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SYNTHESIS OF METHYL 3-*O*-(α- AND β-D-GALACTOPYRANOSYL)-4-*O*-(β-D-GLUCOPYRANOSYLURONIC ACID)-α-L-RHAMNOPYRANOSIDE USING PHOTOLABILE METHYL 2,3-*O*-(2-NITROBENZYLIDENE)-α-L-RHAMNOPYRANOSIDE*

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ABSTRACT

The title branched-trisaccharide derivatives (**9** and **13**) have been synthesised from methyl 2,3-*O*-(2-nitrobenzylidene)-α-L-rhamnopyranoside (**2**) using the 2-nitrobenzylidene residue as a temporary blocking-group. Condensation of **2** with methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate afforded the blocked disaccharide **3** which, on sequential photolysis and oxidation, gave methyl 4-*O*-(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-2-*O*-(2-nitrobenzoyl)-(**4**) and -3-*O*-(2-nitrobenzoyl)-α-L-rhamnopyranoside (**5**) as a 4:1 mixture. Galactosylation of HO-3 of **4** gave two fully protected trisaccharides having βαα and ββα configuration which, on deacylation, saponification, and treatment with ion-exchange resins, gave the free acids **9** and **13**.

INTRODUCTION

We have shown that, in sugar chemistry, 2-nitrobenzaldehyde acetals are good temporary blocking-groups, which, on u.v. irradiation followed by oxidation of the resulting nitroso derivatives, give 2-nitrobenzoyl sugars with a free hydroxyl group. This has led to a useful partial-blocking, partial-deblocking sequence, which is an essential prerequisite for satisfactory syntheses of branched oligosaccharides[2]. In our work, 3,4- and 2,3-*O*-(2-nitrobenzylidene)hexopyranosides with, respectively, the *galacto*[3] and *manno*[1] configurations have been used to prepare neutral trisaccharides. We now report the use of this protecting group in the synthesis of two trisaccharide methyl glycosides which contain uronic acid residues. These are related to the aldotriouronic acid α-D-Gal*p*-(1→3)[β-D-Glc*p*A-(1→4)]-L-Rha*p*, which Lindberg and co-workers[4] have shown is part of the tetrasaccharide repeating-unit found in the *Klebsiella* Type 9 capsular polysaccharide.

*The Photochemistry of Carbohydrate Derivatives, Part 8. For Part 7, see ref. 1.
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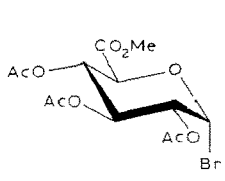
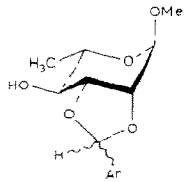
RESULTS AND DISCUSSION

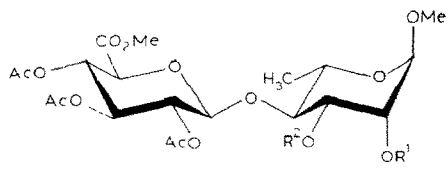
The readily prepared *endo,exo*-2,3-*O*-(2-nitrobenzylidene)rhamnoside mixture[1] (**2**) was glycosylated[5] at O-4 with methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate[6] (**1**) to give, after chromatography, 85% of the disaccharide derivative **3**. Many well-resolved signals in the ^{1}H- and ^{13}C-n.m.r. spectra of **3** were in accord with this structure, but there was some doubling of signals due to the presence of the *endo*- and *exo*-acetal stereoisomers. That the D-glucuronate moiety was β was indicated[7] by the two doublets at δ 4.90 and 5.22 (each split by 8.0 Hz) and the two carbon resonances at δ 99.7 and 99.9.



1



2



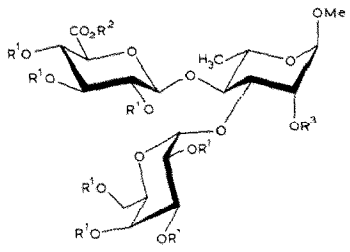
3 R^1, R^2 = CHAr
4 R^1 = COAr, R^2 = H
5 R^1 = H, R^2 = COAr

