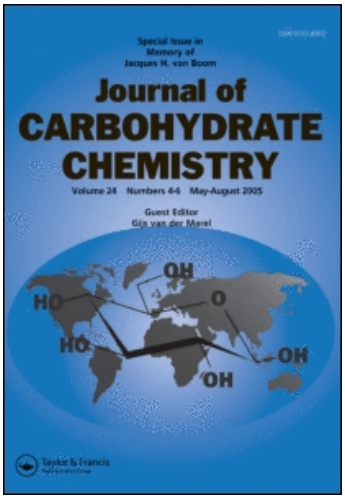

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Synthesis of Non-glucosamino Glucan Oligosaccharides Related to Heparin and Heparan Sulphate

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SYNTHESIS OF NON-GLUCOSAMINO GLUCAN OLIGOSACCHARIDES RELATED TO HEPARIN AND HEPARAN SULPHATE

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ABSTRACT

A series of three oligosaccharides, α-D-Glc-(1→4)-β-D-GlcA-1→OMe, β-D-GlcA-(1→4)-α-D-Glc-(1→4)-β-D-GlcA-1→OMe and α-D-Glc-(1→4)-β-D-GlcA-(1→4)-α-D-Glc-(1→4)-β-D-GlcA-1→OMe was prepared by a short synthetic route, using maltose and glucuronic acid derivatives as starting materials. The oligosaccharides contain glucose residues instead of glucosamines, and have a less complicated structure than the corresponding unsulphated structures found in native heparin and heparan sulphate. This simplification in structure has diminished the number of synthetic steps and raised the total yield compared to the preparation of the corresponding heparin/heparan sulphate structures which have been found to bind acidic and basic FGF.

INTRODUCTION

Heparin and heparan sulphate (HS) are well known polysaccharides which consist of alternating uronic acids and hexosamines joined together by (1→4) glycosidic linkages. Heparin/HS are products of biosynthetic transformations, and since the enzymatic reactions do not operate completely along the polysaccharide chains, the chain sequences are highly irregular.[1] Heparin is most known for its antithrombotic properties and is today one of the best

characterised antithrombotic drugs.[2,3] Additionally, a number of other biological effects such as anti-inflammatory[4], anti-metastatic[5] and angiogenic modulatory activity[6] have been discovered. Several research groups[7-10] are trying to find the smallest fragments, the essential parts, of heparin and heparan sulphate which have these specific effects. An example, where the search for a structure with a particular biological activity has succeeded, is the well-known pentasaccharide[10] which binds to antithrombin III. This pentasaccharide has been synthesised several times by different routes.[10] Considering the costs for the preparations, which often is an important aspect, the pentasaccharide syntheses have one thing in common - too many steps. In order to simplify the synthesis, the investigators have prepared modified structures in which the glucosamino residues have been replaced by glucose residues and methyl groups have been introduced as permanent protective groups.[10] These modifications decreased the number of synthetic steps and raised the total yield without loss of antithrombotic activity.

In an earlier report we described the synthesis of di- and trisaccharide derivatives related to heparin and heparan sulphate and presented results from a study of their interaction with acidic and basic fibroblast growth factor (aFGF and bFGF). It was found that two non-sulphated trisaccharides, β-D-GlcA-(1→4)-α-D-GlcNAc-(1→4)-β-D-GlcA-1→OMe and α-L-IdoA-(1→4)-α-D-GlcNAc-(1→4)-β-D-GlcA-1→OMe, bind to aFGF and bFGF and also induce FGF receptor activation.[11,12]

In order to determine if modified oligosaccharides, in which the glucosamine residues have been replaced by glucose, can interact with aFGF and bFGF we have prepared the following compounds:

α-D-Glc-(1→4)-β-D-GlcA-1→OMe	**6**
β-D-GlcA-(1→4)-α-D-Glc-(1→4)-β-D-GlcA-1→OMe	**13**
α-D-Glc-(1→4)-β-D-GlcA-(1→4)-α-D-Glc-(1→4)-β-D-GlcA-1→OMe	**15**

RESULTS AND DISCUSSION

The strategy employed was to prepare the oligosaccharides by using maltose and glucuronic acid derivatives as building blocks. An advantage of such a procedure is that the α-glycosidic linkage, which can be difficult to form due to stereo selectivity and separation problems, already exists. From earlier attempts we knew that glycosylation of an aglycon with a glucuronic acid glycosyl donor requires that the aglycon be protected by groups which do not lower its reactivity. A suitable protected disaccharide **4**, having several benzyl

