Carbohydrate Research, 246 (1993) 161–172
Elsevier Science Publishers B.V., Amsterdam

Synthesis of the methyl glycosides of a tri- and a tetra-saccharide related to heparin and heparan sulphate

Marianne Nilsson, Carl-Magnus Svahn and Jacob Westman

Organic Chemistry, Kabi Pharmacia AB, S-112 87 Stockholm (Sweden)

(Received December 16th, 1992; accepted February 28th, 1993)

ABSTRACT

The methyl glycoside of a tetrasaccharide isolated from heparin, methyl *O*-(α-L-idopyranosyluronic acid)-(1 → 4)-*O*-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1 → 4)-*O*-(β-D-glucopyranosyluronic acid)-(1 → 3)-*O*-β-D-galactopyranoside disodium salt and a trisaccharide derivative thereof, methyl *O*-(α-L-idopyranosyluronic acid)-(1 → 4)-*O*-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1 → 4)-*O*-β-D-glucopyranosyluronic acid disodium salt, were synthesized using a block-type strategy. A suitable protected disaccharide block of iduronic acid and glucosamine (IdoA-GlcN) was used as a key intermediate for the syntheses and was glycosidated with a protected galactose derivative and a disaccharide block of glucuronic acid and galactose (GlcA-Gal) to give tri- and tetra-saccharide derivatives, respectively. Deprotection gave the target compounds.

INTRODUCTION

Heparin and heparan sulphate are well-known glycosaminoglycans which are composed of alternating uronic acids and hexosamines joined together by (1 → 4) glycosidic linkages. The uronic acid is either glucuronic acid or iduronic acid and the hexosamine is *N*-acetylated or *N*-sulphated glucosamine. Heparin is a product of biosynthetic transformation where most of the glucuronic acids residues originally introduced, have undergone epimerisation to iduronic acids. Most of the *N*-acetyl groups of the glucosamines have been replaced by *N*-sulphate groups, and *O*-sulphate groups occupy many positions of the polysaccharide chain[1,2]. However, nonsulphated GlcA-GlcNAc and IdoA-GlcNAc disaccharide units may nevertheless be found in heparin. As the enzymatic reactions do not operate completely along the chains, the chain sequences are highly irregular. Because of their polyanionic nature, heparin and heparan sulphate bind to many proteins. The binding is mediated mainly by interactions between sulphate and carboxyl groups of the glycosaminoglycan and basic amino acids of the protein. Heparin binds, for example, to several proteins of the extracellular matrix and growth factors[2]. Because of the irregularity of heparin and heparan sulphate, domains having unique sequences for protein binding may exist, such as the unique pentasaccharide fragment of heparin involved in the binding of antithrombin III (AT III)[3].

In our research project aimed at isolating and characterizing fragments from depolymerized heparin and heparan sulphate, an interesting tetrasaccharide having the sequence: IdoA-GlcNAc-GlcA-Gal was isolated from heparin[4]. The structure contains a galactose residue, which indicates that the tetrasaccharide is a part of the linkage region. The linkage region is the sequence GlcA-Gal-Gal-Xyl, which links the polysaccharide chain of the glycosaminoglycan to a peptide core and consists of sugar residues different from those in the rest of the chain. A schematic picture of the isolated tetrasaccharide is shown in **I**.

Syntheses of structures from the linkage region have been described before[5–9]. In order to verify the structure of the particular tetrasaccharide isolated and to test this tetrasaccharide and its corresponding trisaccharide (IdoA-GlcNAc-GlcA) for biological activities, we have synthesized the tetrasaccharide **19** and the trisaccharide **10**.

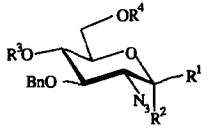
α-L-IdoA-(1 → 4)-α-D-GlcNAc-(1 → 4)-β-D-GlcA-(1 → 3)-D-Gal

I

RESULTS AND DISCUSSION

To synthesize the tri- and the tetra-saccharide using a minimum number of reaction steps a block-type strategy was adopted. The thioglycoside building block **5** was prepared from methyl (2,3,4-tri-*O*-acetyl-β-L-idopyranosyl bromide)uronate[10] and **4**, and was used for preparing both the trisaccharide and the tetrasaccharide. Glycosidating **5** with **8** gave the trisaccharide **9** and glycosidating **5** with the disaccharide block **17** afforded the tetrasaccharide **18**. The tri- and the tetra-saccharide were then deblocked to give the target structures. The following steps were performed:

Compound **1** was synthesized from 1,5-anhydro-3-*O*-benzyl-2-deoxy-4,6-*O*-benzylidene-D-*arabino*-hex-1-enitol[11] in order to attain a maximum yield of the D-*gluco* configuration in the azidonitration reaction[12,13]. The β product from the azidonitration, 2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl nitrate[12], was treated with tetraethylammonium chloride in acetonitrile and the α-chloride **1** was obtained in 73% yield. Treatment of **1** with sodium thioacetate[14,15] in *N*,*N*-dimethylformamide gave the β-acetylthio derivative **2** in 52% yield. *S*-Deactylation of **2** with methanolic sodium methoxide at room temperature gave the corresponding 1-thiol, which was directly alkylated with ethyl iodide to give **3** in 79% yield. Reductive opening of the 4,6-benzylidene acetal in **3** was performed by treatment with sodium cyanoborohydride in tetrahydrofuran and hydrogen chloride in diethyl ether[16] to give the OH-4 compound **4** in 83% yield. Glycosylation of **4** with methyl (2,3,4-tri-*O*-acetyl-β-L-idopyranosyl bromide)uronate[10], using silver triflate as promoter and 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) as acid acceptor gave the disaccharide **5** in 60% yield.

