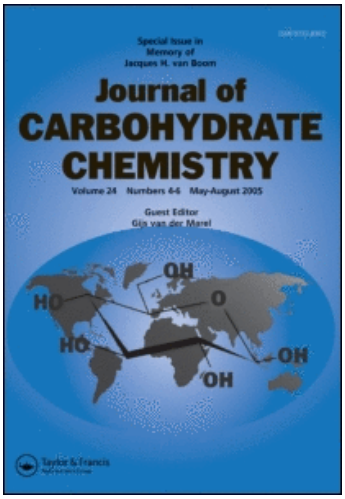

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Synthesis of Tri-And Tetrasaccharides Present in the Linkage Region of Heparin and Heparan Sulphate

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SYNTHESIS OF TRI- AND TETRASACCHARIDES PRESENT IN THE LINKAGE REGION OF HEPARIN AND HEPARAN SULPHATE

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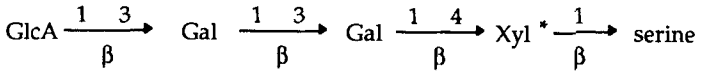
ABSTRACT

The methyl glycosides of the the tri- and tetrasaccharides present in the linkage region of heparin, methyl *O*-(β-D-galactopyranosyl)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→4)-β-D-xylopyranoside and methyl *O*-(β-D-glucopyranosyluronic acid)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→4)-β-D-xylopyranoside sodium salt, were synthesized together with their phosphate containing analogues, methyl *O*-(β-D-galactopyranosyl)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→4)-β-D-xylopyranoside 2-(disodium phosphate) and methyl *O*-(β-D-glucopyranosyluronic acid)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→4)-β-D-xylopyranoside 2-(disodium phosphate) sodium salt, which are glycosides of the structure found in the linkage region of heparan sulphate.

INTRODUCTION

Heparin and heparan sulphate are sulphated glycosaminoglycans widely distributed in animal tissues. They are both composed of repeating disaccharide units of uronic acid (L-iduronic or D-glucuronic acid) and glucosamine (*N*-acetylated or *N*-sulphated).[1] Heparin has a higher *N*-sulphate/*N*-acetyl ratio (usally more than 4:1) than heparan sulphate, which has a ratio of approximately 1:1. The *O*-sulphate content and the iduronic acid/glucuronic acid ratio are higher in heparin than in heparan sulphate. Heparin and heparan sulphate are synthesized in Nature as proteoglycans. The region of



* The xylose residue may be substituted with a phosphate group at C-2.

Figure 1

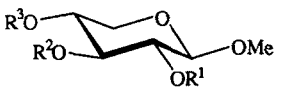
heparin and heparan sulfate which links the carbohydrate chain to a peptide-core is called the linkage region. It consists of sugar residues (galactose and xylose) different from those in the rest of the chain. In heparan sulphate from bovine lung, a phosphate group has been detected at C-2 in the xylose unit.[2] Phosphate was also found in the linkage region of heparin from bovine lung in about one third of the heparin chains,[3] but could not be detected in the linkage region of heparin isolated from porcine intestinal mucosa.[4] A schematic picture of the structure present in the carbohydrate-protein linkage region is shown in figure 1.

In conjunction with our studies of the effect on cell proliferation of oligosaccharides from nitrous acid depolymerized heparin we obtained fractions from the linkage region containing galactose and xylose.[5] Some of these linkage region derived fractions also contained phosphate.[5] It was not established whether these fractions originated from heparin or from contaminating heparan sulphate. We were, therefore, interested in synthesizing linkage region oligosaccharides with and without phosphate in the xylose residue in order to study their potential effect on cell proliferation.

Syntheses of a serine-linked trisaccharide (Gal-Gal-Xyl-Ser)[6,7] and a trisaccharide with a free reducing end (Gal-Gal-Xyl)[8] have been described before by other authors. This paper is a report on the syntheses of the tri- and tetrasaccharides (Gal-Gal-Xyl-OMe and GlcA-Gal-Gal-Xyl-OMe) with and without a phosphate group in the 2-position of xylose. We have prepared the trisaccharides **15** and **16** and the tetrasaccharides **21** and **22**.

RESULTS AND DISCUSSION

The initial idea was to utilize a stepwise strategy, with an excess of the glycosyl donors in the coupling reactions. The trisaccharides **15** and **16** were synthesized in this way and good yields were obtained. When it came to the tetrasaccharides, this strategy did not give satisfactory results and therefore a block type route was adopted. The following steps were performed:



1 $R^1 = R^2 = H$, $R^3 = ClAc$

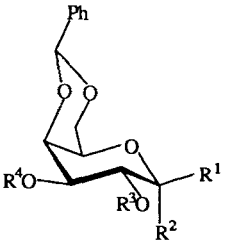
2 $R^1 = R^2 = Ac$, $R^3 = ClAc$

3 $R^1 = R^2 = Ac$, $R^3 = H$

4 $R^1 = H$, $R^2 = Ac$, $R^3 = ClAc$

5 $R^1 = PO(OBn)_2$, $R^2 = Ac$, $R^3 = ClAc$

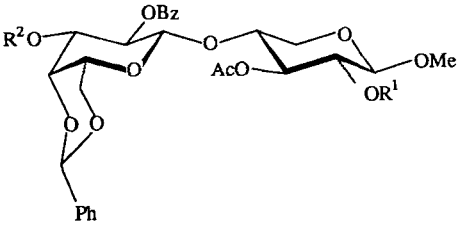
6 $R^1 = PO(OBn)_2$, $R^2 = Ac$, $R^3 = H$