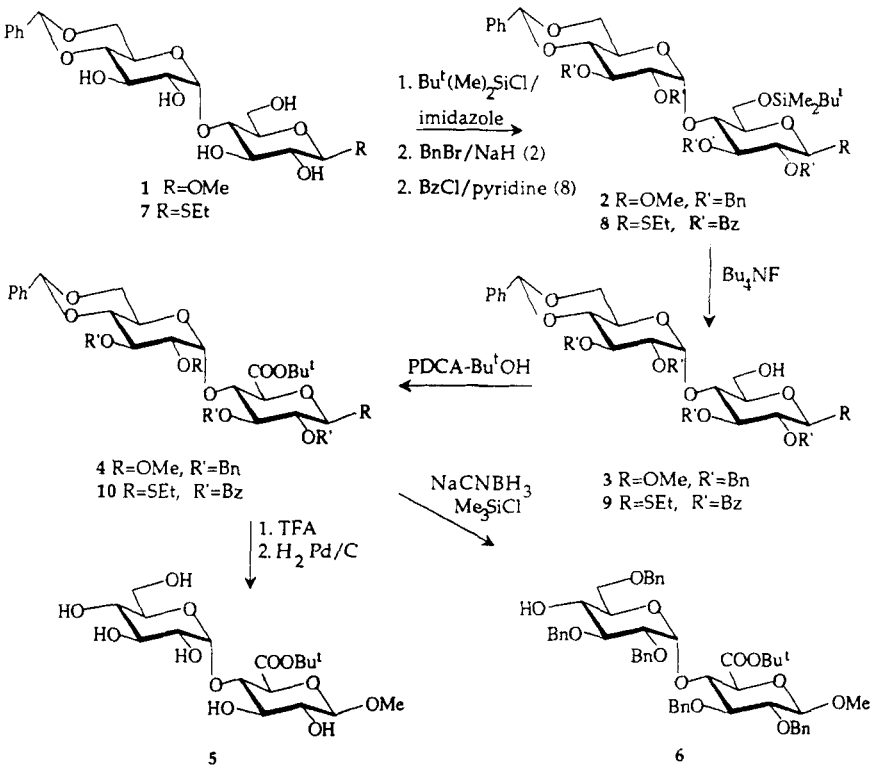
groups was therefore used as a key-intermediate. Complete deprotection of **4** gave the disaccharide **5**. Selective deprotection of **4** in the 4-position gave the disaccharide **6**, which was coupled to a glucuronic acid glycosyl donor. The trisaccharide obtained was then converted to the target trisaccharide **13**. For the preparation of the tetrasaccharide **15**, compound **6** was glycosylated with the benzoylated disaccharide **10**. Subsequent deprotection gave the tetrasaccharide **15**. The following steps were performed:

Methyl 4',6'-*O*-benzylidene-β-D-maltopyranoside[14] (**1**) was treated with *tert*-butyldimethylsilyl chloride and imidazole in *N*,*N*-dimethylformamide (DMF) to protect the 6-OH position.[15] The mixture was extracted and directly, without further purification, treated with benzyl bromide and sodium hydride in DMF to protect the remaining hydroxyl groups. This procedure gave after purification the fully protected disaccharide **2** in 82% yield. Removal of the *tert*-butyldimethylsilyl group in **2** was performed with tetrabutylammonium fluoride (1 M) in tetrahydrofuran (THF)[15] giving compound **3** in 91% yield. Oxidation of compound **3** by pyridinium dichromate-acetic anhydride (PDCA) in the presence of *tert*-butyl alcohol in dichloromethane,[16] gave the *tert*-butyl ester **4** in 84% yield. Compound **4** was deprotected, by hydrolysis of the *tert*-butyl ester using trifluoroacetic acid in dichloromethane[17] and subsequent hydrogenolysis over Pd-C, to give compound **5** in 87 % yield.

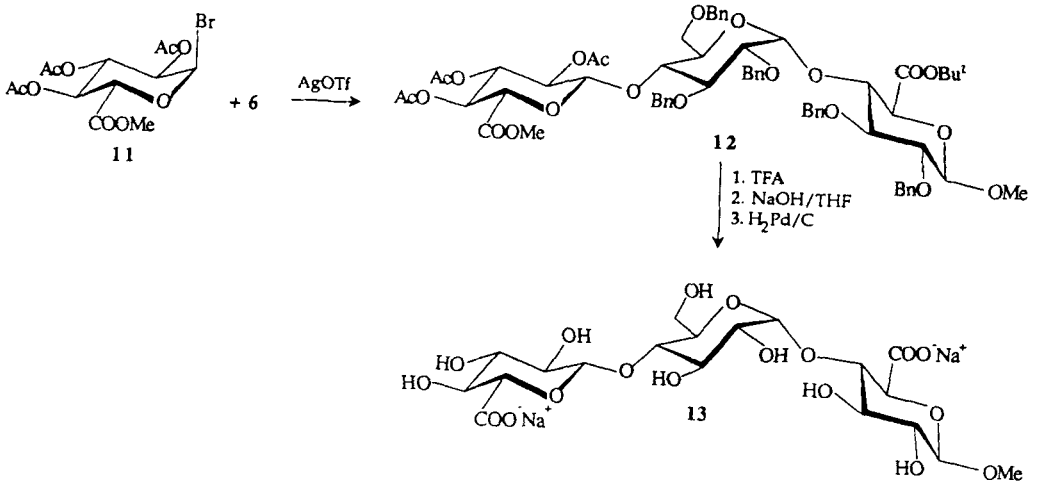
The 4',6'-*O*-benzylidene acetal in compound **4** was subjected to reductive ring-opening by treatment with sodium cyanoborohydride and HCl in diethyl ether[18] to give the OH-4' compound **6** in 87% yield.