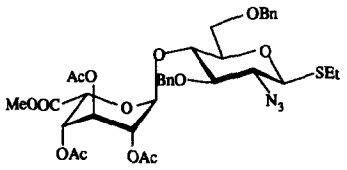


1 R^1 = H, R^2 = Cl, R^3, R^4 = PhCH

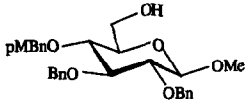
2 R^1 = SAc, R^2 = H, R^3, R^4 = PhCH

3 R^1 = SEt, R^2 = H, R^3, R^4 = PhCH

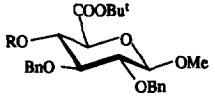
4 R^1 = SEt, R^2 = H, R^3 = H R^4 = Bn



5

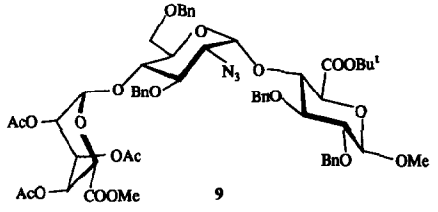


6

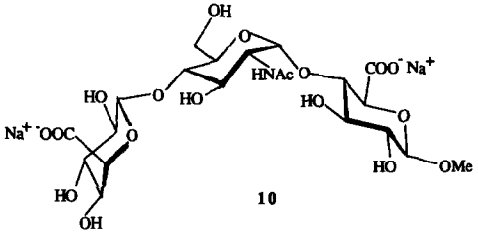


7 R = pMBn

8 R = H



9



10

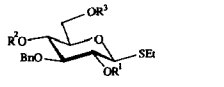
pMBn = *p*-methoxybenzylidene

Methyl 2,3-di-*O*-benzyl-4,6-*O*-*p*-methoxybenzylidene-β-D-glucopyranoside[17] was treated with sodium cyanoborohydride and chlorotrimethylsilane in acetonitrile[18] to give the OH-6 compound **6** in 67% yield. Oxidation of **6** by treatment with pyridinium dichromate–acetic anhydride (PDCA)[19] in the presence of *tert*-butyl alcohol in dichloromethane gave the *tert*-butyl ester **7** in 75% yield. Compound **7**

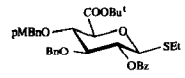
was then treated with ceric ammonium nitrate in acetonitrile to give **8** in 92% yield.

Glycosylation of **8** with **5** using *N*-iodosuccinimide and silver triflate as promoters[20,21] gave the trisaccharide **9** in 73% yield. Deprotection of **9** was performed by hydrolysis of the *tert*-butyl ester using trifluoroacetic acid in dichloromethane followed by deacylation using sodium hydroxide in tetrahydrofuran and hydrogenolysis over Pd–C. Selective acetylation of the amino group, by treatment with acetic anhydride in water–methanol, gave the trisaccharide **10** in 62% yield.

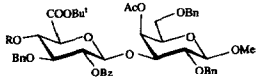
1,2,4,6-Tetra-*O*-acetyl-3-*O*-benzyl-β-D-glucopyranose[22] was treated with ethanethiol and boron trifluoride etherate to give the thioglycoside **11** in 91% yield. Compound **11** was O-deacetylated using sodium methoxide in methanol and treated with *p*-methoxybenzaldehyde dimethyl acetal and *p*-toluenesulphonic acid in tetrahydrofuran to give **12** in 71% yield. Treatment of **12** with benzoyl chloride and pyridine gave **13** in 73% yield. Compound **13** was treated with sodium cyanoborohydride and chlorotrimethylsilane in acetonitrile to give the OH-6



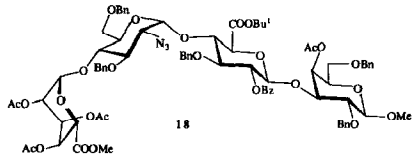
11 R^1, R^2, R^3 = Ac
12 R^1 = H, R^2, R^3 = (pMeO)PhCH
13 R^1 = Bz, R^2, R^3 = (pMeO)PhCH
14 R^1 = Bz, R^2 = pMBn, R^3 = H



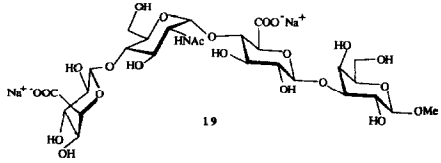
15



16 R = pMBn
17 R = H



18



19

derivative **14** in 70% yield. Compound **14** was then converted into the corresponding *tert*-butyl ester **15** in 68% yield using the method just described.

Glycosylation of methyl 4-*O*-acetyl-2,6-di-*O*-benzyl-β-D-galactopyranoside[23] with **15** using dimethyl(methylthio)sulfonium triflate (DMTST) as promoter gave the disaccharide **16** in 87% yield. This reaction was performed without using an acid acceptor in order to prevent orthoester formation. The *p*-methoxybenzyl group was removed by treatment with ceric ammonium nitrate in acetonitrile to give **17** in 80% yield.

Compound **17** was glycosylated with **5** using DMTST as promoter and DTBMP as acid acceptor to give the tetrasaccharide **18** in 79% yield. Compound **18** was then deprotected and *N*-acetylated in the same way as already described to give **19** in 64% yield.

EXPERIMENTAL

General methods.—Melting points were measured using a Mettler FP5 melting-point apparatus and are uncorrected. Concentrations were performed under diminished pressure at < 40°C (bath). Optical rotations were recorded for 0.5–0.6% solutions at room temperature (22–25°C) using a Perkin–Elmer 241 polarimeter. NMR spectra were recorded in $CDCl_3$ at 30°C, using JEOL EX-400 and Varian 600 MHz instruments, chemical shifts are given in ppm relative to internal Me_4Si, unless otherwise stated. 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 often presented as tables, in which the sugar residues are given as Gal, GlcA, GlcN, and IdoA. 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 μm, Amicon). Organic solutions were dried over $MgSO_4$. Molecular sieves (Fluka) were desiccated at 300°C overnight. Elemental analyses were performed by Analytische Laboratorien, Germany and by Mikro Kemi AB, Sweden. The purity of the target compounds was ascertained by HPLC and by NMR spectroscopy.