Isolation of these isomers was unnecessary since the direction of the photochemical opening of the dioxolane ring is not dependent upon the configuration at the acetal carbon. Therefore, the *endo,exo* mixture **3** in methanol containing 3.6% of acetic acid was irradiated for 1.7 h with light from a 450-watt mercury lamp. The nitrosobenzoates formed were oxidised at 0° with trifluoroperacetic acid in dichloromethane to give 92% of a 4:1 mixture of the 2- (**4**) and 3-nitrobenzoate (**5**), which were isolated by chromatography to afford 62% of the desired 2-nitrobenzoate **4**. The ^{13}C-n.m.r. spectrum of **4** confirmed the presence of a β-linked methyl tri-*O*-acetyl-D-glucuronate residue, as shown by comparison with spectra of known β-D-glucosiduronic acids[7] (see Table I). The ^{1}H-n.m.r. spectrum showed two doublets for anomeric protons at δ 5.00 (*J* 8.0 Hz) and 4.85 (*J* 1.5 Hz). The former signal corroborated the β linkage of the disaccharide. The lowest-field non-aromatic signal was a doublet of doublets at δ 5.62 (*J* 1.5 and 4.0 Hz) which indicated the nitrobenzoate group to be at position 2 of the rhamnose ring.

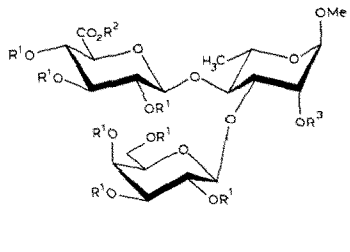
The isomer **5**, isolated as a gum (12%), exhibited its lowest-field saccharide-proton resonance at δ 5.85 as a doublet of doublets (*J* 10.0 and 3.2 Hz) which indicated that this isomer was the 3-nitrobenzoate. The other ^{1}H and ^{13}C signals (see Table I) supported this conclusion.

Condensation[5] of the disaccharide derivative **4** with tetra-*O*-acetyl-α-D-galactopyranosyl bromide (acetobromogalactose) gave a 3:7 mixture of the trisaccharide derivatives **6** and **10**, which were isolated by chromatography and in which the galactosyl groups were α and β, respectively. In the ^{1}H-n.m.r. spectrum of the crystalline isomer **10**, which had $[\alpha]_D$ −44° (chloroform), three doublets for

anomeric protons were observed. The signal at δ 4.57 (J 8.0 Hz) arose from the galactosyl group and indicated that it was β. This conclusion was confirmed by the ^{13}C-n.m.r. spectrum (see Table I), which contained signals similar to those of methyl tetra-*O*-acetyl-β-D-galactopyranoside rather than those of its α anomer[8,9].



6 R^1 = Ac, R^2 = Me, R^3 = COAr
7 R^1 = R^3 = H, R^2 = Me
8 R^1 = R^3 = H, R^2 = Na
9 R^1 = R^2 = R^3 = H



10 R^1 = Ac, R^2 = Me, R^3 = COAr
11 R^1 = R^3 = H, R^2 = Me
12 R^1 = R^3 = H, R^2 = Na
13 R^1 = R^2 = R^3 = H

Ar = 

For the syrupy isomer **6**, which had $[\alpha]_D$ +0.3° (chloroform), the galactosyl group was shown to be α by the ^{13}C-n.m.r. data (see Table I), since the signals matched those of methyl tetra-*O*-acetyl-α-D-galactopyranoside[8,9]. Because the galactosyl H-1 resonance was obscured in the ^{1}H-n.m.r. spectrum, direct coupling-evidence was not available to corroborate this assignment. However, the appearance of a strongly deshielded doublet of doublets at δ 5.81 (J 10.0 and 3.5 Hz), which indicated that the signal was for H-3 of the galactosyl group, was in keeping with the view that the "aglycon" was axial in this component of **6**.

Deacylation of **10** and **6** gave, respectively, the methyl esters of the $\beta\beta$-trisaccharide-α-glycoside **11** and the $\beta\alpha$-trisaccharide-α-glycoside **7**, each of which exhibited peaks at m/z 531.2 for $(M + H)^+$ and at 553.2 for $(M + Na)^+$ in positive ion f.a.b.–m.s.

Crystalline **11**, which had $[\alpha]_D$ −56° (methanol), showed three doublets for anomeric protons (J 1.7, 8.0, and 7.0 Hz) for one α and two β links. This inference was corroborated by the ^{13}C-n.m.r. spectrum which showed signals for galactopyranose and methyl glucopyranuronate that were similar to those of the methyl β-glycosides of these two sugars[8,10,11] (see Table I), and also a third anomeric signal with a chemical shift similar to that of methyl α-L-rhamnopyranoside[12]. The structure of trisaccharide **7**, which had $[\alpha]_D$ +10° (water–ethanol), was established similarly. It exhibited, in the p.m.r. spectrum, splittings of 7.8, 3.8, and 1.7 Hz for the doublets of the three anomeric protons, consistent with the proposed β and α linkages, and the ^{13}C-n.m.r. spectrum showed signals for the glucuronate and galactose residues that were in close agreement with those of β- and α-pyranosides of these sugars, respectively.