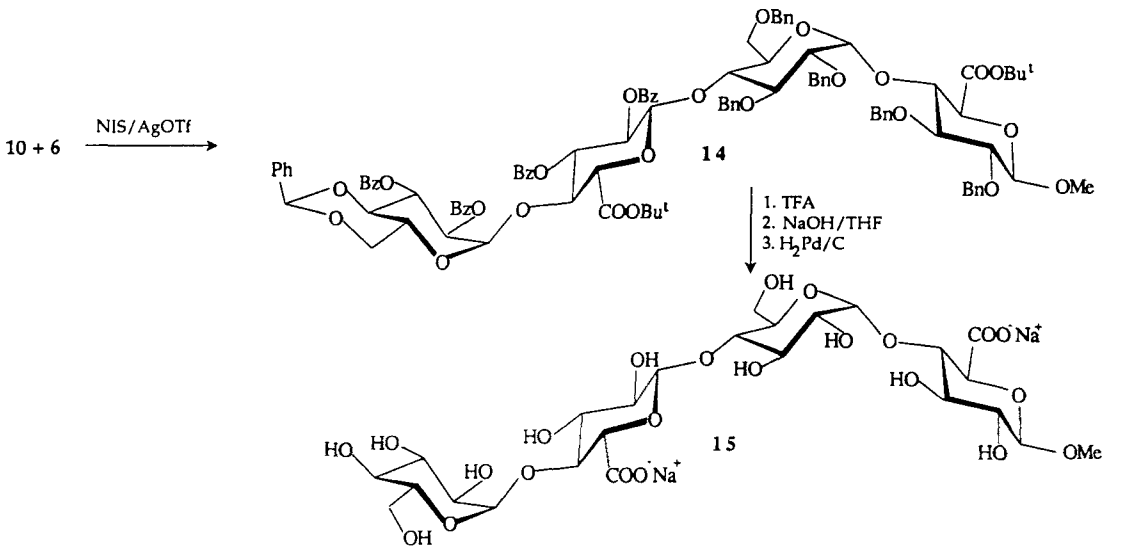
The 4',6'-*O*-benzylidene acetal compound **7** was prepared from ethyl 1-thio-β-D-maltopyranoside[19] which was treated with α,α-dimethoxytoluene and *p*-toluenesulfonic acid in DMF[20] to give **7** in 63% yield. Compound **7** was treated as described above with *tert*-butyldimethylsilyl chloride and imidazole to protect the 6-OH position and after extraction, directly benzoylated with benzoyl chloride in pyridine giving the fully protected disaccharide block **8** in 84% yield. Benzoyl groups were used instead of acetyl groups in order to minimize migration of ester groups during the deprotection of the silyl group.[21] The 6-OH position was deprotected in the same way as described above for **2**, to give compound **9** in 85% yield. Compound **9** was then converted into the corresponding *tert*-butyl ester **10** in 80% yield using the method described above.

Compound **6** was glycosylated with methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate[22] (**11**) using silver triflate as promoter giving the trisaccharide **12** in 46% yield and with compound **10** using *N*-iodosuccinimide and silver triflate[23] as promoters giving the tetrasaccharide **14** in 74% yield. Deprotection of compounds **12** and **14** was performed by



hydrolysis of the *tert*-butyl esters using trifluoracetic acid in dichloromethane followed by deacetylation using 2 M sodium hydroxide in tetrahydrofuran, (to prevent β-elimination of the glucuronic acid) and hydrogenolysis over Pd-C, to give the trisaccharide **13** in a 83% yield and the tetrasaccharide **15** in a 81% yield. The biological testing results from these substances will be described elsewhere.