2-Azido-3-O-benzyl-4,6-O-benzylidene-2-deoxy α-D-glucopyranosylchloride (**1**).—Tetraethylammonium chloride (20 g, 120 mmol) was added to a solution of 2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl nitrate[12] (9.30 g, 21.7 mmol) in MeCN (50 mL) and the mixture was stirred at room temperature for 5 h. The mixture was then diluted with CH_2Cl_2, washed with water, dried and concentrated. Column chromatography of the residue, using toluene as eluent, gave **1** (6.40 g, 15.9 mmol, 73%) having $[\alpha]_{578}$ +20° (*c* 0.5, $CHCl_3$); R_f 0.42 (6:1 petroleum ether–EtOAc); NMR data: ^{13}C, δ 64.2, 65.6, 68.1, 75.3, 76.2, 81.9 (C-2,3,4,5,6 and 2 CH_2Ph), 92.9 (C-1) and 101.6 (Ph*C*H); ^1H, δ 3.72 (dd, $J_{1,2}$ 4.0,

$J_{2,3}$ 9.6 Hz, H-2) 3.74 (t, $J_{5,6a} = J_{6a,6b} = 10.1$ Hz, H-6a), 3.75 (t, $J_{3,4} = J_{4,5}$ 9.6 Hz, H-4), 4.10 (t, H-3), 4.20 (m, H-5), 4.30 (dd, $J_{5,6b}$ 4.9 Hz, H-6b), 6.01 (d, H-1).

1-S-Acetyl-2-azido-3-O-benzyl-4,6-O-benzylidene-2-deoxy-1-thio-β-D-glucopyranoside (**2**).—Potassium thioacetate (1.22 g, 10.7 mmol) was added to a stirred solution of **1** (2.15 g, 5.35 mmol) in DMF (5.0 mL) and the mixture was stirred at room temperature for 1 h. Toluene was added and the mixture was washed with water, dried and concentrated. The residue was purified by column chromatography (15 : 1 toluene–EtOAc) to give **2** (1.23 g, 2.78 mmol, 52%). Crystallization from diethyl ether–petroleum ether gave material having mp 139°C; $[\alpha]_{578}$ −56° (*c* 0.5, $CHCl_3$); R_f 0.71 (4:1 petroleum ether–EtOAc); NMR data: ^{13}C, δ 30.9 (Me *S*-acetyl), 101.6 (PhCH), 191.7 (C=O *S*-acetyl); ^1H, δ 5.06 (d, $J_{1,2}$ 10.8 Hz, H-1). Anal. Calcd for $C_{22}H_{23}N_3O_5S$: C, 59.8; H, 5.2; N, 9.5; S, 7.3 Found: C, 60.0; H, 5.4; N, 9.7; S, 7.2.

Ethyl 2-azido-3-O-benzyl-4,6-O-benzylidene-2-deoxy-1-thio-β-D-glucopyranoside (**3**).—Sodium methoxide in MeOH (10 mL, 1 M) was added to a stirred solution of **2** (5.7 g, 13 mmol) in CH_2Cl_2 (140 mL). After 30 min at room temperature, EtI (4.2 mL, 52 mmol) was added and the mixture was stirred until TLC indicated that the reaction was complete. The mixture was then washed with water, dried, and concentrated. The residue was purified by column chromatography (8 : 1 petroleum ether–EtOAc) to give **3** (4.37 g, 10.2 mmol, 79%). Crystallization from EtOAc–petroleum ether gave material having mp 86°C; $[\alpha]_{578}$ −118° (c 0.5, $CHCl_3$); R_f 0.52 (6:1 petroleum ether–EtOAc); NMR data: ^{13}C, δ 15.0 (Me ethyl), 24.9 (CH_2S), 84.9 (C-1), 101.3 (PhCH); ^1H, δ 3.43 (m, H-5), 3.46 (dd, $J_{1,2}$ 10.2, $J_{2,3}$ 8.9 Hz, H-2), 3.64 (t, $J_{3,4}$ 8.9 Hz, H-3), 3.73 (t, $J_{4,5}$ 9.2 Hz, H-4), 3.77 (t, $J_{5,6a} = J_{6a,6b} =$ 10.4 Hz, H-6a), 4.34 (dd, $J_{5,6b}$ 5.1 Hz, H-6b), 4.37 (d, H-1). Anal. Calcd for $C_{22}H_{25}N_3O_4S$: C, 61.8; H, 5.9; S, 7.5; N, 9.8. Found: C, 61.6; H, 5.9; S, 7.5; N, 9.8.

Ethyl 2-azido-3,6-di-O-benzyl-2-deoxy-1-thio-β-D-glucopyranoside (**4**).—Diethyl ether saturated with HCl was added, at room temperature, to a stirred mixture of **3** (2.5 g, 5.8 mmol), $NaCNBH_3$ (2.2 g, 35 mmol) and 3A molecular sieves in THF (50 mL) until the mixture was acidic (as determined with indicator paper). The mixture was stirred for 10 min and was then diluted with CH_2Cl_2 and filtered. The solution was washed with aq $NaHCO_3$, water, dried and concentrated. The residue was purified by column chromatography (4:1 toluene–EtOAc) to give **4** (2.1 g, 4.9 mmol, 83%), isolated as a syrup, having $[\alpha]_{578}$ −73° (*c* 0.6, $CHCl_3$); R_f 0.47 (4:1 petroleum ether–EtOAc); NMR data: ^{13}C, δ 15.0 (Me ethyl), 24.6 (CH_2S), 84.5 (C-1); ^1H, δ 4.29 (d, $J_{1,2}$ 9.5 Hz, H-1).