TABLE I

^{13}C-CHEMICAL SHIFT DATA FOR METHYL 4-*O*-(D-GLUCOSYLURONIC ACID)- AND METHYL 3-*O*-D-GALACTOSYL-4-*O*-(D-GLUCOSYLURONIC ACID)-L-RHAMNOSIDES[a]

Compound[b]	*GlcA/Gal*						*Rha*						*OMe*
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	
4[c]	101.3	72.7	72.7	71.2	69.6	167.7	97.9	74.9	69.9	81.3	66.7	17.5	52.9 55.1
5[c]	100.5	72.5	72.1	70.9	69.5	167.3	100.5	68.8	76.5	76.8	66.6	17.5	52.9 55.1
6[c]	99.6 94.8	72.5 67.7[f]	72.5 67.3[f]	71.7 67.3[f]	70.1 68.2[f]	167.9 62.0	97.7	71.5	75.0	76.0	68.4[f]	17.6	52.7 55.0
10[c,e]	99.6 101.7	72.4 69.8	72.2 70.9	71.3 67.0	69.9 71.9	167.4 61.5	97.7	74.1	76.0	78.3	66.8	17.9	52.5 54.9
14[c]	101.0	72.6	72.1	71.4	69.9	167.6							
15[c] α	96.5	67.6	67.6	67.0	65.7	61.2							
16[c] β	101.5	68.5	70.2	66.8	70.6	61.0							
7[d]	103.1 94.1	73.9 68.4	76.7 70.2	72.9 69.0	74.0[f] 71.9	177.0 62.1	101.4	70.2[f]	76.5[f]	77.3[f]	65.8	17.8	49.6 55.7
11[d]	103.3 104.8	74.0 71.9	76.3 73.6	72.3 69.5	75.4 76.0	172.1 61.7	101.2	70.8	79.0	81.2	67.8	17.6	53.8 55.5
8[d]	103.1 94.3	73.9 68.4	76.7 70.3	72.9 69.0	74.3 71.9	176.7 62.1	101.3	70.3	76.8	77.2	66.0	17.9	— 55.7
12[d,e]	103.1 104.8	73.6 72.0	76.7 74.3	72.9 69.5	77.3 75.9	176.9 61.8	101.2	70.7	78.8	81.1	67.9	17.7	— 55.6
17[d] α	100.8	71.9	73.7	72.4	71.9	—							54.2
18[d] β	104.6	73.7	76.3	72.4	75.7	—							56.5
19[d] α	100.7	71.9	73.8	72.5	71.9	—							
20[d] β	104.3	73.8	76.5	72.5	75.6	—							
21[d] α	100.1	69.2	70.5	70.2	71.6	62.2							
22[d] β	104.5	71.7	73.8	69.7	76.0	62.0							

[a]Signals for acetate and nitrobenzoate residues are recorded in the Experimental. [b]Key: **14**, β-glycosides of methyl 2,3,4-tri-*O*-acetyl-D-glucopyranosiduronate[9] and the 2-phenylethyl β-glycoside; **15** and **16**, methyl 2,3,4,6-tetra-*O*-acetyl-α- and -β-D-galactopyranoside[8,9]; **17** and **18**, methyl (methyl α- and β-D-glucopyranosid)uronate[11]; **19** and **20**, methyl α- and β-D-glucopyranosiduronic acid[11]; **21** and **22**, methyl α- and β-D-galactopyranoside[8,10]. [c]In $CDCl_3$ (internal Me_4Si). [d]In D_2O (internal 1,4-dioxane, δ_{Me_4Si} 67.4). [e]Rhamnoside signal assignment from ref. 1. [f]Signal assignments may be interchanged.

Compounds **11** and **7** were readily saponified with the stoichiometric amount of aqueous sodium hydroxide to give, respectively, the crystalline sodium salts of the $\beta\beta$-trisaccharide-α-glycoside **12** and the $\beta\alpha$-trisaccharide-α-glycoside **8**, each of which gave f.a.b.–m.s. peaks at *m/z* 539.1 and 561.0 for $(M + H)^+$ and $(M + Na)^+$, respectively. Their ^{13}C-n.m.r. spectra (Table I) were very similar to those of the methyl esters from which they were derived.

The free-acid forms **13** and **9** were obtained, as their monohydrates, by treating the salts with Amberlite IR-120 (H^+) resin. The ^{1}H-n.m.r. spectra clearly indicated the $\beta\beta\alpha$-configuration for the material with $[\alpha]_D$ −29° (water) and the $\beta\alpha\alpha$-configuration for the material with $[\alpha]_D$ +12° (water). Further hydrolysis of these compounds was not selective. Lindberg and his co-workers[4] found the assignment of anomeric configuration to the galactosyl group in the trisaccharide D-Gal*p*-(1→3)[β-D-Glc*p*A(1→4)]-L-Rha, which they isolated from *Klebsiella* type 9, rather troublesome. They eventually deduced, from oxidation studies, that it was α-linked to rhamnose. Their conclusion is now corroborated by the optical rotations of the isomeric, synthetic glycosides **9** and **13**, since that of **9** containing an α-D-galactosyl group had an optical rotation in closer agreement with the value (+22°) for the material they isolated from the bacterial cells.