EXPERIMENTAL

General Methods. Melting points were measured using an Electrochemical AI 9200 melting point apparatus and are reported uncorrected. Concentrations were performed under diminished pressure at < 40 °C (bath). Optical rotations were recorded for 0.5% solutions at room temperature (22-25 °C) using a Perkin-Elmer 241 polarimeter. NMR spectra were recorded either in $CDCl_3$ with Me_4Si as internal standard or in D_2O with sodium 4,4-dimethyl-4-silapentanoate-2,2',3,3'-d_4 as internal standard at 30 °C, using a JEOL EX-400 instrument. All ^1H NMR assignments were based on 2D experiments. NMR spectra recorded for all new compounds, were in agreement with the postulated structures, and only selected data are reported. ^1H NMR shift values and coupling constants (values in parentheses) are presented as tables, in which the sugar residues are given as Glc, Glc', GlcA and GlcA'. TLC was performed on Silica gel F254 (Merck) with detection by UV and/or by charring with H_2SO_4. Column chromatography was performed on Silica gel (Matrex Silica Si 60A, 35-70 mm, Amicon). Organic solutions were dried over magnesium sulphate. Molecular sieves were desiccated at 300 °C overnight. The purity of the target compounds was ascertained by NMR spectroscopy.

Methyl *O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl)-(1→4)-2,3-di-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranoside (2). Imidazole (1.55 g, 22.4 mmol) and *tert*-butyldimethylsilyl chloride (2.02 g, 13.5 mmol) were added to a stirred solution of methyl *O*-(4,6-*O*-benzylidene-α-D-glucopyranosyl)-(1→4)-β-D-glucopyranoside[14] (1) (5.00 g, 11.2 mmol) in DMF (80

mL). The mixture was stirred for several hours at room temperature. The solution was hydrolysed in ice-water, and then the mixture was repeatedly extracted with CH_2Cl_2. The combined organic phases were washed with ammonium sulphate (aq) and water, dried and concentrated. The residue was then dissolved together with benzyl bromide (11 mL, 90 mmol) in DMF (100 mL), and added to a cooled mixture of sodium hydride (4.0 g, 55%, 92 mmol) and DMF (15 mL). The mixture was stirred at room temperature for 4 h. Methanol was then added (to destroy the excess of sodium hydride) and the solution was poured into ice-water. The mixture was extracted with toluene, dried and concentrated. The resulting syrup was purified by column chromatography (toluene-ethyl acetate 8:1) to give **2** (8.40 g, 9.14 mmol, 82%), isolated as a syrup having $[\alpha]_{578}$ 6° (*c* 0.5, $CHCl_3$), R_f 0.61 (toluene-ethyl acetate, 8:1). NMR data ($CDCl_3$): ^{13}C, δ -4.9 (Me silyl), 26.1 (Me *tert*-butyl), 56.7 (OMe), 97.3 (C'-1), 101.3 (PhCH), 104.3 (C-1). ^1H NMR data are shown in the following table.

	H-1	H-2	H-3	H-4	H-5
Glc	4.22 (7.8)	3.30 (8.8)	3.66 (9.3)	3.86	ND
Glc'	5.56 (3.9)	3.40 (9.8)	3.88 (9.3)	3.50 (9.3)	3.80

Methyl *O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl)-(1→4)-2,3-di-*O*-benzyl-β-D-glucopyranoside (3). A mixture of **2** (1.50 g, 1.63 mmol) and Bu_4NF in THF (1 M, 10 mL) was stirred at room temperature for 2 h. The solution was hydrolysed in ice-water, and the mixture was repeatedly extracted with CH_2Cl_2. The combined organic phases were washed with ammonium sulphate (aq) and water, dried and concentrated. The residue was purified by column chromatography (toluene-ethyl acetate 4:1) to give **3** (1.20 g, 1.49 mmol, 91%), isolated as an amorphous solid having $[\alpha]_{578}$ 3° (*c* 0.5, $CHCl_3$), R_f 0.33 (toluene-ethyl acetate, 4:1). NMR data ($CDCl_3$): ^{13}C, δ 57.2 (OMe), 97.8 (C'-1), 101.1 (PhCH), 104.7 (C-1). ^1H NMR data are shown in the following table.

	H-1	H-2	H-3	H-4	H-5
Glc	4.37 (7.8)	3.43 (8.8)	3.80 (9.3)	4.02	ND
Glc'	5.68 (3.9)	3.52 (9.3)	4.00 (9.3)	3.61 (9.3)	3.86

Anal. Calcd for $C_{48}H_{52}O_{11}$: C, 71.6; H, 6.5. Found: C, 71.6; H, 6.5.

