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THE STEPWISE SYNTHESIS OF AN ALDOPENTAOURONIC ACID DERIVATIVE RELATED TO BRANCHED (4-*O*-METHYLGLUCURONO)XYLANS*

PAVOL KOVÁČ, JÁN HIRSCH, VLADIMÍR KOVÁČIK, AND PAVOL KOČIŠ
Institute of Chemistry, Slovak Academy of Sciences, 809 33 Bratislava (Czechoslovakia)
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ABSTRACT

Benzylation of methyl 2,3-anhydro-4-*O*-[2-*O*-benzyl-3,4-di-*O*-(β-D-xylopyranosyl]-β-D-xylopyranosyl]-β-D-ribopyranoside (**1**) afforded the crystalline, fully benzylated tetrasaccharide derivative **2**. The octa-*O*-benzyl derivative **3**, having only HO-2 unsubstituted, obtained by treatment of **2** with benzyl alcoholate anion in benzyl alcohol, was allowed to react in dichloromethane with methyl 2,3-di-*O*-benzyl-1-chloro-1-deoxy-4-*O*-methyl-α,β-glucopyranuronate in the presence of silver perchlorate and triethylamine to give the branched, 4-*O*-methyl-α-D-glucuronic acid-containing pentasaccharide derivative **4a** as the major product. Subsequent debenzylation afforded the aldopentaouronic acid derivative **5a**, which contains all the basic structural features of branched, hardwood (4-*O*-methylglucurono)xylans. The structure of **5a** was confirmed by analysis of its ^{13}C-n.m.r. spectrum and the mass-spectral fragmentation pattern of the corresponding fully methylated derivative **6a**.

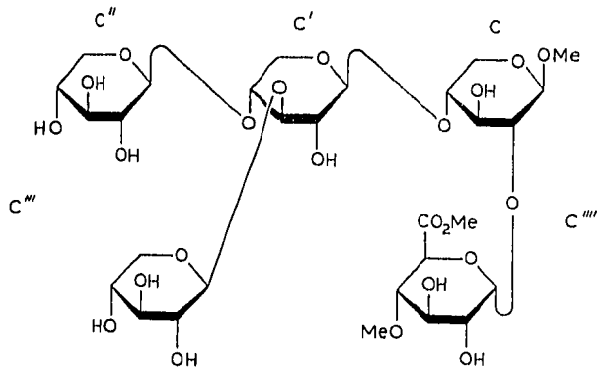
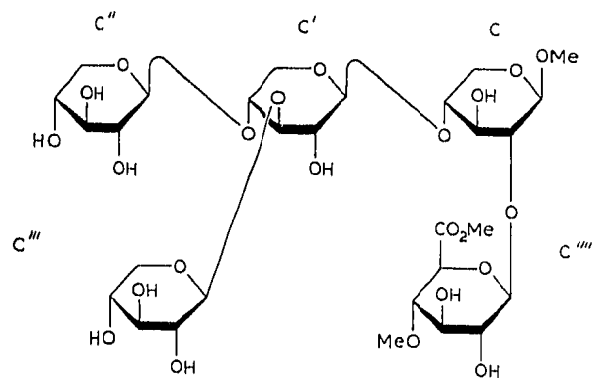
INTRODUCTION