EXPERIMENTAL

^{1}H-N.m.r. spectra were recorded for solutions in $CDCl_3$ (internal Me_4Si) with a Jeol FX200 FT instrument. Natural-abundance ^{13}C-n.m.r. spectra were recorded with a Jeol FX60 FT instrument operating at 15 MHz, usually for solutions in $CDCl_3$ (internal Me_4Si) or in D_2O (internal 1,4-dioxane). All δ_c values were recorded with reference to Me_4Si. The low-resolution mass spectra were measured by the PCMU, Harwell, with a VG Micromass ZAB IF spectrometer using an f.a.b. source. Optical rotations were measured with an Optical Activity polarimeter Model A100. Flash chromatography[13] was carried out with Kieselgel 60 (230–400 mesh, Merck 9395).

*Methyl 4-*O*-(methyl 2,3,4-tri-*O*-acetyl-β-*D*-glucopyranosyluronate)-2,3-*O*-*endo,exo*-(2-nitrobenzylidene)-α-*L*-rhamnopyranoside* (**3**). — Methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate[14] (**1**, 3.5 g) was added to a 1.4:1.0 *exo,endo* mixture of the nitrobenzylidene-rhamnoside **2**[15] (2.1 g) in anhydrous benzene (2 mL) and nitromethane (2 mL) containing mercury(II) cyanide (2.2 g). The mixture was stirred at 22° for 18 h, toluene (100 mL) was added, and the solution was washed successively with aqueous sodium hydrogencarbonate and water, dried, and concentrated. The resulting gum (5.0 g) was purified by flash chromatography, using light petroleum (b.p. 40–60°)–ethyl acetate (3:1), to give **3** as a 1.4:1 *exo,endo* mixture (3.6 g, 85% from **2**). N.m.r. data: ^{1}H ($CDCl_3$), δ 3.38, 3.40 (2 s, 1.8 H and 1.3 H, CO_2Me), 3.70, 3.79 (2 s, 1.3 H and 1.8 H, OMe), 6.56, 6.76 (2 s, 0.42 H and 0.58 H, C*H*Ar), 1.18, 1.34 (2 d, 1.3 H and 1.8 H, *J* 6.5 Hz, HC*Me*), 1.96–2.08 (9 H, 3 Ac), 4.70 (d, 0.6 H, $J_{1,2}$ 8.0 Hz, H-1 GlcA, *endo* or *exo*

form), 4.02 (d, 1 H, $J_{5,4}$ 5.8 Hz, H-5 GlcA), 4.96 (d, ~0.5 H, $J_{1,2}$ 1.5 Hz, H-1 Rha, *endo* or *exo* form), plus m for all other protons; (C_6D_6), δ 2.94, 2.97 (2 s, 1.3 H and 1.8 H, CO_2Me), 3.29, 3.39 (2 s, 1.3 H and 1.8 H, OMe), 6.75, 6.50 (2 s, 0.58 H and 0.42 H, C*H*Ar), 1.28, 1.47 (2 d, 1.3 H and 1.8 H, *J* 6.5 Hz, HC*Me*), 1.66–1.84 (9 H, 3 Ac), 4.90 and 5.22 (2 d, 0.4 H and 0.6 H, each $J_{1,2}$ 8.0 Hz, H-1 GlcA, *endo* and *exo* forms), 4.82 and 4.85 (2 d, 0.4 and 0.6 H, each $J_{1,2}$ 1.5 Hz, H-1 Rha, *endo* and *exo* forms), 5.25–5.70 (m, 3 H, H-2,3,4 of GlcA), plus signals for all other protons; ^{13}C (resonances for *endo* and *exo* forms, respectively, are presented in parentheses), uronic acid moiety, δ (99.93, 99.67) C-1, (72.52, 72.25), 72.20, (71.54, 71.51), 69.66 C-5, (167.64, 167.31) C-6, 52.73 (OMe), 20.57, 170.18, 169.70, and 169.74 (3 Ac); rhamnoside moiety, δ 97.60 C-1, (79.42, 78.32), (77.86, 77.66), (76.23, 76.95), 63.67 C-5, 17.38 C-6, (99.28, 99.67), (148.82, 149.21), (133.13, 133.38), 133.40, 130.20, (127.34, 127.53) (125.00, 124.60) C-Ar, and 54.94 (OMe).