Methyl *O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl)-(1→4)-*O*-(*tert*-butyl 2,3-di-*O*-benzyl-β-D-glucopyranoside)uronate (4) Pyridinium dichromate (1.10 g, 2.92 mmol), Ac_2O (1.40 mL, 14.9 mmol), and *tert*-butyl alcohol (2.90 mL, 30.7 mmol) were added to a stirred solution of **3** (1.20 g, 1.49

mmol) in CH_2Cl_2 (20 mL). The mixture was stirred for 4 h at room temperature and then applied on top of a silica gel column in ethyl acetate, with a 5 cm layer of ethyl acetate on top of the gel. The chromium compounds precipitated in the presence of ethyl acetate, and after 15 min the product was eluted with ethyl acetate. After evaporating the solvent, the product was purified by column chromatography (toluene-ethyl acetate 8:1) to give **4** (1.10 g, 1.26 mmol, 84%), isolated as a syrup, having $[\alpha]_{578}$ 2° (*c* 0.5, $CHCl_3$), R_f 0.64 (toluene-ethyl acetate 6:1). NMR data ($CDCl_3$): ^{13}C, δ 28.1 (Me *tert*-butyl), 56.9 (OMe), 97.7 (C'-1), 101.2 (PhCH), 104.7 (C-1), 167.2 (C-6). ^1H NMR data are shown in the following table.

	H-1	H-2	H-3	H-4	H-5
GlcA	4.36 (7.8)	3.47 (8.8)	3.72 (8.8)	4.25 (8.8)	3.82
Glc	5.41 (3.9)	3.49 (9.3)	3.97 (9.3)	3.57 (9.3)	3.78

Methyl *O*-(β-D-glucopyranosyl)-(1→4)-*O*-β-D-glucopyranosyluronic acid sodium salt (5). Compound **4** (80.0 mg, 91.4 μmol) was dissolved in a solution of CF_3CO_2H in CH_2Cl_2 (20%, 2 mL) and stirred at room temperature for 2 h. The mixture was diluted with CH_2Cl_2, washed with $NaHCO_3$ (aq) and water, dried and concentrated. The residue was dissolved in water-ethanol (1:1, 10 mL) and hydrogenolyzed over Pd-C for 24 h. After filtering through Celite and concentrating the residue was dissolved in water and passed through a column of Dowex Na^+. The eluate was concentrated and purified on a column of P2 Biogel, using water (containing 1% 1-butanol) as eluent, to give **5** (30.1 mg, 80.0 μmol, 87%) isolated as a solid, having $[\alpha]_{578}$ 24° (*c* 0.5, H_2O), R_f 0.50 (EtOAc-EtOH-AcOH-H_2O, 4:3:3:2). NMR data (D_2O): ^{13}C, δ 60.1 (OMe), 63.0, 72.1, 74.5, 74.6, 75.6, 75.9, 79.2, 79.4, 79.6 (ring C), 101.2 (C'-1), 106.0 (C-1), 178.0 (C-6). ^1H NMR data are shown in the following table.

	H-1	H-2	H-3	H-4	H-5
Glc	4.39 (7.8)	3.34 (8.5)	3.75 (8.8)	3.78	ND
Glc'	5.47 (3.9)	3.51 (9.7)	3.69 (9.5)	3.40	ND

Methyl *O*-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-*O*-(*tert*-butyl 2,3-di-*O*-benzyl-β-D-glucopyranoside)uronate (6). Diethyl ether saturated with HCl was added, at room temperature, to a stirred mixture of **4** (1.02 g, 1.16 mmol), $NaCNBH_3$ (430 mg, 6.85 mmol) and 3A molecular sieves in THF (20 mL) until the mixture was acidic (as determined with indicator paper). The mixture was stirred for 30 min and was then diluted with CH_2Cl_2 and filtered. The solution was washed with $NaHCO_3$ (aq) and water, dried and concentrated. The residue was purified by column chromatography (toluene-ethyl acetate 4:1) to give **6**

(890 mg, 1.01 mmol, 87%) isolated as a syrup, having $[\alpha]_{578}$ 22° (*c* 0.5, $CHCl_3$), R_f 0.58 (toluene-ethyl acetate 4:1). NMR data ($CDCl_3$): ^{13}C, δ 28.0 (Me *tert*-butyl), 56.9 (OMe), 96.8 (C'-1), 104.7 (C-1), 167.4 (C-6). ^1H NMR data are shown in the following table.