Ethyl 2-azido-3,6-di-O-benzyl-2-deoxy-4-O-(methyl 2,3,4-tri-O-acetyl-α-L-idopyranosyluronate)-1-thio-β-D-glucopyranoside (**5**).—A mixture of **4** (650 mg, 1.51 mmol), freshly prepared methyl (2,3,4-tri-*O*-acetyl-β-L-idopyranosyl bromide)uronate[10] (750 mg, 1.81 mmol), 4A molecular sieves and 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) (71 mg, 0.30 mmol) 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 (580 mg, 2.3 mmol) was added and the mixture was stirred at this temperature for 15

min. Triethylamine (1.2 mL) was added and the mixture was allowed to attain room temperature. The mixture was filtered through Celite, concentrated, and purified by column chromatography (3:1 toluene–EtOAc) to give **5** (665 mg, 0.89 mmol, 60%), isolated as an amorphous solid, having $[\alpha]_{578}$ +50° (*c* 0.6 $CHCl_3$); R_f 0.54 (4:1 toluene–EtOAc); NMR data: ^{13}C, δ 15.2 (Me ethyl), 20.6, 20.8, 21.0 (Me acetyl) 24.4 (CH_2S), 52.2 (MeO), 84.4 (C-1), 97.0 (C-1′), 168.0 (C-6′), 169.0, 169.4 169.6 (C=O acetyl). ^1H NMR data are shown in the following table. Anal. Calcd for $C_{35}H_{43}N_3O_{13}S$: C, 56.5; H, 5.85; S, 4.3. Found: C, 56.4; H, 6.0; S, 4.1.

	H-1	H-2	H-3	H-4	H-5
GlcN	4.27 (10.1)	3.49 (9.4)	3.35 (9.2)	4.03 (10.0)	3.34
IdoA	5.34 (3.0)	4.79 (4.7)	5.16 (4.7)	5.05 (3.4)	4.90

*Methyl 2,3-di-*O*-benzyl-4-*O*-p-methoxybenzyl-β-*D*-glucopyranoside* (**6**).—A cooled solution of Me_3SiCl (12 ml, 72 mmol) in MeCN (70 mL) was added dropwise to a stirred mixture of methyl 2,3-di-*O*-benzyl-4,6-*O*-*p*-methoxybenzylidene-β-D-glucopyranoside[17] (6.0 g, 12 mmol), $NaCNBH_3$ (4.6 g, 72 mmol), and 3A molecular sieves in MeCN (240 mL) of 0°C. The mixture was stirred at room temperature for 6 h and was then filtered through Celite and diluted with CH_2Cl_2. The solution was washed with aq $NaHCO_3$ and water, dried, and concentrated. Purification of the product by column chromatography (3:1 toluene–EtOAc) gave **6** (4.0 g, 8.0 mmol, 67%), isolated as an amorphous solid, having $[\alpha]_{578}$ −1° (*c* 0.5, $CHCl_3$); R_F 0.50 (1:1 toluene-EtOAc); NMR data: ^{13}C, δ 55.3 (MeO *p*-methoxybenzyl), 57.3 (MeO), 104.7 (C-1), 113.9, 159.4 (aromatic C *p*-methoxybenzyl); ^1H, δ 3.34 (m, H-5), 3.39 (dd, $J_{1,2}$ 7.8, $J_{2,3}$ 9.1 Hz, H-2), 3.54 (t, $J_{3,4}$ = 9.1 Hz, H-4), 3.65 (t, H-3), 3.71 (m, H-6a), 3.86 (m, H-6b), 4.34 (d, H-1). Anal. Calcd for $C_{29}H_{34}O_7$: C, 70.4; H, 6.9 Found: C, 70.4; H, 6.9.

tert-*Butyl (methyl 2,3-di-*O*-benzyl-4-*O*-p-methoxybenzyl-β-*D*-glucopyranosid)uronate* (**7**).—Pyridinium dichromate (6.1 g, 16 mmol), Ac_2O (7.6 mL, 80.4 mmol), and *tert*-butyl alcohol (15 mL, 160 mmol) were added to a stirred solution of **6** (4.0 g, 8.0 mmol) in CH_2Cl_2 (100 mL). The mixture was stirred for 6 h at room temperature and was then applied on the top of a silica gel column in EtOAc, with a 5-cm layer of EtOAc on top of the gel. The chromium compounds precipitated in the presence of EtOAc and after 15 min the product was eluted with EtOAc. After evaporating the solvent, the product was purified by column chromatography (6:1 toluene–EtOAc) to give **7** (3.4 g, 6.0 mmol, 75%). Crystallization from diethyl ether–petroleum ether gave material having mp 70°C; $[\alpha]_{578}$ −30° (*c* 0.6, $CHCl_3$); R_f 0.45 (6:1 petroleum ether–EtOAc); NMR data: ^{13}C, δ 28.0 (Me *tert*-butyl), 55.3 (MeO *p*-methoxybenzyl), 57.3 (MeO), 105.0 (C-1), 113.8, 159.3 (aromatic C *p*-methoxybenzyl), 167.8 (C-6); ^1H δ 3.45 (dd, H-2), 3.63 (t, $J_{2,3} = J_{3,4} = 9.0$ Hz, H-3), 3.75 (d, $J_{4,5}$ 9.5 Hz, H-5), 3.82 (dd, H-4), 4.34 (d, $J_{1,2}$ 7.6 Hz, H-1). Anal. Calcd for $C_{33}H_{40}O_8$: C, 70.2; H, 7.1 Found: C, 70.3; H, 7.3.

tert-*Butyl (methyl 2,4-di-*O*-benzyl-β-*D*-glucopyranosid)uronate* (**8**).—A solution of ceric ammonium nitrate (2.3 g, 4.2 mmol) in (10:1 MeCN–water (11 mL) was

added to a solution of **7** (1.2 g, 2.1 mmol) in CH_2Cl_2 (15 mL) of 0°. The mixture was stirred at room temperature for 2 h and then extracted with aq $NaHCO_3$ and water, dried and concentrated. The residue was purified by column chromatography (6:1 toluene–EtOAc) to give **8** (880 mg, 2.0 mmol, 92%), isolated as a syrup, having $[\alpha]_{578}$ −7° (*c* 0.5, $CHCl_3$); R_f 0.62 (4:1 petroleum ether–EtOAc); NMR data: ^{13}C, δ 27.9 (Me *tert*-butyl), 57.2 (MeO), 104.7 (C-1), 168.4 (C-6); ^1H, δ 3.04 (d, $J_{OH,4}$ 2.2 Hz, OH), 3.42 (dd, $J_{1,2}$ 7.6, $J_{2,3}$ 9.0 Hz, H-2), 3.51 (t, $J_{3,4}$ 8.7 Hz, H-3), 3.69 (d, $J_{4,5}$ 9.8 Hz, H-5), 3.83 (m, H-4), 4.34 (d, H-1).