Anal. Calc. for $C_{27}H_{33}NO_{16}$: C, 51.67; H, 5.30; N, 2.23. Found: C, 51.76; H, 5.55; N, 2.14.

Methyl 4-O-(methyl 2,3,4-tri-O-acetyl-β-D-glucopyransyluronate)-2-O-(2-nitrobenzoyl)- and -3-O-(2-nitrobenzoyl)-α-L-rhamnopyranoside (**4** *and* **5**). — A solution of **3** (3.3 g, 5.3 mmol) in methanol (320 mL) containing acetic acid (12 mL) was agitated by the passage of nitrogen and irradiated for 1.7 h through Pyrex with a 450-W, medium-pressure mercury lamp in the annular space of a conventional photochemical well[1]. Evaporation of the solvent below 5° gave a mixture of nitrosobenzoates as a dark-green syrup, a solution of which in dichloromethane (200 mL) was oxidised at 0° for 1 h with trifluoroperacetic acid (8.0 mmol). The usual work-up gave a 4:1 mixture (3.1 g, 93%) of **4** and **5**, R_F 0.53 and 0.39 (benzene–ethyl acetate, 2:1). Flash chromatography (same solvent) gave a mixture (0.6 g, 18%), **4** (2.1 g, 62%), and **5** (0.4 g, 12%).

The 2-nitrobenzoate **4** had m.p. 153–154°, $[\alpha]_D$ −73° (*c* 1.4, chloroform). N.m.r. data: ^1H (C_6D_6), δ 5.00 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1'), 5.24–5.52 (m, 3 H, H-2',3',4'), 3.77 (d, 1 H, $J_{5',4'}$ 6.5 Hz, H-5'), 2.96 (s, 3 H, CO_2Me), 1.68, 1.70, 1.84 (3 s, each 3 H, 3 Ac), 4.85 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 5.62 (dd, 1 H, $J_{2,1}$ 1.5, $J_{2,3}$ 4.0 Hz, H-2), 3.72–3.90 (m, 2 H, H-3,4), 4.26 (m, 1 H, H-5), 1.54 (d, 3 H, $J_{6,5}$ 7.0 Hz, *Me*CH), 2.60 (d, 1 H, *J* 6.0 Hz, OH), 3.32 (s, 3 H, OMe), 7.54 (dd, 1 H, *J* 8.0 and 1.7 Hz, Ar), 7.04 (dd, 1 H, *J* 8.0 and 1.7 Hz, Ar), 6.72 (td, 1 H, *J* 8.0, 8.0, and 1.7 Hz, Ar), 6.56 (td, 1 H, *J* 8.0, 8.0, and 1.7 Hz, Ar); ^1H ($CDCl_3$), δ 2.03, 2.04, 2.10, 3.38, and 3.77 (5 s, each 3 H, 3 Ac, CO_2Me, and OMe); ^{13}C (see Table I for signals of the sugar carbons), δ 164.8, 149.0, 133.1, 133.2, 131.1, 126.4, 124.0 ($COC_6H_4NO_2$), 170.4, 169.9, 169.92, and 20.6 (3 C, 3 Ac).

Anal. Calc. for $C_{27}H_{33}NO_{17}$: C, 50.38; H, 5.17; N, 2.18. Found: C, 50.56; H, 5.39; N, 2.28.

The 3-nitrobenzoate **5** had $[\alpha]_D$ +12.7° (*c* 1, chloroform). N.m.r. data: ^1H (C_6D_6), δ 4.85 (second-order m, 1 H, H-1'), 5.20–5.50 (m, 3 H, H-2',3',4'), 3.64 (d, 1 H, $J_{5',4'}$ 9.5 Hz, H-5'), 2.98 (s, 3 H, CO_2Me), 1.64, 1.66, 1.88 (3 s, each 3 H,

3 Ac), 4.52 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 4.38 (bdd, 1 H, $J_{2,1}$ 1.5, $J_{2,3}$ 3.5 Hz, H-2), 5.85 (dd, 1 H, $J_{3,2}$ 3.5, $J_{3,4}$ 10.0 Hz, H-3), 4.19 (t, 1 H, $J_{4,3}$ 10.0, $J_{4,5}$ 10.0 Hz, H-4), 3.86 (dq, 1 H, $J_{5,4}$ 10.0, $J_{5,6}$ 6.5 Hz, H-5), 1.56 (d, 3 H, $J_{6,5}$ 6.5 Hz, *Me*CH), 3.32 (s, 3 H, OMe), 8.07 (dd, 1 H, Ar), 7.24 (m, 2 H, Ar), and 6.76 (dd, 1 H, Ar); ^{13}C (see Table I for signals of the sugar carbons), δ 164.8, 147.6, 134.4, 132.4, 129.6, 128.6, 124.5 ($COC_6H_4NO_2$), 170.6, 169.5, 169.6, and 20.6 (3 C, 3 Ac).