	H-1	H-2	H-3	H-4	H-5
Glc	4.38 (7.8)	3.47 (8.8)	3.72 (8.8)	4.25 (8.8)	3.83
Glc'	5.44 (3.9)	3.45 (9.3)	3.70 (9.3)	ND	ND

Ethyl *O*-(4,6-*O*-benzylidene-α-D-glucopyranosyl)-(1→4)-1-thio-β-D-glucopyranoside (7). Ethyl 1-thio-β-D-maltopyranoside heptaacetate[19] (22.0 g, 32.3 mmol) was dissolved in CH_2Cl_2-MeOH (100 mL, 1:1) and methanolic sodium methoxide (1 M) was added to the stirred solution until the mixture was basic (as determined with indicator paper). The solution was stirred for 2 h at room temperature and was then neutralized with acetic acid and concentrated. The residue was dissolved in dry DMF (60 mL) containing benzaldehyde dimethyl acetal (9.8 mL, 65 mmol) and *p*-toluenesulphonic acid was added until the mixture was acidic. The mixture was stirred for 24 h on a rotary evaporator at a bath temperature of 40 °C. The mixture was then cooled, neutralized with triethylamine, and coevaporated with toluene. The resulting syrup was purified by column chromatography (chloroform-methanol 5:1) to give **7** (9.70 g, 20.4 mmol, 63%). Crystallisation from methanol-diethyl ether gave material, having mp 190 °C, $[\alpha]_{578}$ 46° (*c* 0.5, MeOH), R_f 0.30 (chloroform-methanol, 10:1). NMR data (CD_3OD): ^{13}C, δ 15.4 (Me ethyl), 24.9 (CH_2S), 62.4, 62.7, 71.5, 73.9, 74.1, 74.7, 75.1, 79.3, 80.6 (ring C), 86.8 (C-1), 102.8 (PhCH), 104.6 (C'-1); ^1H, δ 3.28 (dd $J_{1,2}$ 9.8, $J_{2,3}$ 8.9, H-2), 3.45 (dd $J_{1',2'}$ 3.9, $J_{2',3'}$ 9.8, H'-2), 3.63 (t, H-3), 3.65 (t H'-3), 4.39 (d, H-1), 5.18 (d, H'-1).

Anal. Calcd for $C_{21}H_{30}O_{10}S$: C, 53.2; H, 6.4; S, 6.7. Found: C, 53.1; H, 6.3; S 6.5.

Ethyl *O*-(2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranosyl)-(1→4)-2,3-di-*O*-benzoyl-6-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (8). Imidazole (920 mg, 13.3 mmol) and *tert*-butyldimethylsilyl chloride (1.20 g, 8.00 mmol) were added to a stirred solution of **7** (3.16 g, 6.66 mmol) in DMF (50 mL). The mixture was stirred for 24 h at 40 °C. The solution was hydrolysed in ice-water, and the mixture was repeatedly extracted with CH_2Cl_2. The combined organic phases were washed with ammonium sulphate (aq) and water, dried and concentrated. The residue was then dissolved in pyridine (50 mL), cooled in an ice-bath and treated with benzoyl chloride (6.3 mL, 53 mmol). After stirring for 24 h at 50 °C the solution was poured into ice-water, and the mixture was extracted several times with CH_2Cl_2. The combined organic phases

were washed with $NaHCO_3$ (aq) and water, dried and concentrated. The resulting syrup was purified by column chromatography (toluene:ethyl acetate 8:1) to give **8** (5.60 g, 5.57 mmol, 84%). Crystallisation from diethyl ether-petroleum ether gave crystalline material, having mp 69-71 °C, $[\alpha]_{578}$ 35° (*c* 0.5, $CHCl_3$), R_f 0.61 (toluene-ethyl acetate, 8:1). NMR data ($CDCl_3$): ^{13}C, δ -4.7 (Me silyl), 14.8 (Me ethyl), 25.7 (CH_2S), 26.2 (Me *tert*-butyl), 83.0 (C-1), 96.6 (C'-1), 101.7 (PhCH). ^1H NMR data are shown in the following table.

	H-1	H-2	H-3	H-4	H-5
Glc	4.72 (9.8)	5.24 (9.3)	5.68 (9.3)	4.38 (9.3)	3.80
Glc'	5.62 (3.9)	5.17 (10.2)	5.91 (9.8)	3.83 (9.8)	4.12

Anal. Calcd for $C_{55}H_{60}O_{14}SSi$: C, 65.7; H, 6.0; S, 3.2. Found: C, 65.5; H, 5.8; S, 2.9.

Ethyl *O*-(2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranosyl)-(1→4)-2,3-di-*O*-benzoyl-1-thio-β-D-glucopyranoside (9). Compound **8** (1.40 g, 1.39 mmol) was treated as described for the preparation of **3** to give **9** (1.05 g, 1.18 mmol, 85%). Crystallisation from diethyl ether-petroleum ether gave material, having mp 101 °C, $[\alpha]_{578}$ 32° (*c* 0.5, $CHCl_3$), R_f 0.43 (toluene-ethyl acetate 4:1). NMR data ($CDCl_3$): ^{13}C, δ 14.8 (Me ethyl), 24.4 (CH_2S), 83.6 (C-1), 97.1 (C'-1), 101.4 (PhCH). ^1H NMR data are shown in the following table.