Methyl O-*(methyl 2,3,4-tri-*O*-acetyl-α-*L*-idopyranosyluronate)-(1 → 4)-*O*-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-*D*-glucopyranosyl)-(1 → 4)-*O*-(tert-butyl 2,3-di-*O*-benzyl-β-*D*-glucopyranosid)uronate* (**9**).—*N*-Iodosuccinimide (48 mg, 0.22 mmol), immediately followed by a solution of silver triflate (37 mg, 0.15 mmol) in toluene (0.5 mL) was added at −20°C to a stirred mixture of **5** (140 mg, 0.19 mmol) and **8** (65 mg, 0.15 mmol) in CH_2Cl_2 (5 mL) containing 4A molecular sieves. The mixture was stirred at this temperature for 15 min and was then allowed to attain room temperature. When TLC indicated complete reaction, the mixture was diluted with CH_2Cl_2 and filtered through Celite. The filtrate was extracted with 10% aq $Na_2S_2O_3$ and water, dried, and concentrated. The residue was purified by column chromatography (4:1 toluene–EtOAc) to give **9** (120 mg, 0.11 mmol, 73%), isolated as an amorphous solid, having $[\alpha]_{578}$ +6° (*c* 0.6, $CHCl_3$); R_f 0.58 (4:1 toluene–EtOAc); NMR data: ^{13}C, δ 20.6, 20.7, 21.0 (Me acetyl), 28.1 (Me *tert*-butyl), 52.1, 57.0 (MeO), 96.8, 96.9 (C-1′, C-1″), 104.8 (C-1), 167.4, 167.9 (C-6, C-6″) 169.0, 169.4, 169.6 (C=O acetyl). ^1H NMR data are shown in the following table.

	H-1	H-2	H-3	H-4	H-5
GlcA	4.38 (7.4)	3.48 (8.9)	3.75 (8.9)	4.16 (9.0)	3.68
GlcNAc	5.65 (3.8)	3.32 (10.4)	3.71 (9.5)	4.07 (9.5)	3.58
IdoA	5.33 (3.4)	4.74 (5.0)	5.16 (5.0)	5.03 (3.7)	4.8

Methyl O-*(α-L-idopyranosyluronic acid)-(1 → 4)-*O*-2-acetamido-2-deoxy-α-*D*-glucopyranosyl)-(1 → 4)-*O*-β-*D*-glucopyranosyluronic acid disodium salt* (**10**). Compound **9** (75 mg, 66 μmol) was dissolved in a solution of CF_3CO_2H in CH_2Cl_2 (2 mL, 20%) and stirred for 1 h. The mixture was then diluted with CH_2Cl_2, washed with aq $NaHCO_3$ and water, dried, and concentrated. The residue was dissolved in THF (4 mL) and cooled to +8°C. Cold aq NaOH (2 mL, 1 M) was added dropwise and the mixture was stirred at +8°C until TLC indicated complete reaction. The mixture was neutralized with Dowex H^+ and concentrated. The residue was dissolved in 1:1 water–EtOH (10 mL) and hydrogenolyzed over Pd–C for 24 h. After filtering through Celite and concentrating, the residue was dissolved in 4:1 water–MeOH (4 mL) and the pH of the solution was adjusted to 7.5 with satd aq $NaHCO_3$. Acetic anhydride (40 μL) was added in small portions and the mixture was stirred for 30 min at room temperature. The mixture was concentrated and 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 **10** (26 mg, 41 μmol, 62%), isolated as an amorphous solid, having $[\alpha]_{578}$ + 29° (*c* 0.5, H_2O); R_f 0.53 (4:3:3:2 EtOAc–EtOH–AcOH–H_2O); water NMR data (D_2O; sodium 4,4-dimethyl-4-silapentanoate-2,2′,3,3′-d_4, δ 0.00): ^{13}C, δ 24.7 (Me *N*-acetyl), 56.5 (MeO), 60.0, 62.4 (C-2 and C-6′), 72.2, 73.8, 74.0, 74.3, 74.4, 75.3, 76.2, 78.9, 79.5, 79.7, 80.4 (ring C) 99.6 (C-1′), 104.1, 106.0 (C-1 and C-1″), 177.2, 177.8, 178.6 (C-6, C-6″, and C=O *N*-acetyl). ^1H NMR data are shown in the following table:

	H-1	H-2	H-3	H-4	H-5
GlcA	4.38 (7.9)	3.31 (9.3)	3.68 (8.7)	3.77	ND
GlcN	5.39 (3.9)	3.94 (10.5)	3.79 (8.7)	ND	ND
IdoA	4.80 (5.9)	3.50 (8.1)	3.67 (7.2)	3.86 (4.7)	4.54

Ethyl 2,4,6-tri-O-acetyl-3-O-benzyl-1-thio-β-D-glucopyranoside (**11**). – A solution of $BF_3 \cdot OEt_2$ (6.2 ml, 50 mmol) in CH_2Cl_2 (10 mL) was added dropwise to a stirred solution of 1,2,4,6-tetra-*O*-acetyl-3-*O*-benzyl-β-D-glucopyranoside[22] (6.0 g, 14 mmol), EtSH (1.2 mL, 16 mmol) and 4A molecular sieves in anhyd CH_2Cl_2 (50 mL) at 0°C. The mixture was stirred for 2 h at room temperature and was then filtered through Celite, washed with water, aq $NaHCO_3$ and water, dried and concentrated. The product was purified by column chromatography (4:1 toluene–EtOAc) and then crystallized from EtOAc–petroleum ether to give **11** (5.5 g, 13 mmol, 91%) having mp 93°C; $[\alpha]_{578}$ −34° (*c* 0.5, $CHCl_3$); R_f 0.70 (1:1 toluene–EtOAc); NMR data: ^{13}C, δ 14.8 (Me ethyl), 20.75, 20.77, 20.9 (Me acetyl), 24.0 (CH_2S), 83.7 (C-1), 169.29, 169.33, 170.7 (C=O acetyl); ^1H, δ 3.60 (m, H-5), 3.71 (t, $J_{2,3} = J_{3,4} = 9.3$ Hz, H-3), 4.12 (dd, $J_{5,6a}$ 2.6, $J_{6a,6b}$ 12.3 Hz, H-6a), 4.19 (dd, $J_{5,6b}$ 5.2 Hz, H-6b), 4.40 (d, $J_{1,2}$ 10.0 Hz, H-1), 5.08 (dd, H-2), 5.10 (t, $J_{4,5}$ 9.7 Hz, H-4). Anal. Calcd for $C_{21}H_{28}O_8S$: C, 57.2; H, 6.4; S, 7.3 Found: C, 57.2; H, 6.4; S, 7.3.