7 $R^1 = SEt$, $R^2 = H$, $R^3 = Bz$, $R^4 = ClAc$

8 $R^1 = H$, $R^2 = Br$, $R^3 = Bz$, $R^4 = ClAc$

17 $R^1 = SEt$, $R^2 = H$, $R^3 = Ac$, $R^4 = H$

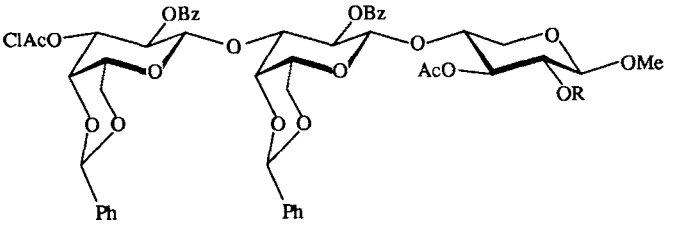


9 $R^1 = Ac$, $R^2 = ClAc$

10 $R^1 = PO(OBn)_2$, $R^2 = ClAc$

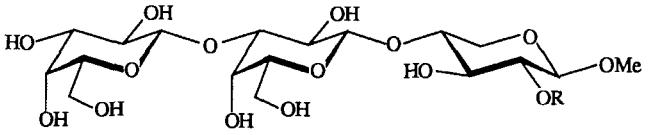
11 $R^1 = Ac$, $R^2 = H$

12 $R^1 = PO(OBn)_2$, $R^2 = H$

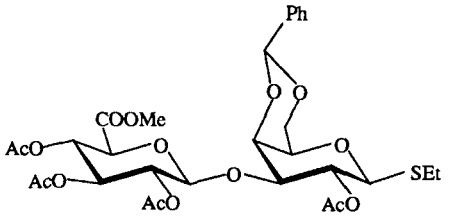


13 R = OAc

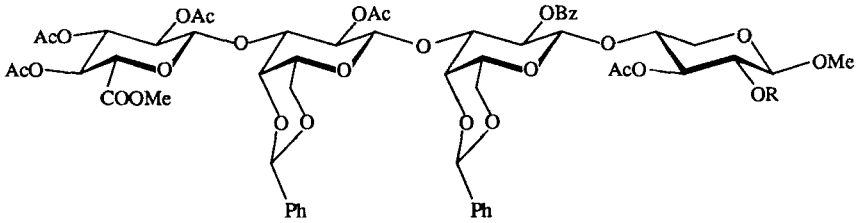
14 $R = PO(OBn)_2$



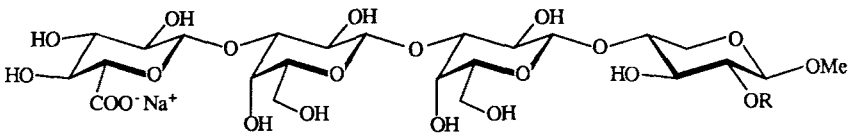
15 R = H
16 R = PO_3Na_2



18



19 R = Ac
20 R = $PO(OBn)_2$



21 R = H
22 R = PO_3Na_2

Methyl β-D-xylopyranoside was selectively chloroacetylated in the 4-position by treatment with dibutyltin oxide followed by chloroacetyl chloride giving compound **1** in 78% yield. Acetylation of **1** using acetyl chloride and pyridine gave compound **2** in 92% yield. Treatment of **2** with hydrazine acetate gave the 4-OH compound **3**[9] compound in 77% yield. Compound **1** was also subjected to selective acetylation giving the 3-*O*-acetyl compound **4** in 68% yield. Phosphorylation of **4** using phosphorus trichloride-imidazole, followed by treatment with benzyl alcohol and oxidation by *m*-chloroperbenzoic acid *in situ* gave the phosphotriester **5** in 72% yield. Removal of the chloroacetyl group in **5** gave the 4-OH compound **6** in 91% yield.

Ethyl 4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside[10] was selectively chloroacetylated, using the same method as for the preparation of **1**, after which it was benzoylated by benzoyl chloride-pyridine to give compound **7** in 69% yield. Preparation of **7** has been described before by Norberg *et al.*[11] Treatment of **7** as described by the same authors gave the glycosyl halide **8**.[11]

Glycosidation of **3** with **8**, using silver triflate-collidine as promoter, gave the disaccharide **9** in 80% yield. The corresponding phosphorylated disaccharide **10**, was prepared in 67% from **6** with the same method. The chloroacetyl groups in **9** and **10** were removed by hydrazine acetate giving the 3′-OH compounds **11** and **12** in 92% and 78% yield, respectively. Silver triflate-collidine promoted glycosidation of **11** with **8** gave the trisaccharide **13** in 85% yield. Glycosidation of **12** by the same method gave **14** in 79% yield. Treatment of **13** with aqueous acid followed by methanolic sodium methoxide gave the deprotected trisaccharide **15** in 91% yield. Deprotection of **14** was performed by hydrogenolysis over Pd/C followed by deacylation using sodium hydroxide to give the trisaccharide **16** in 67% yield.

In order to produce tetrasaccharides, the trisaccharides **13** and **14** were dechloroacetylated giving the 3′′-OH derivatives, which were planned to be glycosidated with methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide) uronate[12] to give tetrasaccharides. However, the tetrasaccharides could not be produced in satisfactory and reproducible yields by this route.

To avoid the problem, we decided to synthesize a disaccharide block of glucuronic acid and galactose. We prepared the thioethyl glycoside **18**, which was reacted with **11** and **12** giving the protected tetrasaccharides **19** and **20** respectively.

Treatment of ethyl 2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-1-thio-β-D-galactopyranoside[13] by hydrazine acetate gave the 3-OH compound **17** in 78% yield. Glycosidation of **17** with methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide) uronate[9] using silver triflate as promoter gave the disaccharide **18** in 50% yield together with the acetylated aglycon (39%), which was identified by ^{13}C NMR as ethyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside.

Glycosidation of **11** with **18** using dimethyl(methylthio)sulfonium triflate (DMTST)[14] as promoter and 2,6-di-*tert*-butylpyridine as acid acceptor gave the tetra-

saccharide **19** in 64% yield. Compound **20** was prepared in 58% yield from **12** and **18** with the same method. Treatment of **19** with aqueous acetic acid followed by sodium hydroxide gave the deprotected tetrasaccharide **21** in 90% yield. Deprotection of **20** was performed by hydrogenolysis over Pd/C followed by deacylation using sodium hydroxide to give the phosphate containing tetrasaccharide **22** in 65% yield.

EXPERIMENTAL

General methods. Melting points are reported corrected. Concentrations were performed under reduced pressure at < 40 °C (bath). Optical rotations were recorded for 0.4-0.7% solutions at room temperature (22-25 °C) using a Perkin-Elmer 241 MC polarimeter. NMR spectra were recorded in $CDCl_3$ at 30 °C, using a JEOL GX/EX-400 instrument, and chemical shifts are given in ppm relative to internal tetramethylsilane, unless otherwise stated. All ^1H 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 shift values and coupling constants (values in parantheses) are often presented as tables, in which the sugar residues are given as Xyl, XylP, Gal, and GlcA. The designations between the two galactose units are arbitrary. TLC was performed on Silica Gel F254 (Merck) with detection by UV and/or by charring with sulfuric acid. Column chromatography was performed on silica gel (Matrex Silica Si 60A, 35-70μ, Amicon). Organic solutions were dried over magnesium sulfate. Molecular sieves (4Å, Fluka) were desiccated at 300 °C overnight. Elemental analyses were performed by Mikro Kemi AB, Sweden and by Analytische Laboratorien, Germany. The purity of the target compounds was ascertained by HPTLC and by NMR spectroscopy.