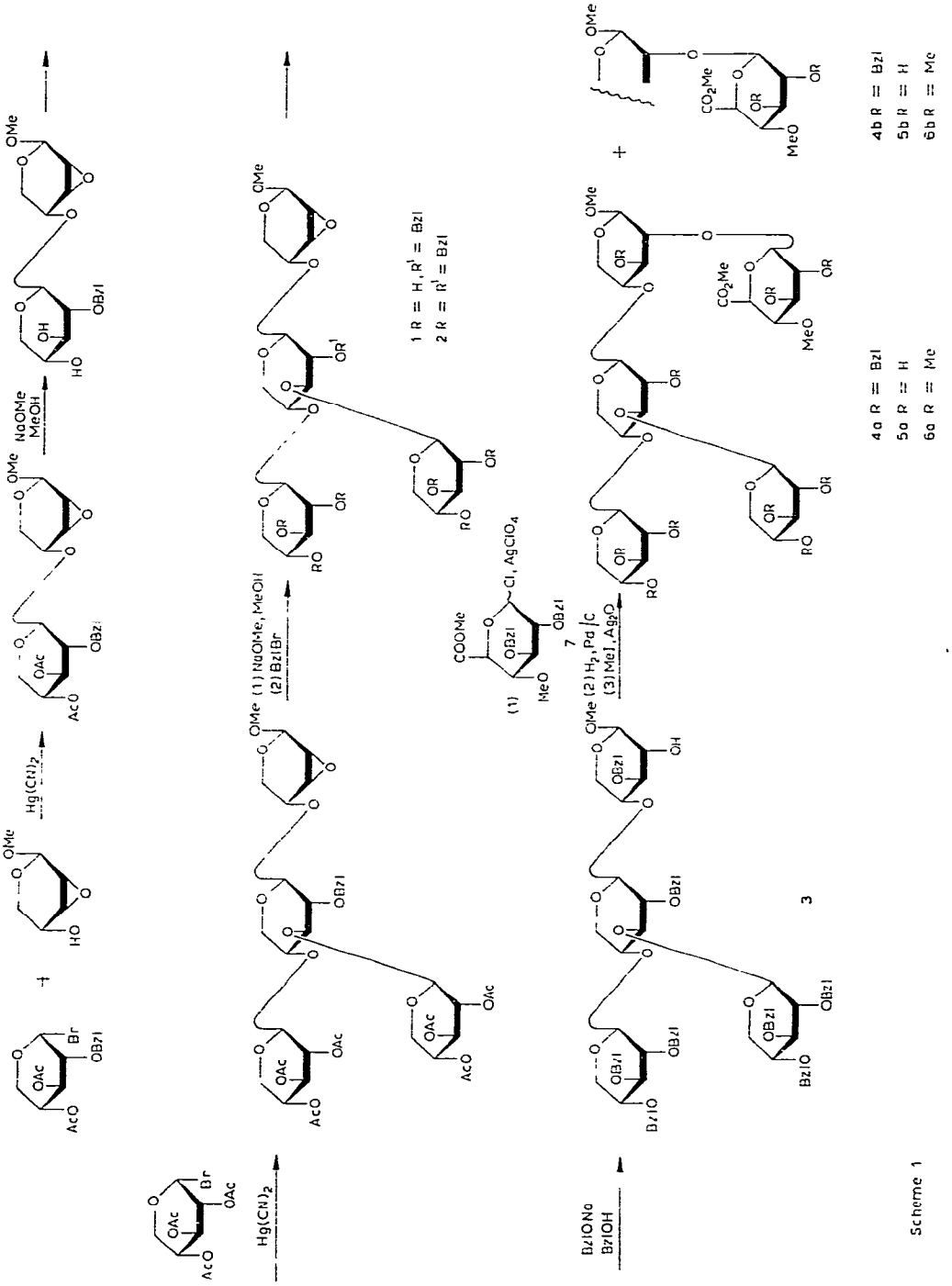
Oligosaccharides are important model compounds for studies of various properties of polysaccharides. The structures of oligosaccharides, and the amount in which they are formed on acid-catalyzed, partial hydrolysis of polysaccharides, reflect the sensitivity of various types of glycosidic linkage towards the hydrolytic conditions applied. Therefore, the partial hydrolyzate contains preferentially certain types of oligosaccharide, and their amounts in the hydrolyzate do not necessarily reflect the population of the same sequences in the natural polymer. For example, chemical or enzymic depolymerization of hardwood (4-*O*-methylglucurono)xylans (for basic structural features of this type of polysaccharide, see the accompanying formula) yields[2–6], in addition to D-xylose and β-(1→4)-linked xylo-oligosaccharides, a homologous series of linear, oligoglycuronic acids of the type G→C→D→E,

*Synthesis and Reactions of Uronic Acid Derivatives, Part XIX. For Part XVIII, see ref. 1.