Ethyl-3-O-benzyl-4,6-O-p-methoxybenzylidene-1-thio-β-D-glucopyranoside (**12**).—Compound **11** (12 g, 27 mmol) was dissolved in CH_2Cl_2–MeOH (200 mL, 1:1) and methanolic NaOMe (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 temperature of 50°C and was then neutralized with AcOH and concentrated. The residue was dissolved in THF (150 mL) containing *p*-methoxybenzaldehyde dimethyl acetal (9.5 mL, 55 mmol) and *p*-toluenesulphonic acid was added until the mixture was acidic. The mixture was stirred at room temperature for 5 h and was then neutralized with pyridine. The pyridine–*p*-toluenesulphonic acid salt precipitated in the refrigerator overnight and was removed by filtering. The filtrate was concentrated and the product was purified by column chromatography (10:1 toluene–EtOAc). Crystallization from EtOAc–petroleum ether gave **12** (8.4 g, 20 mmol, 71%) having mp 128°C; $[\alpha]_{578}$ −53° (*c* 0.5, $CHCl_3$); R_f 0.61 (4:1 toluene–EtOAc); NMR data: ^{13}C, δ 15.2 (Me ethyl), 24.5 (CH_2S), 55.3 (MeO), 86.6 (C-1), 101.3 (Ph*C*H), 113.6, 160.1 (aromatic C *p*-methoxybenzyl); ^1H, δ 2.53 (d, $J_{OH,2}$ 2.0 Hz, OH), 3.48 (m, H-5), 3.57 (m, H-2), 3.66 (t, $J_{2,3} = J_{3,4} = 9.0$

Hz, H-3), 3.69 (t, $J_{4,5}$ 9.0 Hz, H-4), 3.75 (t, $J_{5,6a} = J_{6a,6b} = 10.4$ Hz, H-6a), 4.33 (dd, $J_{5,6b}$ 4.9 Hz, H-5), 4.45 (d, $J_{1,2}$ 9.8 Hz, H-1). Anal. Calcd for $C_{23}H_{28}O_6S$: C, 63.9; H, 6.5; S, 7.4. Found: C, 63.9; H, 6.5; S, 7.5.

Ethyl 2-O-benzoyl-3-O-benzyl-4,6-O-p-methoxybenzylidene-1-thio-β-D-glucopyranoside (**13**).—Benzoyl chloride (6.8 mL, 58 mmol) in CH_2Cl_2 (140 mL) was added to a stirred solution of **12** (12.6 g, 29.1 mmol) and pyridine (50 mL) in CH_2Cl_2 (200 mL) at 0°C. The mixture was stirred at room temperature overnight, and then MeOH was added. The mixture was concentrated, purified by column chromatography (10:1 tolulene–EtOAc) and the product was crystallized from EtOAc–petroleum ether to give **13** (11.4 g, 21.2 mmol, 73%) having mp 141°C; $[\alpha]_{578}$ +18° (*c* 0.5, $CHCl_3$); R_f 0.52 (4:1 petroleum ether–EtOAc); NMR data: ^{13}C, δ 14.8 (Me ethyl), 24.0 (CH_2S), 55.3 (MeO), 84.3 (C-1), 101.3 (Ph*C*H), 113.6, 160.1 (aromatic C *p*-methoxybenzyl), 165.1 (C=O benzoyl); ^1H, δ 3.54 (m, H-5), 3.80 (t, $J_{5,6a} = J_{6a,6b} = 10.4$ Hz, H-6a), 3.82 (t, $J_{3,4} = J_{4,5} = 8.9$ Hz, H-4), 3.88 (t, H-3), 4.38 (dd, $J_{5,6b}$ 5.1 Hz, H-6b), 4.61 (d, $J_{1,2}$ 10.0 Hz, H-1), 5.32 (dd, $J_{2,3}$ 8.5 Hz, H-2). Anal. Calcd for $C_{30}H_{32}O_7S$: C, 67.1; H, 6.0; S, 6.0. Found: C, 67.3; H, 6.1; S, 5.9.

Ethyl 2-O-benzoyl-3-O-benzyl-4-O-p-methoxybenzyl-1-thio-β-D-glucopyranoside (**14**).—Compound **13** (2.0 g, 3.7 mmol) was treated as described for the preparation of **6**. The product was purified by column chromatography (6:1 toluene–EtOAc) to give **14** (1.4 g, 2.6 mmol 70%). Crystallization from EtOAc–petroleum ether gave material having mp 110°C; $[\alpha]_{578}$ +27° (*c* 0.5, $CHCl_3$); R_f 0.55 (2:1 toluene– EtOAc); NMR data: ^{13}C, δ 14.9 (Me ethyl), 24.1 (CH_2S), 55.3 (MeO), 84.2 (C-1), 114.0, 159.5 (aromatic C *p*-methoxybenzyl), 165.3 (C=O benzoyl); ^1H, δ 3.46 (m, H-5), 3.68 (dd, $J_{3,4}$ 9.0, $J_{4,5}$ 9.5 Hz, H-4), H-4), 3.84 (t, $J_{2,3}$ 9.0 Hz, H-3), 4.57 (d, $J_{1,2}$ 10.0 Hz, H-1), 5.26 (t, H-2). Anal. Calcd for $C_{30}H_{34}O_7S$: C, 66.9; H, 6.4; S, 5.9. Found: C, 66.8 H, 6.4; S, 5. 9.