Methyl 4-*O*-Chloroacetyl-β-D-xylopyranoside (1). A mixture of methyl β-D-xylopyranoside (12.0 g, 73.1 mmol) and dibutyltin oxide (25 g, 100 mmol) in methanol (600 mL) was heated under reflux for 1.5 h. The solvent was evaporated and the residue was dissolved in dichloromethane (600 mL). A solution of chloroacetyl chloride (6.1 mL, 76 mmol) in dichloromethane (50 mL) was added dropwise and the mixture was stirred in room temperature for 30 min. The solvent was evaporated and the product was purified by column chromatography (toluene-ethyl acetate, 2:3) to give **1** (13.7 g, 57.0 mmol, 78%). Crystallization from acetone-petroleum ether gave material having mp 113 °C, $[\alpha]_{578}$ -93.8° (*c* 0.5, chloroform), R_f 0.36 (toluene-ethyl acetate, 1:4). NMR data: ^{13}C, δ 40.7 (CH_2Cl), 56.9 (MeO), 61.3 (C-5), 72.2, 72.4, 72.8 (C-2,3,4), 103.5 (C-1) and 166.8 (C=O acetyl); ^1H δ 3.38 (dd, $J_{4,5a}$ = 8.1 Hz, $J_{5a,5b}$ = 12.2 Hz, H-5a), 3.49 (dd, $J_{1,2}$ = 6.1 Hz, H-2), 3.75 (dd, H-3), 4.11 (dd, $J_{4,5b}$ = 4.8 Hz, H-5b), 4.30 (d, H-1), 4.91 (m, H-4).

Anal. Calcd for $C_8H_{13}O_6Cl$: C, 39.9; H, 5.5; Cl 14.7. Found: C, 39.8; H, 5.5; Cl, 14.7.

Methyl 2,3-Di-*O*-acetyl-4-*O*-chloroacetyl-β-D-xylopyranoside (2). Acetyl chloride (14.2 mL, 200 mmol) in dichloromethane (100 mL) was added to a stirred solution of **1** (12.0 g, 49.9 mmol) and pyridine (50 mL) in dichloromethane (300 mL) at 0 °C. The

mixture was stirred 20 min at this temperature. Ice was then added and the mixture was washed with sulfuric acid (1M, aq) and water, dried and concentrated. The residue was crystallized from ethyl acetate-petroleum ether giving **2** (14.9 g, 45.9 mmol, 92%) having mp 127 °C, $[\alpha]_{578}$ -61.4° (*c* 0.6, chloroform), R_f 0.82 (toluene-ethyl acetate, 1:1). NMR data: ^{13}C, δ 20.67, 20.70 (Me acetyl), 40.5 (CH_2Cl), 56.7 (MeO), 61.6 (C-5), 70.56, 70.62, 71.2 (C-2,3,4), 101.5 (C-1), 166.5 (C=O chloroacetyl), 169.4, 170.0 (C=O acetyl); ^1H δ 3.44 (dd, $J_{4,5a}$ = 8.3 Hz, $J_{5a,5b}$ = 11.9 Hz, H-5a), 4.16 (dd, $J_{4,5b}$ = 5.0 Hz, H-5b), 4.43 (d, $J_{1,2}$ = 6.6 Hz, H-1), 4.92 (dd, $J_{2,3}$ = 8.4 Hz, H-2), 5.01 (m, H-4), 5.19 (t, $J_{3,4}$ = 8.3 Hz, H-3).

Anal. Calcd for $C_{12}H_{17}O_8Cl$: C, 44.4; H, 5.3. Found: C, 44.4; H, 5.4.

Methyl 2,3-Di-*O*-acetyl-β-D-xylopyranoside (3).[9] A solution of hydrazine acetate (7.7 g, 84 mmol) in methanol (200 mL) was added to **2** (13.6 g, 42.0 mmol) in dichloromethane (200 mL). The mixture was stirred at room temperature for 2 h and then evaporated. The product was purified by chromatography (toluene-ethyl acetate, 1:1) to give **3** (8.06 g, 32.5 mmol, 77%). Crystallization from ethyl acetate-petroleum ether gave material having mp 84 °C, $[\alpha]_{578}$ -82.6° (*c* 0.5, chloroform), R_f 0.44 (toluene-ethyl acetate, 1:1). NMR data: ^{13}C, δ 20.8, 20.9 (2 Me acetyl), 56.7 (MeO), 64.9, 68.5, 70.7, 75.4 (C-2, 3, 4, 5), 101.7 (C-1), 169.6 and 171.4 (2 C=O acetyl); ^1H, δ 2.76 (d, $J_{OH,4}$ = 5.9 Hz, OH), 3.36 (dd, $J_{4,5a}$ = 8.8 Hz, $J_{5a,5b}$ = 11.8 Hz, H-5a), 3.83 (m, H-4), 4.07 (dd, $J_{4,5b}$ = 5.0 Hz, H-5b), 4.38 (d, $J_{1,2}$ = 6.6 Hz, H-1), 4.88 (dd, $J_{2,3}$ = 8.5 Hz, H-2), 4.91 ($J_{3,4}$ = 8.4 Hz, H-3).

Anal. Calcd for $C_{10}H_{16}O_7$: C, 48.4; H, 6.5. Found: C, 48.2; H, 6.6.