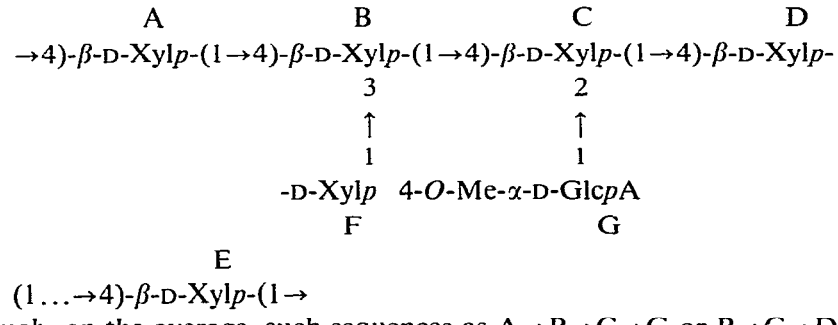
TABLE I

^{13}C-N.M.R. SPECTRAL DATA FOR **5a** AND **5b** IN D_2O

Compound	*Ring*	*chemical shifts*								
		C-1	*C-2*	*C-3*	*C-4*	*C-5*	*CO*	*OMe*	*OMe (4)*	*OMe (6)*
	C	105.1	78.8	73.3	78.8	63.4		58.3		
	C′	102.1	73.0	80.1	74.0	63.4				
	C″	102.1	73.3	76.2	70.1	66.1				
	C‴	103.6	74.0	76.2	70.1	66.1				
	C⁗	99.2	71.8	73.2	82.3	70.11	72.8		60.9	54.2
5a										
	C	103.9	82.1	73.4	77.0	66.1		57.7		
	C′	102.1	73.4	80.3	74.8	63.5				
	C″	102.1	73.4	76.2	70.1	66.1				
	C‴	103.4	74.0	76.2	70.1	66.1				
	C⁗	103.9	70.1	74.8	82.1	70.11	71.7		60.9	54.2
5b										


Hg(CN)2
BzlONa
BzlOH
(1) NaOMe, MeOH
(2) BzlBr
(1) Cl, AgClO4
7
(2) H2, Pd/C
(3) MeI, Ag2O
1 R = H, R1 = Bzl
2 R = R1 = Bzl
3
4a R = Bzl
5a R = H
6a R = Me
4b R = Bzl
5b R = H
6b R = Me

Scheme 1



although, on the average, such sequences as A→B→C→G or B→C→D are equally

abundant in the natural polysaccharide. Other types of oligoglycuronic acid are only rarely isolated[7-10] from partial hydrolyzates of (4-*O*-methylglucurono)xylans and only in very low yield.

Preparation of oligosaccharides not found among the products of partial hydrolysis of polysaccharides requires planned syntheses. We have previously reported the synthesis of the aldotriouronic acid that constitutes sequence[1] B→C←G, and we now describe a stepwise synthesis of the pentasaccharide derivative **5a** containing the sequence A→B→C→G (for the overall reaction-pathway, see Scheme 1).

Compound **5a** is an important model for the title polysaccharide, in that it contains all of its basic structural features, namely, three (1→4)-linked β-D-xylose residues constituting the main chain of the polysaccharide, a (1→3)-linked β-D-xylosyl group comprising the branch point, and a (1→2)-linked, terminal 4-*O*-methyl-α-D-glucuronic acid group. Furthermore, the β-linked aglycon imitates the situation in the main backbone of the polysaccharide.

RESULTS AND DISCUSSION

The starting point in the present synthesis was the 2-*O*-benzyl tetrasaccharide derivative **1**, obtained[11] as an intermediate in the course of the stepwise synthesis of a branched methyl β-D-xylotetraoside. Benzylation of **1** gave the fully substituted product **2**, which was also isolated crystalline. Compound **2**, when treated with benzyl alcoholate anion in benzyl alcohol, yielded the key intermediate (**3**) in the present synthesis, having only HO-2 unsubstituted.

We have previously prepared the glycosyl chloride **7** and demonstrated its use in stereoselective synthesis[1,12,13] of the 4-*O*-methyl-α-D-glucopyranosyluronic acid linkage. The original procedure[13], which required treatment of methyl 2,3-di-*O*-benzyl-4-*O*-methyl-α-D-glucopyranuronate with thionyl chloride for 24 h, has now been improved in that an amount of an organic base equimolar with respect to the sugar derivative is used in the reaction. In this way, the reaction time may be sub-

stantially shortened (1 h). The ^{1}H-n.m.r. spectrum of compound **7** thus obtained was almost identical to that of **7** prepared in the absence of the base, the difference being merely in the intensity of signals that reflect the anomeric composition ($\alpha:\beta$ = 1:2.5, compare[12,13] $\alpha:\beta$ = 1:3.5).