tert-*Butyl (ethyl 2-O-benzoyl-3-O-benzyl-4-O-p-methoxybenzyl-1-thio-β-D-glucopyranosid)uronate* (**15**).—Compound **14** (800 mg, 1.48 mmol) was treated as described for the preparation of **7**. The product was purified by column chromatography (8:1 toluene-EtOAc) to give **15** (615 mg, 1.01 mmol, 68%). Crystallization from diethyl ether–petroleum ether gave material having mp 80°C; $[\alpha]_{578}$ −8° (*c* 0.5, $CHCl_3$); R_f 0.61 (4:1 petroleum ether–EtOAc); NMR data: ^{13}C, δ 14.9 (Me ethyl), 23.8 (CH_2S), 28.0 (Me *tert*-butyl), 55.3 (MeO), 84.0 (C-1), 113.8, 159.4 159.4 (aromatic C *p*-methoxybenzyl), 165.2 (C=O) benzoyl), 167.2 (C-6); ^1H, δ 3.81 (t, $J_{2,3} = J_{3,4} = 9.0$ Hz, H-3), 3.85 (d, $J_{4,5}$ 9.6 Hz, H-5), 3.95 (dd, H-4), 4.54 (d, $J_{1,2}$ 10.0 Hz, H-1), 5.32 (dd, H-2). Anal. Calcd for $C_{34}H_{40}O_8S$: C, 67.1; H, 6.6; S, 5.9. Found: C, 67.2; H, 6.7; S, 5.9.

*Methyl 4-O-acetyl-2,6-di-O-benzyl-3O-(*tert*-butyl 2-O-benzoyl-3-O-benzyl-4-O-p-methoxybenzyl-β-D-glucopyranosyluronate)-β-D-galactopyranoside* (**16**).—DMTST (460 mg, 1.8 mmol) was added to a stirred mixture of compound **15** (363 mg, 0.60 mmol), methyl 4-*O*-acetyl-2,6-di-*O*-benzyl-β-D-galactopyranoside[16] (260 mg, 0.62 mmol) and 4A molecular sieves in dry CH_2Cl_2 (5 mL) at 0°C under N_2. The mixture was allowed to attain room temperature and the stirring was continued for

3 h. The mixture was then cooled to 0°C, Et_3N (0.75 mL, 5.4 mmol) was added and the stirring was continued for 30 min. The mixture was filtered through Celite, concentrated, and purified by column chromatography (6:1 toluene–EtOAc) to give **16** (500 mg, 0.52 mmol, 87%), isolated as an amorphous solid, having $[\alpha]_{578}$ +24° (*c* 0.6, $CHCl_3$); R_f 0.60 (6:1 toluene–EtOAc); NMR data: ^{13}C, δ 20.7 (Me acetyl), 28.0 (Me *tert*-butyl), 55.3 (MeO *p*-methoxybenzyl), 57.2 (MeO), 100.6 (C-1′), 104.6 (C-1), 113.8, 159.3 (aromatic C *p*-methoxybenzyl), 164.8 (C=O benzoyl), 167.4 (C-6′), 170.1 (C=O acetyl). ^1H NMR data are shown in the following table: Anal. Calcd for $C_{55}H_{62}O_{15}$: C, 68.6; H, 6.5. Found: C, 69.1; H 6.5.

	H-1	H-2	H-3	H-4	H-5
Gal	4.26 (7.9)	3.46 (9.7)	3.90 (3.6)	5.46 (0.9)	3.72
GlcA	5.05 (7.1)	5.25 (8.5)	3.72 (8.9)	4.02 (10.0)	3.79

*Methyl 4-O-acetyl-2,6-di-O-benzyl-3-O-(*tert*-butyl-2-O-benzoyl-3-O-benzyl-β-*D*-glucopyranosyluronate)-β-*D*-galactopyranoside* (**17**).—Ceric ammonium nitrate (400 mg, 0.73 mmol) dissolved in 5:1 MeCN–water (6 mL) was added to a solution of **16** (320 mg, 0.33 mmol) in CH_2Cl_2 (10 mL) at 0°C. The mixture was stirred for 2 h at room temperature and then extracted with aq $NaHCO_3$ and water, dried and concentrated. The residue was purified by column chromatography (4:1 toluene–EtOAc) to give **17** (225 mg, 0.27 mmol, 80%), isolated as a syrup, having $[\alpha]_{578}$ +25° (*c* 0.5, $CHCl_3$); R_f 0.53 (4:1 toluene–EtOAc); NMR data: ^{13}C, δ 20.7 (Me acetyl), 28.0 (Me *tert*-butyl), 100.9 (C-1′), 104.6 (C-1), 164.8 (C=O benzoyl), 168.4 (C-6′), 170.2 (C=O acetyl). ^1H NMR data are shown in the following table:

	H-1	H-2	H-3	H-4	H-5
Gal	4.25 (7.8)	3.45 (9.5)	3.90 (3.5)	5.44 (1.7)	3.71
GlcA	5.00 (7.6)	5.20 (9.0)	3.62 (9.0)	4.03 (9.8)	3.68

*Methyl O-(methyl 2,3,4-tri-O-acetyl-α-L-idopyranosyluronate)-(1 → 4)-O-(2-azido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranosyl)-(1 → 4)-O-(*tert*-butyl 2,3-di-O-benzyl-α-D-glucopyranosyluronate)-(1 → 3)-4-O-acetyl-2,6-di-O-benzyl-β-D-galactopyranoside* (**18**).—A mixture of **17** (330 mg, 390 μmol), **5** (351 mg, 471 μmol), 2,6-di-*tert*-butyl-4-methylpyridine (50 mg, 200 μmol), and 4A molecular sieves in CH_2Cl_2 (2 mL) was treated with DMTST (360 mg, 1.4 mmol) as described for the preparation of **16**. The product was purified by column chromatography (2:1 petroleum ether–EtOAc) to give **18** (472 mg, 309 μmol, 79%) having $[\alpha]_{578}$ +15° (*c* 0.5, $CHCl_3$); R_f 0.38 (2:1 petroleum ether–EtOAc); NMR data: ^{13}C, δ 20.58, 20.61, 20.9 (Me acetyl), 28.0, 28.1, 29.0 (Me *tert*-butyl), 52.1, 57.1 (MeO), 96.6, 97.0 C-1′, C-1‴), 100.7 (C-1″), 104.6 (C-1), 164.7 (C=O benzoyl), 166.8, 168.0 (C-6′, C-6‴), 169.9, 169.3, 169.6, 170.0 (C=O acetyl). ^1H NMR data are shown in the following table: Anal. Calcd for $C_{80}H_{91}N_3O_{27}$: C, 62.9; H, 6.0. Found: C, 62.7; H 6.1.