Methyl 3-*O*-Acetyl-4-*O*-chloroacetyl-β-D-xylopyranoside (4). Acetyl chloride (3.7 mL, 52 mmol) in dichloromethane (50 mL) was added to a stirred solution of **1** (11.3 g, 47.0 mmol) and pyridine (50 mL) in dichloromethane (1.0 L) at 0 °C. The mixture was stirred 20 min at this temperature. Ice was then added and the mixture was washed with sulfuric acid (1M, aq) and water, dried and concentrated. The residue was crystallized from acetone-petroleum ether giving **4** (9.04 g, 32.0 mmol, 68%) having mp. 165 °C $[\alpha]_{578}$ -26° (*c* 0.5, chloroform), R_f 0.43 (toluene-ethyl acetate, 1:1). NMR data: ^{13}C, δ 20.9 (Me acetyl), 40.5 (CH_2Cl), 57.2 (MeO), 62.1 (C-5), 70.8, 71.7, 73.3 (C-2,3,4), 104.1 (C-1), 166.6 (C=O chloroacetyl) and 170.7 (C=O acetyl). ^1H, δ 3.55 (m, H-2), 3.39 (dd, $J_{4,5a}$ = 9.5 Hz, $J_{5a,5b}$ = 11.6 Hz, H-5a), 4.12 (dd, $J_{4,5b}$ = 5.5 Hz, H-5b), 4.27 (d, $J_{1,2}$ = 7.3, H-1), 5.00 (m, H-4), 5.12 (dd, $J_{2,3}$ = $J_{3,4}$ 9.0 Hz, H-3).

Anal. Calcd for $C_{10}H_{15}ClO_7$: C, 42.5; H, 5.4; Cl, 12.5. Found: C, 42.5; H, 5.3; Cl, 12.4.

Methyl 3-*O*-Acetyl-4-*O*-chloroacetyl-2-*O*-dibenzyloxyphosphoryl-β-D-xylopyranoside (5). Phosphorus trichloride (5.0 mL, 137 mmol) in dichloromethane (30 mL) was added to a solution of imidazole (12 g, 170 mmol) in dichloromethane (120 mL) at 0 °C. The mixture was stirred at this temperature for 10 min and then triethylamine (16 mL, 12 mmol) was added. After additional 10 min compound **4** (4.78 g, 16.9 mmol) dissolved in acetonitrile-dichloromethane (1:1, 140 mL) was added. The mixture was stirred at 0 °C for 30 min. Benzyl alcohol (16 mL, 154 mmol) in dichloromethane (30 mL) was added and the reaction was stirred at room temperature for 1 h. After cooling to 0 °C a solution of *m*-chloroperbenzoic acid (12.5 g , 72 mmol) in dichloromethane (150 mL)

was added. The reaction mixture was stirred for 1 h at 0 °C and then sodium thiosulfate (120 mL, 10%, aq) and sodium hydrogen carbonate (120 mL, aq) were added. The organic layer was separated, washed with water, dried and concentrated. The residue was purified twice by chromatography (toluene-ethyl acetate, 2:1) to give **5** (6.56 g, 12.1 mmol, 72%) having $[\alpha]_{578}$ 2.4° (*c* 0.6, chloroform), R_f 0.61 (toluene-ethyl acetate, 1:1). NMR data: ^{13}C, δ 20.6 (Me acetyl), 40.4 (CH_2Cl), 56.7 (MeO), 61.9 (C-5), 69.3 ($J_{C,P}$ 6.1 Hz, CH_2Ph), 69.5 ($J_{C,P}$ = 6.1 Hz, CH_2Ph), 70.7, 71.1, 75.9 (C-2,3,4), 102.2 ($J_{C,P}$ = 4.8 Hz, C-1), 166.5 (C=O chloroacetyl) and 170.2 (C=O acetyl). ^1H, δ 3.41 (dd, $J_{4,5a}$ = 9.2 Hz, $J_{5a,5b}$ = 11.8 Hz, H-5a), 4.13 (dd, $J_{4,5b}$ = 5.2 Hz, H-5b), 4.36 (dd, $J_{1,2}$ = 6.9 Hz, H-2), 4.42 (d, H-1), 4.98 (m, H-4), 5.29 (dd, $J_{2,3}$ = $J_{3,4}$ = 8.6 Hz, H-3).

Methyl 3-*O*-Acetyl-2-*O*-dibenzyloxyphosphoryl-β-D-xylopyranoside (6). A solution of hydrazine acetate (3.7 g, 40 mmol) in methanol (120 mL) was added to **5** (7.29 g, 13.4 mmol) in dichloromethane-ethyl acetate (1:1, 200 mL). The mixture was stirred at room temperature for 2 h and then concentrated. The product was purified twice by chromatography (toluene-ethylacetate, 2:3) to give **6** (5.77 g, 12.2 mmol, 91%) having $[\alpha]_{578}$ -7.5° (*c* 0.6, chloroform), R_f 0.47 (toluene-ethyl acetate, 1:4). NMR data: ^{13}C, δ 20.8 (Me acetyl), 56.7 (MeO), 65.5 (C-5), 68.7, 76.0, 76.3 (C-2,3,4), 102.3 ($J_{C,P}$ 4.8 Hz, C-1), 171.7 (C=O acetyl). ^1H, δ 3.31 (dd, $J_{4,5a}$ = 9.7 Hz, $J_{5a,5b}$ = 11.8 Hz, H-5a), 3.78 (m, H-4), 4.02 (dd, $J_{4,5b}$ = 5.4 Hz, H-5b), 4.29 (dd, $J_{1,2}$ = 7.0 Hz, H-2), 4.36 (d, H-1), 5.01 (dd, $J_{2,3}$ = $J_{3,4}$ = 8.8 Hz, H-3).

Ethyl 2-*O*-Benzoyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-1-thio-β-D-galactopyranoside (7).[11] A mixture of ethyl 4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside[10] (20.0 g, 64 mmol) and dibutyltin oxide (24 g, 96 mmol) in dichloromethane-methanol (800 mL, 2:1) was heated under reflux for 2 h. The solvent was evaporated and the residue was dissolved in dichloromethane (800 mL). A solution of chloroacetyl chloride (6.0 mL, 75 mmol) in dichloromethane (50 mL) was added dropwise and the mixture was stirred at room temperature for 10 min. Ice was then added and the mixture was washed with sulfuric acid (1M, aq), water, dried and concentrated. To the residue, dissolved in dichloromethane (400 mL) and pyridine (30 mL), was added benzoyl chloride (16 mL, 140 mmol) at 0 °C. The mixture was stirred at room temperature for 4 h, then methanol (30 mL) was added and the solvent was evaporated. The residue was purified by column chromatography (toluene-ethyl acetate, 14:1) and then crystallized from dichloromethane-petroleum ether to give **7** (21.7 g, 43.9 mmol, 69%) having mp 164 °C, $[\alpha]_{578}$ 47.6° (*c* 0.5, chloroform), R_f 0.71 (toluene-ethyl acetate, 3:1). NMR data: ^{13}C, δ 14.8 (Me ethyl), 23.0 (CH_2S), 40.6 (CH_2Cl), 67.1, 69.2, 69.8, 73.6, 74.9 (C-2,3,4,5,6), 83.9 (C-1), 101.3 (CHPh), 165.2 (C=O benzoyl) and 167.2 (C=O chloroacetyl); ^1H δ 3,65 (m, $J_{4,5}$ = 1.7 Hz, H-5), 4.06 (dd, $J_{5,6a}$ = 1.8 Hz, $J_{6a,6b}$ = 12.4 Hz, H-6a), 4.39 (dd, $J_{5,6b}$ = 1.7 Hz, H-6b, H-6b), 4.50 (dd, $J_{3,4}$ = 3.6 Hz, H-4), 4.65 (d, $J_{1,2}$ = 10 Hz, H-1), 5.25 (dd, $J_{2,3}$ = 10.0 Hz, H-3), 5.76 (t, H-2).