The method applied in previous syntheses[1,12,13] of 4-*O*-methyl-α-D-glucuronic acid-containing aldouronic acids was that developed by Igarashi *et al.*[14], who showed that glucosyl chlorides having a nonparticipating group at C-2 react with nucleophiles in dilute ethereal solution, in the presence of silver perchlorate and *sym*-collidine, to afford α-linked glycosides with pronounced stereoselectivity and in high yield. Our results[1,12,13] agreed with observations of the original authors and we were able to show for the first time the possibility for synthesis of 4-*O*-methyl-α-D-glucuronic acid-containing oligosaccharides. In this work, better results were obtained when the reaction was performed in the manner described by Paulsen and Stenzel[15], namely, by adding a concentrated solution of the glycosyl halide in dichloromethane to a stirred mixture of the nucleophile, organic base (in our case, comparable results were obtained with either *sym*-collidine, triethylamine, or diisopropylethylamine), and solid silver perchlorate in a little dichloromethane (for details, see Experimental section). Because of side reactions of **7**, three molar proportions had to be used to bring the reaction of **3** practically to completion. The main side-reaction of **7** was hydrolysis and eventually the formation of non-reducing disaccharides[16], the presence of which in the crude product complicated the isolation of the desired products **4a** and **4b**. In fact, as the pentasaccharide derivatives **4a** and **4b** could not be obtained crystalline, their separation from the by-products was the critical step in the synthesis. Chromatographic systems *A* and *D* (see Experimental section) which satisfactorily resolved the α- and β-linked pentasaccharide derivatives formed, did not separate **4a** and **4b** from the by-products. On the other hand, solvent *E* did not separate **4a** and **4b** from each other, but may be used to remove the by-products from either of the two pentasaccharides. Therefore, monitoring of the reaction by t.l.c. did not provide a true picture of the composition of the mixture; it could be used for checking the conversion of **3** but not for evaluation of the stereoselectivity of the condensation. As use of the aforementioned excess of halide **7** in the coupling reaction caused the nucleophile **3** to react almost completely, the difference between the theoretical and isolated combined yield of **4a** and **4b** (78.2%) may be accounted for through manipulative losses, occurring mainly during repeated purification of products by chromatography. Owing to manipulative losses, the ratio of **4a** to **4b** (2.7:1) isolated does not necessarily reflect the stereoselectivity of the reaction of **3** and **7**.

The desired glycoside **5a** and its isomer **5b** were obtained by hydrogenolytic deprotection of their precursors **4a** and **4b**. Compounds **6a** and **6b**, obtained from **5a** and **5b** by conventional methylation, produced qualitatively identical mass spectra, which confirmed the sequence of monosaccharides in the pentasaccharides studied.

For convenience of interpretation, the component sugars are designated as follows:

$$a(1\rightarrow4)b(1\rightarrow4)c$$
$$\begin{matrix} 3 & 2 \\ \uparrow & \uparrow \\ 1 & 1 \\ d & e \end{matrix}$$

where *a*, *b*, *c*, and *d* are D-xylopyranose, and *e* methyl 4-*O*-methyl-D-glucopyranuronate residues (or groups). Based on the known fragmentation-characteristics of isomeric xylo-oligosaccharides[17] and aldobiouronic acids[18], the following information on the structures of **6a** and **6b** could be extracted from their 12-eV mass spectra. The molecular weights of the products were calculated from the *m/z* values of the $bcdeA_1$ (713) and aA_1 (175) ion-peaks according to the equation $M = aA_1 + bcdeA_1 + 16 = 904$, and the identity of ions having *m/z* 713 confirmed their precursor, $abcdJ_1$ (*m/z* 773) ion-peak. The formation of aA_1 (identical to those of the dA_1 series) and eA_1 ions at *m/z* 175 and 233, respectively, proved that *a* and *d* xylopyranose groups, as well as the uronic acid residue, were present as non-reducing end-groups of the molecule. The intense peak present at *m/z* 129 in the spectra of **6a** and **6b** was also found[17] in the spectra of fully methylated xylotriose and xylotetraose containing the arrangement $a(1\rightarrow4)b(3\rightarrow1)d$, and it appears that formation of this fragment is characteristic of the arrangement in related substances.

The structures of **5a** and **5b** were finally confirmed by their ^{13}C-n.m.r. spectra (Table I), the analysis of which was based on interpreted spectra of a series of isomeric xylo-oligosaccharides[19]. Chemical shifts for carbon atoms of the C′, C″, and C‴ rings are very close or identical to those found[19] in the ^{13}C-n.m.r. spectra of a branched methyl β-D-xylotetraoside obtained[11] by hydrogenolytic cleavage of the benzyl group from **3**. The difference in the ^{13}C chemical-shifts for ring C, as compared with the chemical shift for carbon atoms of the ring C in the branched methyl β-D-xylotetraoside, is consistent with the expected α- and β-effects arising from the attached 4-*O*-methyl-α- or -β-D-glucuronic acid groups. Chemical shifts observed in the ^{13}C-n.m.r. spectra of **5a** and **5b** for carbon atoms of the methyl 4-*O*-methyl-D-glucuronate residue are consistent with data in the literature[20].