Anal. Found: C, 50.46; H, 5.41; N, 2.14.

*Methyl 4-*O*-(methyl 2,3,4-tri-*O*-acetyl-β-*D*-glucopyranosyluronate)-2-*O*-(2-nitrobenzoyl)-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α- and -β-*D*-galactopyranosyl)-α-*L*-rhamnopyranoside* (**6** *and* **10**). — A mixture of **4** (1.7 g), benzene (0.6 mL), and nitromethane (0.6 mL) was treated at room temperature with acetobromogalactose (1.7 g) and mercury(II) cyanide (1.0 g) and stirred for 5 h. Two successive additions of the same quantities of the glycosyl bromide and catalyst were made during a further 10 h. The mixture was subsequently stirred for a further 18 h. After the usual work-up, the crude, syrupy product [R_F 0.80 (acetobromogalactose), 0.51 (**4**), 0.40–0.29 (trisaccharide derivatives); benzene–ethyl acetate (1:1)] was partially purified by flash chromatography in the same solvent to give **4** (0.13 g) and a 3:7 (^{1}H-n.m.r.) mixture (2.1 g, 87%) of **6** and **10**. Further flash chromatography with light petroleum–ether (1:3) gave **6** (0.3 g, 12%), R_F 0.13; **10** (1.3 g, 54%), R_F 0.09; and a mixture (0.35 g).

The αβ-compound **6** had $[\alpha]_D$ +0.3° (*c* 1.1, chloroform). N.m.r. data (prime and double prime refer to Gal and GlcA moieties, respectively): ^{1}H (C_6D_6, 400 MHz), δ 4.74 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 5.73 (dd, 1 H, $J_{2,1}$ 1.7, $J_{2,3}$ 3.5 Hz, H-2), 4.24 (dd, 1 H, $J_{3,2}$ 3.5, $J_{3,4}$ 9.5 Hz, H-3), 4.15 (t, 1 H, $J_{4,3}$ 9.5, $J_{4,5}$ 9.5 Hz, H-4), 3.73 (dq, 1 H, $J_{5,4}$ 9.5, $J_{5,6}$ 6.2 Hz, H-5), 1.58 (d, 3 H, $J_{6,5}$ 6.2 Hz, *Me*CH), 3.46 (s, 3 H, OMe), 7.68 (dd, 1 H, Ar), 7.09 (dd, 1 H, Ar), 6.92 (dt, 1 H, Ar), 6.72 (dt, 1 H, Ar), 5.81 (dd, 1 H, $J_{3',2'}$ 10.0, $J_{3',4'}$ 3.5 Hz, H-3′), 4.59 (dt, 1 H, $J_{5',4'}$ 1.5, $J_{5',6'a}$ 6.5, $J_{5',6'b}$ 6.5 Hz, H-5′), 4.34 (dd, 1 H, $J_{6'a,5'}$ 6.5, $J_{6'a,6'b}$ 11.5 Hz, H-6′a), 4.21 (dd, 1 H, $J_{6'b,5'}$ 6.5, $J_{6'b,6'a}$ 11.5 Hz, H-6′b), 4.39 (d, 1 H, $J_{5'',4''}$ 10.0 Hz, H-5″), 2.96 (s, 3 H, CO_2Me), 1.72, 1.73, 1.75, 1.77, 1.78, 1.80, 2.06 (7 s, each 3 H, 7 Ac), 5.35–5.41, 5.47–5.53, and 5.66–5.72 (3 m, 2 H, 3 H, 2 H, H-1′,2′,4′,1″,2″,3″,4″); ^{13}C (see Table I for signals of the sugar carbons), δ 164.0, 148.8, 132.6, 132.4, 131.1, 126.1, 123.7 ($COC_6H_4NO_2$), 169.5, 169.6 (2 C), 169.8, 170.1, 170.3, 170.4, 20.4, 20.5, and 20.6 (5 C, 7 Ac).

Anal. Calc. for $C_{41}H_{51}NO_{26}$: C, 50.56; H, 5.28. Found: C, 50.37; H, 5.24.