Anal. Calcd for $C_{24}H_{25}O_7SCl$: C, 58.5; H, 5.1; S, 6.5; Cl, 7.2. Found: C, 58.2; H, 5.2; S, 6.4; Cl, 7.4.

Methyl 2,3-Di-*O*-acetyl-4-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-β-D-galactopyranosyl)-β-D-xylopyranoside (9). A solution of silver triflate (2.98 g, 11.6 mmol) and 2,4,6-trimethylpyridine (540 μL, 4.0 mmol) in dichloromethane-toluene (3:2, 10 mL) was added to a stirred mixture of **3** (2.0 g, 8.06 mmol), **8** (4.95 g, 9.67 mmol) and molecular sieves in dry dichloromethane (40 mL) at -40 °C under nitrogen. Stirring was continued for 15 min at this temperature, then aqueous sodium thiosulfate (10%, 30 mL) and pyridine (2 mL) were added and the mixture was allowed to attain room temperature. After filtering through Celite, the organic layer was washed with water, dried and concentrated. The residue was purified by chromatography (toluene-ethyl acetate, 3:2) to give **9** (4.38 g, 6.45 mmol, 80%). Crystallization from ethyl acetate-petroleum ether gave material having mp 211 °C, $[\alpha]_{578}$ 6.6° (*c* 0.5, chloroform), R_f 0.74 (toluene-ethyl acetate, 1:1). NMR data: ^{13}C, δ 20.7, 20.8 (Me acetyl), 40.6 (CH_2Cl), 56.7 (MeO), 62.8 (C-5), 66.4, 68.7, 69.2, 70.9, 71.7, 73.1, 73.6, 75.7 (C-2, 3, 4, 2′, 3′, 4′, 5′, 6′), 101.1, 101.3, 101.7 (C-1, 1′, PhCH), 164.7 (C=O benzoyl), 167.2 (C=O chloroacetyl), 169.9, 170.1 (C=O acetyl). ^1H NMR data are shown in the table;

	H-1	H-2	H-3	H-4	H-5
Xyl	4.28 (6.8)	4.81 (8.7)	5.15 (7.8)	3.84 (8.5)	3.20, 3.88
Gal	4.75 (8.0)	5.55 (10.4)	5.17 (3.7)	4.43 (1.8)	3.60

Anal. Calcd for $C_{32}H_{35}O_{14}Cl$: C, 56.6; H, 5.2. Found: C, 56.2; H, 5.2.

Methyl 3-*O*-Acetyl-4-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-β-D-galactopyranosyl)-2-*O*-dibenzyloxyphosphoryl-β-D-xylopyranoside (10). Compound **6** (1.25 g, 2.68 mmol) was glycosylated with **8** (1.78 g, 3.48 mmol) using silver triflate (1.3 g, 5.2 mmol) and 2,4,6-trimethylpyridine (180 μL, 1.4 mmol) in dichloromethane (14 mL) as described for the preparation of **9**. Purification by chromatography (toluene-ethyl acetate, 1:1) gave the disaccharide **10** (1.60 g, 1.78 mmol, 67%) having $[\alpha]_{578}$ 21° (*c* 0.5, chloroform), R_f 0.66 (toluene-ethyl acetate, 1:4). NMR data: ^{13}C, δ 20.7 (Me acetyl), 40.6 (CH_2Cl), 56.9 (MeO), 63.5 (C-5), 100.8, 101.3 (C-1′ and CHPh), 102.5 ($J_{C,P}$ = 4.9 Hz, C-1), 164.6 (C=O benzoyl), 167.2 (C=O chloroacetyl) and 170.3 (C=O acetyl). ^1H NMR data are shown in the table;

	H-1	H-2	H-3	H-4	H-5
XylP	4.24 (7.6)	4.31 (9.5)	5.20 (8.8)	3.86	3.16, 3.86
Gal	4.71 (8.0)	5.52 (10.4)	5.17 (3.6)	4.41 (1.6)	3.56

Methyl 2,3-Di-*O*-acetyl-4-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-β-D-xylopyranoside (11). A solution of hydrazine acetate (1.2 g, 13 mmol) in methanol (40 mL) was added to **9** (3.00 g, 4.42 mmol) in dichloromethane-ethyl acetate (1:1, 80 mL). The mixture was stirred at room temperature for 1.5 h and then concentrated. The product was purified by chromatography (toluene-ethylacetate, 1:1) to give **11**

(2.45 g, 4.07 mmol, 92%). Crystallization from ethyl acetate-petroleum ether gave material having mp 190 °C, $[\alpha]_{578}$ -49.6° (*c* 0.5, chloroform), R_f 0.61 (toluene-ethyl acetate, 1:4). NMR data: ^{13}C, δ 20.7, 20.8 (Me acetyl), 56.7 (MeO), 62.9 (C-5), 66.7, 68.8, 70.9, 71.8, 71.9, 73.0, 75.5, 75.6 (C-2, 3, 4, 2′, 3′, 4′, 5′, 6′), 100.9, 101.6, 101.7 (C-1, 1′ and CHPh), 165.9 (C=O benzoyl), 169.8 and 170.2 (C=O acetyl). ^1H NMR data are shown in the table;

	H-1	H-2	H-3	H-4	H-5
Xyl	4.30 (6.8)	4.83 (8.5)	5.15 (8.5)	3.84 (9.0)	3.26, 3.96
Gal	4.86 (8.1)	5.26 (10.0)	3.86 (3.8)	4.24	3.54

Anal. Calcd for $C_{30}H_{34}O_{13}$: C, 59.8; H, 5.7. Found: C, 59.5; H, 5.8.