	H-1	H-2	H-3	H-4	H-5
Glc	4.75 (9.8)	5.29 (9.3)	5.73 (9.3)	4.44	3.80
Glc'	5.61 (3.9)	5.19 (10.2)	5.92 (9.8)	3.83 (9.8)	4.09

Anal. Calcd for $C_{49}H_{46}O_{14}S$: C, 66.1; H, 5.2; S, 3.6. Found: C, 65.8; H, 5.2; S, 3.3.

Ethyl *O*-(2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranosyl)-(1→4)-*O*-(*tert*-butyl 2,3-di-*O*-benzoyl-1-thio-β-D-glucopyranoside)uronate (10). Compound **9** (4.65 g, 5.22 mmol) was treated as described for the preparation of **4** to give **10** (4.00 g, 4.16 mmol, 80%). Crystallisation from diethyl ether-petroleum ether gave pure compound, having mp 115 °C, $[\alpha]_{578}$ 33° (*c* 0.5, $CHCl_3$), R_f 0.74 (toluene-ethyl acetate 4:1). NMR data ($CDCl_3$): ^{13}C, δ 15.1 (Me ethyl), 24.4 (CH_2S), 84.3 (C-1), 96.6 (C'-1) 101.7 (PhCH), 166.4 (C-6). ^1H NMR data are shown in the following table.

	H-1	H-2	H-3	H-4	H-5
GlcA	4.76 (9.8)	5.34 (9.3)	5.71 (9.3)	4.60 (9.3)	4.10
Glc	5.61 (3.9)	5.13 (10.2)	5.90 (9.8)	3.81 (9.8)	3.98

Anal. Calcd for $C_{53}H_{52}O_{15}S$: C, 66.2; H, 5.4; S, 3.3. Found: C, 66.4; H, 5.3; S, 3.3.

Methyl *O*-(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-*O*-(*tert*-butyl 2,3-di-*O*-benzyl-β-D-glucopyranoside)uronate (12). A mixture of **6** (150 mg, 170 μmol), methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate[22] (**11**) (88.0 mg, 220 μmol) and 4A molecular sieves in CH_2Cl_2 (2 mL) was stirred under N_2 at room temperature for 10 min and was then cooled to -15 °C. Silver triflate (120 mg, 470 μmol) was added and the mixture was stirred at this temperature for 1 hour. Triethylamine (0.4 mL) was added and the reaction mixture was allowed to attain room temperature. The mixture was then filtered through Celite, concentrated, and purified twice by column chromatography (toluene-ethyl acetate 12:1 and 4:1) to give **12** (93 mg, 78 μmol, 46%) isolated as a syrup, having $[\alpha]_{578}$ 13° (*c* 0.5, $CHCl_3$), R_f 0.29 (toluene-ethyl acetate 4:1). NMR data ($CDCl_3$): ^{13}C, δ 20.5, 20.6, 20.8 (Me acetyl), 28.0 (Me *tert*-butyl), 52.6, 57.0 (MeO) 96.4 (C'-1), 99.9 (C''-1), 104.8 (C-1), 166.9, 167.6 (C''-6, C-6), 169.0, 169.3, 170.0 (C=O, acetyl). ^1H NMR data are shown in the following table.

	H-1	H-2	H-3	H-4	H-5
GlcA	4.37 (7.8)	3.46 (8.8)	3.72(8.8)	4.18(9.3)	3.81
Glc	5.42 (3.9)	3.41 (9.8)	3.74 (9.8)	3.91 (9.8)	3.67
GlcA'	4.54 (8.3)	4.86 (9.3)	4.96 (9.3)	5.12 (9.8)	3.65

Methyl *O*-(β-D-glucopyranosyluronic acid)-(1→4)-*O*-(α-D-glucopyranosyl)-(1→4)-*O*-β-D-glucopyranosyluronic acid disodium salt (13). Compound **12** (90 mg, 75 μmol) was deprotected in three steps. Hydrolysis of the *tert*-butyl ester was performed with CF_3CO_2H in CH_2Cl_2 (20%) according to the method described for compound **5**. Subsequent treatment with cold aqueous NaOH (1 mL, 2M) in THF (5 mL) followed by hydrogenolysis over Pd-C in water-EtOH (1:1, 10 mL) gave the deprotected trisaccharide **13** (37 mg, 63 μmol, 83%), isolated as a solid having $[\alpha]_{578}$ 87° (*c* 0.5, H_2O), R_f 0.25 (ethyl acetate-ethanol-acetic acid-water 4:3:2:2). NMR data (D_2O): ^{13}C, δ 58.0 (MeO), 62.2, 73.3, 74.0, 74.2, 74.5, 75.1 75.8, 76.9, 78.0, 78.7, 79.6, 79.9, 80.9 (ring C), 102.1 (C'-1), 105.1 (C''-1), 106.0 (C-1), 177.9, 178.5 (C-6, C''-6). ^1H NMR data are shown in the following table.