The ββ-isomer **10** had m.p. 103–105°, $[\alpha]_D$ −44° (*c* 4.4, chloroform). N.m.r. data: ^{1}H (C_6D_6), δ 5.02 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 5.83 (dd, 1 H, $J_{2,1}$ 1.7, $J_{2,3}$ 3.5 Hz, H-2), 4.36 (dd, 1 H, $J_{3,2}$ 3.5, $J_{3,4}$ 9.5 Hz, H-3), 4.23 (t, 1 H, $J_{4,3}$ 9.5, $J_{4,5}$ 9.5 Hz, H-4), 3.84 (dq, 1 H, $J_{5,4}$ 9.5, $J_{5,6}$ 6.2 Hz, H-5), 1.59 (d, 3 H, $J_{6,5}$ 6.2 Hz, *Me*CH), 3.48 (s, 3 H, OMe), 7.69 (dd, 1 H, Ar), 7.10 (dd, 1 H, Ar), 6.76 (dt, 1 H, Ar), 6.52 (dt, 1 H, Ar), 4.57 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1′), 5.06 (d, 1 H, $J_{1'',2''}$ 8.0 Hz, H-1″), 3.97 (d, 1 H, $J_{5'',4''}$ 9.5 Hz, H-5″), 2.97 (s, 3 H, CO_2Me), 1.63, 1.64, 1.67, 1.71, 1.79, 1.93, and 2.29 (7 s, each 3 H, 7 Ac); ^{13}C (see Table I for signals of the sugar

carbons), δ 164.3, 148.6, 132.9, 132.3, 130.6, 127.4, 124.1 ($COC_6H_4NO_2$), 169.3, 169.7, 170.3 (4 C), 170.8, 20.4 (5 C), 20.8, and 21.3 (7 Ac).

Anal. Found: C, 49.90; H, 5.21.

Methyl 3-O-(α- and β-D-galactopyranosyl)-4-O-(methyl β-D-glucopyranosyluronate)-α-L-rhamnopyranoside (**7** *and* **11**). — A solution of **6** (100 mg) in anhydrous methanol (5 mL) was treated with a catalytic quantity of sodium methoxide for 5 h at 22°. T.l.c. then showed that **6** (R_F 0.82) had been converted into **7** (R_F 0.28; 1-butanol–methanol–water, 3:1:1). The solution was neutralised and concentrated to give **7** (44 mg, 90%), $[\alpha]_D$ +10° (*c* 2.7, ethanol–water, 1:1). ^{1}H-N.m.r. data (D_2O): δ 5.02 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1 Rha), 4.55 (d, 1 H, $J_{1',2'}$ 3.8 Hz, H-1′ Gal), 4.49 (d, 1 H, $J_{1'',2''}$ 7.8 Hz, H-1″ GlcA), 3.17 (s, 3 H, CO_2Me), 3.23 (s, 3 H, OMe), and 1.17 (d, 3 H, $J_{6,5}$ 6.5 Hz, *Me*CH). For ^{13}C-n.m.r. data, see Table I. Positive ion f.a.b.–m.s. in glycerol showed, as the only peaks of significant intensity at high mass, *m/z* 531.2 [9.0%, $(M + H)^+$; calc. for $C_{20}H_{34}O_{16}$ (M), *m/z* 530.18], 553.2 [3.5%, $(M + Na)^+$], and 115.0 (100%).

Anal. Calc. for $C_{20}H_{34}O_{16}$: C, 45.28; H, 6.46. Found: C, 44.88; H, 6.81.

Deacylation of **10** (1.0 g) gave **11** (500 mg, 90%), m.p. 147–149° (crude), $[\alpha]_D$ −56° (*c* 2.8, methanol). ^{1}H-N.m.r. data (D_2O): δ 4.49 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1 Rha), 4.69 and 4.41 (2 d, *J* 8.0 and 7.0 Hz, H-1 of GlcA and Gal), 3.20 (s, 3 H, CO_2Me), 3.63 (s, 3 H, OMe), and 1.12 (d, 3 H, *J* 6.5 Hz, MeCH). For ^{13}C-n.m.r. data, see Table I. Positive ion f.a.b.–m.s. showed *m/z* 531 $[(M + H)^+]$ and 553 $[(M + Na)^+]$; the intensity of the base peak was not recorded.

Anal. Found: C, 45.44; H, 6.53.

Methyl 3-O-(α- and β-D-galactopyranosyl)-4-O-(sodium β-D-glucopyranosyluronate)-α-L-rhamnopyranoside (**8** *and* **12**). — To a solution of **11** (223 mg, 4.39 mmol) in water (1.0 mL) was added 0.1M sodium hydroxide (4.4 mL). The pH of the solution decreased from 10.92 to 8.25 during 7 h and remained thereat for 2 days. The solution was concentrated to give **12**, m.p. 165–168°, $[\alpha]_D$ −7° (*c* 2.3, water–methanol, 1:2). For ^{13}C-n.m.r. data, see Table I. Positive ion f.a.b.–m.s. showed, as the only peaks of significant intensity at high mass, *m/z* 539.1 [14.1%, $(M + 1)^+$; calc. for $C_{19}H_{31}NaO_{16}$ (M), *m/z* 538.15], 561.0 [2.0%, $(M + 23)^+$], and 93.0 (100%).