Methyl 3-*O*-Acetyl-4-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-2-*O*-dibenzyloxyphosphoryl-β-D-xylopyranoside (12). Compound **10** (1.91 g, 2.13 mmol) was treated with hydrazine acetate (590 mg, 6.4 mmol) as described for the preparation of **11**. The product was purified by chromatography (toluene-ethylacetate, 1:1) to give **12** (1.36 g, 1.66 mmol, 78%) having $[\alpha]_{578}$ -11° (*c* 0.6, chloroform), R_f 0.44 (toluene-ethyl acetate, 1:6). NMR data: ^{13}C, δ 20.7 (Me acetyl), 56.9 (MeO), 100.7, 101.7 (C-1′ and CHPh), 102.6 ($J_{C,P}$ = 4.9 Hz, C-1), 165.8 (C=O benzoyl) and 170.3 (C=O acetyl).

Anal. Calcd for $C_{42}H_{45}O_{15}P$: C, 61.5; H, 5.5. Found: C, 61.7; H, 5.6.

Methyl *O*-(2-*O*-Benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-*O*-(1→4)-2,3-di-*O*-acetyl-β-D-xylopyranoside (13). A solution of silver triflate (2.3 g, 9.0 mmol) and 2,4,6-trimethylpyridine (265 μL, 2.0 mmol) in dichloromethane-toluene (3:2, 10 mL) was added to a stirred mixture of **11** (2.4 g, 3.98 mmol), **8** (3.06 g, 5.97 mmol) and molecular sieves in dry dichloromethane (20 mL) at -40 °C under nitrogen. Stirring was continued for 10 min at this temperature, then pyridine (2 mL) and aqueous sodium thiosulfate (10%, 30 mL) were added and the mixture was allowed to attain room temperature. After filtering through Celite, the organic layer was washed with water, dried and concentrated. The residue was purified by chromatography (toluene-ethyl acetate, 1:3) to give **13** (3.50 g, 3.39 mmol, 85%). Precipitation from dichloromethane-petroleum ether gave material having $[\alpha]_{578}$ 3.7° (*c* 0.5, chloroform), R_f 0.60 (toluene-ethyl acetate, 1:4). NMR data: ^{13}C, δ 20.7, 20.8 (Me acetyl), 40.5 (CH_2Cl), 56.6 (MeO), 62.9 (C-5), 66.5, 66.9, 68.7, 68.9, 70.9, 71.0, 71.9, 73.1, 73.8, 75.2, 75.5, 75.9, 77.2 (C-2, 3, 4, 2′, 3′, 4′, 5′, 6′, 2″, 3″, 4″, 5″, 6″), 99.9, 100.9, 101.0, 101.5, 101.7 (C-1, 1′, 1″ and 2 CHPh), 164.5, 165.1 (C=O benzoyl), 167.2 (C=O chloroacetyl), 169.9 and 170.1 (C=O acetyl). ^1H NMR data are shown in the table;

	H-1	H-2	H-3	H-4	H-5
Xyl	4.22 (7.0)	4.77 (8.8)	5.07 (8.0)	3.76	3.09, ND
Gal	4.64 (7.9)	5.40 (10.3)	4.25 (3.5)	4.35 (1.5)	3.43
Gal	5.00 (8.1)	5.60 (10.2)	5.02 (3.6)	4.36 (1.8)	3.47

Anal. Calcd for $C_{52}H_{53}O_{20}Cl$: C, 60.4; H, 5.2. Found: C, 60.4; H, 5.3.

Methyl *O*-(2-*O*-Benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-*O*-(1→4)-3-*O*-acetyl-2-*O*-dibenzyloxyphosphoryl-β-D-xylopyranoside (14). A mixture of **8** (748 mg, 1.46 mmol) and **12** (800 mg, 975 mmol) was treated with silver triflate (560 mg, 2.2 mmol) and 2,4,6-trimethylpyridine (66 μL, 500 mmol) in dichloromethane (15 mL) as described for the preparation of **13**. The residue was purified by chromatography (toluene-ethyl acetate-dichloromethane, 1:2:1) to give **14** (970 mg, 775 mmol, 79%) having $[\alpha]_{578}$ 45° (*c* 0.5, chloroform), R_f 0.31 (toluene-ethyl acetate, 1:6). NMR data: ^{13}C, δ 20.6 (Me acetyl), 40.5 (CH_2Cl), 56.8 (MeO), 99.9, 100.9, 101.0, 101.2 (C-1′,1″ and 2 CHPh), 102.5 ($J_{C,P}$ = 4.8 Hz, C-1), 164.5, 165.0 (C=O benzoyl), 167.1 (C=O chloroacetyl) and 170.3 (C=O acetyl). ^1H NMR data are shown in the table;

	H-1	H-2	H-3	H-4	H-5
XylP	4.19 (7.7)	4.27 (9.5)	5.12 (8.6)	3.78	3.78, 3.07
Gal	4.61 (7.9)	5.37 (10.3)	4.25 (3.6)	4.35	3.42
Gal	4.99 (8.0)	5.59 (10.3)	5.01 (3.6)	4.37	3.46

Anal. Calcd for $C_{64}H_{64}ClO_{22}P$: C, 61.4; H, 5.2; Cl, 2.8. Found: C, 61.3; H, 5.2; Cl, 2.6.

Methyl *O*-(β-D-Galactopyranosyl)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→4)-*O*-β-D-xylopyranoside (15). Compound **13** (1.78 g, 1.72 mmol) was suspended in aqueous acetic acid (90%, 50 mL) and heated at 100 °C for 1 h. The solvent was evaporated and the residue was dissolved in methanol (40 mL) and treated with sodium methoxide in methanol (1M, 4 mL) for 3 h. The solution was then neutralized with acetic acid and the solvent was evaporated. The residue was purified on a P2 Biogel column, using water (containing 1% 1-butanol) as eluent, giving compound **15** (766 mg, 1.57 mmol, 91%) having $[\alpha]_{578}$ -8.2° (*c* 0.5, water), R_f 0.64 (ethyl acetate-methanol-acetic acid-water, 4:3:3:2). NMR data (D_2O; Me_2CO, δ_H= 2.225; 1,4-dioxane, δ_C= 67.4): ^{13}C, δ 58.0 (MeO), 61.8, 61.9 (C-6′, 6″), 63.8, 69.3, 69.5, 70.7, 71.9, 73.4, 73.6, 74.8, 75.8, 75.9, 77.4, 82.9, 102. 3 (C-1), 104.7, and 105.2 (C-1′ and C-1″). ^1H NMR data are shown in the table;