	H-1	H-2	H3	H-4	H-5
Gal	4.26 (7.8)	3.46 (9.7)	3.84 (3.6)	5.41	3.68
GlcA	5.08 (7.1)	5.30 (8.4)	3.91 (8.4)	4.31 (9.7)	3.82
GlcN	5.57 (3.7)	3.34 (10.1)	3.73 (9.8)	4.08 (9.8)	3.67
IdoA	5.37 (3.4)	4.77	5.19	5.04 (3.9)	4.81

Methyl O-(α-L-idopyranosyluronic acid)-(1 → 4)-O-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1 → 4)-O-(β-D-glucopyranosyluronic acid)-(1 → 3)-O-β-D-galactopyranoside disodium salt (**19**).—Compound **18** (75 mg, 49 μmol) was treated as described for the preparation of **10** to give **19** (25 mg, 32 μmol, 64%), isolated as an amorphous solid, having $[\alpha]_{578}$ +16° (*c* 0.5, H_2O); R_f 0.33 (4:3:3:2 EtOAc–EtOH–AcOH–H_2O); NMR data (D_2O; sodium 4,4-dimethyl-4-silapentanoate-*2,2',3,3'-d*$_4$, δ (0.00): ^{13}C, δ 24.7, (Me *N*-acetyl), 56.5 (MeO), 59.0, 62.3, 63.8, 71.0, 72.2, 72.5, 73.8, 74.0, 74.3, 74.4, 75.3, 76.5, 77.7, 78.9, 79.2, 79.5, 80.4, 85.8, (ring C), 99.7 (C-1″), 104.1, 106.2, 106.6 (C-1, C-1′, C-1‴), 177.2, 178.1, 178.7 (C-6′, C-6‴, and C=O N-acetyl). ^1H NMR data are shown in the following table:

	H-1	H-2	H-3	H-4	H-5
Gal	4.38 (8.1)	3.66 (9.8)	3.77	4.16	3.68
GlcA	4.65 (7.8)	3.43 (9.3)	3.70 (8.7)	3.78	ND
GlcN	5.39 (3.9)	3.94 (10.4)	3.79	3.83	ND
IdoA	4.80 (6.0)	3.50 (7.9)	3.67 (7.2)	3.86 (4.7)	4.54

REFERENCES

1 D. Lane and U. Lindahl, *Heparin; Chemical and Biological Properties, Clinical Applications*, Edward Arnold, London, 1989.
2 T. Barrowcliffe, E. Johnson, and D. Thomas, *Low Molecular Weight Heparin*, Wiley, 1992.
3 U. Lindahl, G. Bäckström, and L. Thunberg, *Carbohydr. Res.*, 100 (1982) 393–410.
4 C-M. Svahn, A. Ansari, and T. Wehler, unpublished results, Kabi Pharmacia AB.
5 B. Lindberg, L. Rodén, and B.-G. Silvander, *Carbohydr. Res.*, 2 (1966) 413–417.
6 P.J. Garegg, B. Lindberg, and T. Norberg, *Acta Chem. Scand., Ser. B*, 33 (1979) 449–452.
7 G. Ekborg, T. Curenton, N.R. Krishna and L. Rodén, *J. Carbohydr. Chem.*, 9 (1990) 15–37.
8 M. Nilsson, J. Westman, and C-M. Svahn, *J. Carbohydr. Chem.*, 12 (1993) 23–37.
9 S. Rio, J.-M. Beau, and J.C. Jacquinet, *XVIth Int. Carbohydr. Symp.*, Paris, 1992.
10 T. Chiba and P. Sinaÿ, *Carbohydr. Res.*, 151 (1986) 379–389.
11 A. Fernandez-Mayoralas, A. Marra, M. Trumtel, A. Veyrières, and P. Sinaÿ, *Carbohydr. Res.*, 188 (1989) 81–95.
12 P. Sinaÿ, *Pure Appl. Chem.*, 63 (1991) 519–528.
13 R.U. Lemieux and R.M. Ratcliffe, *Can. J. Chem.*, 57 (1979) 1244–1251.
14 M. Blanc-Muesser, J. Defaye, and H. Driguez, *Carbohydr. Res.*, 67 (1978) 305–328.
15 M. Haraldsson, H. Lönn, and T. Norberg, *Glycoconjugate J.*, 4 (1987) 225–229.
16 P.J. Garegg, H. Hultberg, and S. Wallin, *Carbohydr. Res.*, 108 (1982) 97–101.
17 J.C. Dennison and O.I. McGilary, *J. Chem. Soc.*, (1951) 1616.
18 R. Johansson and B. Samuelsson, *J. Chem. Soc. Perkin Trans. 1*, (1984) 2371–2374.
19 E.J. Corey and B. Samuelsson, *J. Org. Chem.*, 49 (1984) 4735.
20 P.J. Garegg and C. Hällgren, *J. Carbohydr. Chem.*, 11 (1992) 425–443.
21 P. Konradsson, U.E. Udodong, and B. Fraser-Reid, *Tetrahedron Lett.*, 31 (1990) 4313–4316.
22 S. David, A. Malleron, and C. Dini, *Carbohydr. Res.*, 188 (1989) 193–200.
23 H. Paulsen, T. Hasenkamp, and M. Paal, *Carbohydr. Res.*, 144 (1985) 45–55.