Treatment of **7** (28 mg, 0.054 mmol) with 0.1M sodium hydroxide (0.54 mL) gave a quantitative yield of **8**, m.p. 218–220°, $[\alpha]_D$ +7° (*c* 1.4, water–methanol, 1:2). For ^{13}C-n.m.r. data, see Table I. F.a.b.–m.s. showed, as the only peaks of significant intensity at high field, *m/z* 539.0 [12.4%, $(M + H)^+$; calc. for $C_{19}H_{31}NaO_{18}$ (M), *m/z* 538.15], 561.0 [37.6%, $(M + Na)^+$], and 136.9 (100%).

Methyl 3-O-(α-and β-D-galactopyranosyl)-4-O-(β-D-glucopyranosyluronic acid)-α-L-rhamnopyranoside (**9** *and* **13**). — A solutions of **8** (24 mg) in water (1 mL) was treated with Amberlite IR-120 (H^+) resin, filtered, and concentrated to give **9** (20 mg), m.p. 155–160°, $[\alpha]_D$ +13° (*c* 1, water). ^{1}H-N.m.r. data (D_2O): δ 5.0 (d, 1 H, *J* 3.6 Hz, H-1 Rha), 4.52 (d, 1 H, *J* 4.0 Hz, H-1′ Gal), and 4.52 (d, 1 H, *J* 7.5 Hz, H-1 GlcA).

Anal. Calc. for $C_{19}H_{32}O_{16} \cdot H_2O$: C, 42.70; H, 6.4. Found: C, 42.32; H, 6.71.

Similar treatment of **12** (94 mg) gave **13** (78 mg) as a glass, $[\alpha]_D$ −29° (*c* 0.8, water). ^{1}H-N.m.r. data (D_2O): δ 4.50 (d, 1 H, *J* 2.5 Hz, H-1 Rha), 5.30 and 4.35 (2 d, *J* 7.8 and 7.2 Hz, H-1 of Gal and GlcA).

Anal. Found: C, 42.25; H, 6.68.

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REFERENCES

1 P. M. COLLINS AND V. R. N. MUNASINGHE, *J. Chem. Soc., Perkin Trans. 1,* (1983) 1879–1884.
2 R. U. LEMIEUX, *Chem. Soc. Rev.,* 7 (1978) 423–425.
3 P. M. COLLINS AND V. R. N. MUNASINGHE, *J. Chem. Soc., Perkin Trans. 1,* (1983) 921–926; P. M. COLLINS AND H. EDER, *ibid.,* (1983) 927–931.
4 B. LINDBERG, J. LONNGREN, J. L. THOMPSON, AND W. NIMMICH, *Carbohydr. Res.,* 25 (1972) 49–57.
5 H. W. FLOWERS AND R. W. JEANLOZ, *J. Org. Chem.,* 28 (1963) 1377–1379; B. HELFERICH AND K. WEIS, *Chem. Ber.,* 89 (1956) 314–321.
6 G. N. BOLLENBACK, J. W. LONG, D. G. BENJAMIN, AND J. A. LINDQUIST, *J. Am. Chem. Soc.,* 77 (1955) 3310–3315.
7 M. I. DAWSON AND P. D. HOBBS, *Carbohydr. Res.,* 85 (1980) 121–129.
8 K. BOCK AND C. PEDERSEN, *J. Chem. Soc., Perkin Trans. 2,* (1974) 293–297.
9 J. E. N. SHIN AND A. S. PERLIN, *Carbohydr. Res.,* 76 (1979) 165–176.
10 P. E. PFEFFER, K. M. VALENTINE, AND F. W. PARRISH, *J. Am. Chem. Soc.,* 101 (1979) 1265–1274.
11 P. A. J. GORIN AND M. MAZUREK, *Can. J. Chem.,* 53 (1975) 1212–1223.
12 K. BOCK AND C. PEDERSEN, *Adv. Carbohydr. Chem. Biochem.,* 41 (1983) 27–44.
13 W. C. STILL, M. KAHN, AND A. MITRA, *J. Org. Chem.,* 43 (1978) 2923–2925.
14 D. KEGLEVIC, *Adv. Carbohydr. Chem. Biochem.,* 36 (1979) 57–134.
15 P. M. COLLINS AND N. N. OPARAECHE, *Carbohydr. Res.,* 33 (1974) 35–46.