	H-1	H-2	H-3	H-4	H-5
Xyl	4.35 (7.8)	3.29 (9.3)	3.60 (9.2)	3.85	3.41, 4.11
Gal	4.53 (8.0)	3.68 (10.0)	3.81 (3.4)	4.19	3.72
Gal	4.61 (7.6)	3.60 (10.0)	3.66 (3.4)	3.92	3.69

Methyl *O*-(β-D-Galactopyranosyl)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→4)-β-D-xylopyranoside 2-(disodium phosphate) (16). Compound **14** (400 mg, 320 μmol) was dissolved in ethyl acetate-ethanol-water (15:3:2, 30 mL) and hydrogenated over palladium on charcoal overnight. After filtering through Celite, the solvent was evaporated and

the residue was dissolved in methanol-water (1:2, 6 mL) and treated with sodium hydroxide (2.5M, 2 mL) for 2 h. The reaction mixture was neutralized with Dowex H^+ and concentrated. The residue was dissolved in water and passed through a column of Dowex Na^+. The eluate was concentrated, and purified on a P2 Biogel column, using water (containing 1% 1-butanol) as eluent, giving compound **16** (132 mg, 216 μmol, 67%) having $[\alpha]_{578}$ -20° (*c* 0.6, water), R_f 0.38 (ethyl acetate-methanol-acetic acid-water, 2:3:3:2). ^{31}P NMR data (D_2O; external H_3PO_4, δ_P= 0.00): δ 0.02. ^1H NMR (D_2O; Me_2CO, δ_H= 2.225) data are shown in the table;

	H-1	H-2	H-3	H-4	H-5
XylP	4.48 (7.1)	3.83	3.79	3.91	3.46, 4.10
Gal	4.53 (7.8)	3.68 (10.1)	3.81 (3.3)	4.17	3.70
Gal	4.61 (7.6)	3.60 (10.0)	3.66 (3.4)	3.92 (1.0)	3.68

Ethyl 2-*O*-Acetyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (17). Ethyl 2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-1-thio-β-D-galactopyranoside[13] (10.0 g, 23.2 mmol) was treated with hydrazine acetate (4.2 g, 46 mmol) as described for the preparation of compound **3**. The product was purified by column chromatography (toluene-ethyl acetate, 2:1) to give **17** (6.42 g, 18.1 mmol, 78%). Crystallization from ethyl acetate-petroleum ether gave material having mp 144 °C, $[\alpha]_{578}$ -28.8° (*c* 0.5, chloroform), R_f 0.57 (toluene-ethyl acetate, 1:4). NMR data: ^1H, δ 3.46 (m, H-5), 3.71 (m, $J_{2,3}$ = 9.8 Hz, $J_{3,4}$ = 3.7 Hz, H-3) 3.99 (dd, $J_{5,6a}$ = 1.8 Hz, $J_{6a,6b}$ = 12.2 Hz, H-6a), 4.20 (dd, $J_{4,5}$ = 1.6 Hz, H-4), 4.30 (dd, $J_{5,6b}$ = 1.6 Hz, H-6b), 4.38 (dd, $J_{1,2}$ = 9.8 Hz, H-1), 5.16 (dd, H-2).

Anal. Calcd for $C_{17}H_{22}O_6S$: C, 57.6; H, 6.3; S, 9.0. Found: C, 57.4; H, 6.3; S, 8.7.

Ethyl 2-*O*-Benzoyl-4,6-*O*-benzylidene-3-*O*-(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-1-thio-β-D-galactopyranoside (18). A solution of silver triflate (1.5 g, 5.8 mmol) in toluene (5 mL) was added to a stirred mixture of **17** (1.00 g, 2.82 mmol), methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide) uronate[9] (1.20 g, 1.02 mmol) and molecular sieves in dry nitromethane (10 mL) at -30 °C under nitrogen. Stirring was continued for 30 min at this temperature, then pyridine (3 mL) and aqueous sodium thiosulfate (10%, 30 mL) were added and the mixture was allowed to attain room temperature. After filtering through Celite, the organic layer was washed with water, dried and concentrated. The residue was purified by column chromatography (toluene-ethyl acetate, 1:1) giving two major products; compound **18** (951 mg, 1.42 mmol, 50%) and ethyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (432 mg, 1.09 mmol, 39%). Crystallization of **18** from ethanol gave material having mp 199 °C, $[\alpha]_{578}$ -28° (*c* 0.5, chloroform), R_f 0.73 (toluene-ethyl acetate, 1:4). NMR data: ^{13}C, δ 14.7 (Me ethyl), 20.40, 20.45, 20.6, 21.1 (Me acetyl), 22.6 (CH_2S), 52.9 (MeO), 68.2, 69.1, 69.4, 70.3, 71.3, 72.3, 72.5, 76.0, 78.6) C-2, 3, 4, 5, 6, 2′, 3′, 4′, 5′), 82.8 (C-1), 100.9, 101.0 (C-1′ and CHPh), 167.3 (C-6), 169.1, 169.25, 169.29, 170.2 (C=O acetyl). ^1H NMR data are shown in the table;

	H-1	H-2	H-3	H-4	H-5
Gal	4.34 (9.7)	5.41 (9.8)	3.86 (3.5)	4.35 (1.6)	3.49, ND
GlcA	4.83 (7.6)	4.98 (9.2)	5.20 (9.2)	5.23 (9.8)	4.04

Anal. Calcd for $C_{30}H_{38}O_{16}S$: C, 53.7; H, 5.7; S, 4.8. Found: C, 53.3; H, 5.7; S, 4.6.