	H-1	H-2	H-3	H-4	H-5
GlcA	4.39 (7.8)	3.33 (9.0)	3.79	3.76	ND
Glc	4.82 (3.7)	3.64 (9.8)	3.81	3.85	ND
GlcA'	4.49 (7.8)	3,37 (9.5)	3.52	3.75	ND

Methyl *O*-(2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranosyl)-(1→4)-*O*-(*tert*-butyl 2,3-di-*O*-benzoyl-β-D-glucopyranosyl)uronate-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-*O*-(*tert*-butyl 2,3-di-*O*-benzyl-β-D-glucopyranosyl)uronate (14). *N*-Iodosuccinimide (117 mg, 520 μmol), immediately followed by a solution of silver triflate (134 mg, 520 μmol), was added at -20 °C to stirred mixture of **6** (195 mg, 222 μmol) and **10** (320 mg, 333 μmol) in CH_2Cl_2 (4 mL) containing 4A molecular sieves. The mixture was stirred at this temperature until TLC indicated complete reaction. The mixture was then diluted with CH_2Cl_2 and filtered through Celite. The filtrate was washed with $Na_2S_2O_3$ (10%, aq) and water, dried and concentrated. The residue was purified by column chromatography (toluene-ethyl acetate 8:1) to give **14** (290 mg, 163 μmol, 74%) Crystallisation from diethyl ether-petroleum ether gave pure compound, having mp 98 °C, $[\alpha]_{578}$ 23° (*c* 0.5, $CHCl_3$), R_f 0.51 (toluene-ethyl acetate 8:1). NMR data ($CDCl_3$): ^{13}C, δ 28.2 (Me *tert*-butyl), 56.9 (OMe), 96.3, 97.0 (C'-1, C'''-1), 99.6 (C''-1), 101.7 (PhCH), 104.7 (C-1), 164.7, 164.8 (C-6, C''-6). ^1H NMR data are shown in the following table.

	H-1	H-2	H-3	H-4	H-5
GlcA	4.28 (7.8)	3.40 (8.8)	3.68 (8.8)	4.12 (8.8)	3.52
Glc	5.44 (3.9)	3.40 (9.8)	3.63 (9.8)	3.97 (9.8)	3.63
GlcA'	4.65 (8.3)	5.15 (9.3)	5.38 (9.3)	4.57 (9.8)	3.72
Glc'	5.54 (3.9)	5.10 (10.2)	5.95 (9.8)	3.82 (9.3)	3.98

Anal. Calcd for $C_{103}H_{106}O_{27}$: C, 69.6; H, 6.0. Found: C, 69.4; H, 5.7.

Methyl *O*-(β-D-glucopyranosyl)-(1→4)-*O*-β-D-glucopyranosyluronic acid -(1→4)-*O*-(β-D-glucopyranosyl)-(1→4)-*O*-β-D-glucopyranosyluronic acid di-sodium salt (15). Compound **14** (145 mg, 81.6 μmol) was treated as described for the preparation of **13** to give **15** (49.5 mg, 65.8 μmol, 81%) isolated as a solid, having $[\alpha]_{578}$ 78° (*c* 0.5, H_2O), R_f 0.30 (ethyl acetate-ethanol-acetic acid-water 4:3:2:2). NMR data (D_2O): ^{13}C, δ 57.9 (MeO), 60.1, 62.2, 63.0, 72.1, 73.3, 74.0, 74.2, 74.6, 75.1, 75.6, 75.9, 76.9, 78.8, 79.3, 79.7, 79.9 80.8 (ring C), 101.1, 102.1 (C'-1, C'''-1), 105.2, 106 (C-1, C''-1), 177.8, 177.9 (C-6, C''-6). ^1H NMR data are shown in the following table.

	H-1	H-2	H-3	H-4	H-5
GlcA	4.39 (7.8)	3.33 (8.6)	3.78	3.82	ND
Glc	4.81 (3.9)	3.64 (9.6)	3.90	3.74	ND
GlcA'	4.49 (7.8)	3.38 (9.1)	3.80	ND	ND
Glc'	5.49 (3.9)	3.52 (9.5)	3.68	3.39	ND

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