Methyl *O*-(Methyl 2,3,4-tri-*O*-acetyl-β-*O*-glucopyranosyluronate)-(1→3)-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-*O*-(1→4)-2,3-di-*O*-acetyl-β-D-xylopyranoside (19). DMTST (1.3 g, 5.1 mmol) was added to a stirred mixture of **11** (938 mg, 1.56 mmol), **18** (1.10 g, 1.64 mmol), 2,6-di-*tert*-butylpyridine (370 μL, 1.74 mmol) and molecular sieves in dry dichloromethane (10 mL) at 0 °C under nitrogen. The mixture was allowed to attain room temperature and the stirring was continued for 2.5 h. Pyridine (2.6 mL) was added and after 30 min the mixture was purified, without any further workup, by column chromatography (toluene-ethyl acetate, 1:4) giving compound **19** (1.21 g, 1.00 mmol, 64%). Precipitation from dichloromethane-petroleum ether gave material having $[\alpha]_{578}$ -2.1° (*c* 0.6, chloroform), R_f 0.36 (toluene-ethyl acetate, 1:4). NMR data: ^{13}C, δ 20.4-20.8 (6 Me acetyl), 52.8 (MeO Xyl), 56.7 (MeO GlcA), 63.0 (C-5), 100.5, 100.7 100.9, 101.3, 101.7 (C-1, 1′, 1″, 1‴ and 2 CHPh), 164.4 (C=O benzoyl), 167.2 (C-6‴), 168.9 169.0 169.3, 169.9, 170.1 and 170.2 (C=O acetyl). ^1H NMR data are shown in the table;

	H-1	H-2	H-3	H-4	H-5
Xyl	4.25 (7.0)	4.80 (8.8)	5.10 (7.7)	3.80	3.15, 3.84
Gal	4.63 (7.9)	5.46 (10.2)	4.15 (3.6)	4.36 (1.5)	3.43
Gal	4.60 (8.1)	5.23 (10.3)	3.52 (3.6)	4.20 (1.5)	3.27
GlcA	4.53 (7.7)	4.90 (9.2)	5.10 (9.2)	5.16 (9.6)	3.90

Anal. Calcd for $C_{58}H_{66}O_{28}$: C, 57.5; H, 5.5. Found: C, 57.7; H, 5.6.

Methyl *O*-(Methyl 2,3,4-tri-*O*-acetyl-β-*O*-glucopyranosyluronate)-(1→3)-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-*O*-(1→4)-3-*O*-acetyl-2-*O*-dibenzyloxyphosphoryl-β-D-xylopyranoside (20). A mixture of **12** (420 mg, 512 μmol), **18** (515 g, 767 μmol) and 2,6-di-*tert*-butylpyridine (650 μL, 3.1 mmol) was treated with DMTST (790 mg, 3.1 mmol) in dichloromethane (5 mL) as described for the preparation of **19**. The product was purified by column chromatography (toluene-ethyl acetate, 1:4) giving compound **20** (443 mg, 297 μmol, 58%). Precipitation from dichloromethane-petroleum ether gave material having $[\alpha]_{578}$ 7.1° (*c* 0.6, chloroform), R_f 0.2 (ethyl acetate). NMR data: ^{13}C, δ 20.4-20.6 (5 Me acetyl), 52.8 (MeO Xyl), 56.7 (MeO GlcA), 63.7 (C-5), 100.4, 100.7, 100.9, 100.97, 101.04 (C-1′, 1″, 1‴ and 2 CHPh), 102.5 ($J_{C,P}$ = 4.9 Hz, C-1), 164.4 (C=O benzoyl), 167.2 (C-6‴), 168.9 169.1 169.3, 170.2 and 170.3 (C=O acetyl). ^1H NMR data are shown in the table;

	H-1	H-2	H-3	H-4	H-5
XylP	4.21 (7.6)	4.29 (9.4)	5.16	3.82	3.13, ND
Gal	4.60 (8.0)	5.43 (10.4)	4.15 (3.6)	4.35	3.42
Gal	4.59 (7.9)	5.22 (10.4)	3.51 (3.6)	4.19 (1.5)	3.26
GlcA	4.53 (7.6)	4.90 (9.2)	5.10 (9.2)	5.16 (9.8)	3.9

Methyl *O*-(β-D-Glucopyranosyluronic acid)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→4)-β-D-xylopyranoside sodium salt (21). Compound **19** (310 mg, 256 mmol) was suspended in aqueous acetic acid (90%, 20 mL) and heated at 100 °C for 1 h. The solvent was evaporated and the residue was treated with sodium hydroxide (2 mL, 2.5 M, aq) in methanol-water (1:2, 12 mL) for 2 h at room temperature. The reaction mixture was neutralized with Dowex H^+ and concentrated. The residue was dissolved in water and passed through a column of Dowex Na^+. The eluate was concentrated, and purified on a P2 Biogel column, using water (containing 1% 1-butanol) as eluent, giving compound **21** (158 mg, 230 mmol, 90%) having $[\alpha]_{578}$ -21° (*c* 0.6, water), R_f 0.51 (ethyl acetate-methanol-acetic acid-water, 4:3:3:2). NMR data (D_2O; Me_2CO, δ_H= 2.225; 1,4-dioxane, δ_C= 67.4): ^{13}C, δ 58.0 (MeO), 61.8, 61.9, 63.8, 68.9, 69.3, 70.7, 71.0, 72.6, 73.6, 74.0, 74.8, 75.6, 75.8, 76.2, 77.0, 77.3, 82.8, 83.2 (ring C), 102.2 (C-1), 104.4, 104.6, 104.8 (C-1′, 1″, 1‴) and 176.7 (C=O); ^1H NMR data are shown in the table;

	H-1	H-2	H-3	H-4	H-5
Xyl	4.35 (7.8)	3.29 (9.3)	3.60 (9.2)	3.85	3.41, 4.11
Gal	4.53 (7.8)	3.68 (10.0)	3.83 (3.4)	4.18 (1.6)	ND
Gal	4.68 (7.8)	3.42 (9.9)	3.72 (3.5)	4.18	ND
GlcA	4.67 (7.6)	3.76 (9.9)	ND	ND	ND

Methyl *O*-(β-D-Glucopyranosyluronic acid)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→4)-β-D-xylopyranoside 2-(disodium phosphate) sodium salt (22). Compound **20** (200 mg, 140 μmol) was treated as described for the preparation of **16** giving compound **22** (72 mg, 91 mmol, 65%) having $[\alpha]_{578}$ -25° (*c* 0.7, water), R_f 0.64 (methanol-water, 4:1). ^{31}P NMR data (D_2O; external H_3PO_4, δ_P= 0.00): δ 0.05. ^1H NMR data (D_2O; Me_2CO, δ_H=2.225) are shown in the table;

	H-1	H-2	H-3	H-4	H-5
XylP	4.48 (7.1)	3.84	3.80	3.92	3.45, 4.11
Gal	4.53 (7.8)	3.69 (10.0)	3.82 (3.4)	4.18	ND
Gal	4.68 (7.8)	3.42 (9.6)	3.72	4.18	ND
GlcA	4.66 (7.5)	3.76	ND	ND	ND

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