EXPERIMENTAL

General methods. — Melting points were determined on a Kofler hot-stage. Optical rotations (22°, *c* 1, unless stated otherwise) were measured with a Perkin–Elmer Model 141 automatic polarimeter. Mass spectra (70 and 12 eV) were obtained with a JMS-100D instrument at an emission of 300 μA. The evaporation temperature was 300° and that in the ionizing chamber was 180°. The ^{13}C-n.m.r. spectra were measured at room temperature with a Jeol JNM FX-60 spectrometer in the deuterium-lock mode (internal methanol, for solutions in D_2O). The shift of methanol *versus* tetramethylsilane (49.8 p.p.m.) was confirmed separately. Proton-decoupled f.t.-spectra were measured by using a repetition time of 1.0 s, a pulse-width of 4 s

(45°), 4000-Hz sweep-width, and 4K real data-points. The ^{1}H-n.m.r. spectra (80 MHz, $CDCl_3$, internal tetramethylsilane) were recorded with a Tesla BS 487B spectrometer.

T.l.c. was performed on Silica Gel G, and column chromatography on dry-packed Silica Gel 60, with *A*, 15:1 benzene–acetone; *B*, 4:1 benzene–ethyl acetate; *C*, 7:1 carbon tetrachloride–acetone; *D*, 7:1 benzene–ethyl acetate; *E*, 3:1 heptane–acetone; *F*, 3:1 chloroform–methanol; *G*, 4:1 benzene–acetone; and *H*, 6:1 chloroform–methanol. Detection was effected by charring with 5% (v/v) sulfuric acid in ethanol. Dichloromethane was dried with phosphorus pentaoxide and distilled.

Microanalyses were performed with a Perkin–Elmer Model 240 automatic analyzer. Solutions were dried with anhydrous sodium sulfate and evaporated at 40°/2 kPa.

*Methyl 2,3-di-*O*-benzyl-1-chloro-1-deoxy-4-*O*-methyl-α,β-*D*-glucopyranuronate* (7). — Thionyl chloride (4 mL) was added at 0° dropwise and with stirring to methyl 2,3-di-*O*-benzyl-4-*O*-methyl-α-D-glucopyranuronate[13] (2 g) that had been wetted with *sym*-collidine (0.7 mL), and the mixture was kept for 1 h at room temperature. After evaporation with intervening addition of toluene, dry ether (30 mL) was added to the semisolid residue and, after 15 min at 0°, the mixture was filtered, the solids were washed with dry ether (twice), and the filtrate, containing halide **7** (chromatographically almost pure), was used directly, for the next step (when the condensation was performed in ether); alternatively it was concentrated and used for the preparation of a stock solution in dichloromethane. The stock solutions of **7** in either anhydrous ether or dichloromethane, when kept at 0° with the exclusion of moisture, were stable for at least 1 year (t.l.c., solvent *A*).

*Methyl 3-*O*-benzyl-2-*O*-[methyl 2,3-di-*O*-benzyl-4-*O*-methyl-α-* (**4a**) *and -β-*D*-xylopyranosyl)-β-*D*-xylopyranosyl]-β-*D*-ribopyranoside* (**2**). — A mixture of **1** (4 g), silver oxide (25 g), and benzyl bromide (13 mL) in *N*,*N*-dimethylformamide (80 mL) was stirred for 18 h at room temperature, and the mixture was processed conventionally. The partially benzylated product was rebenzylated by stirring in *N*,*N*-dimethylformamide with sodium hydride (1.5 g) and benzyl bromide (5.5 mL). After conventional isolation, t.l.c. (solvent *B*) showed **2** (R_F 0.5) to be the main product. Crystallization from ether–isopropyl ether gave, after the crystalline material had been washed with ethanol, colorless, chromatographically pure **2** (4.75 g). A further crop (1.4 g; total yield 83%) was obtained by chromatography of the material in the mother liquor. Recrystallization of a portion from isopropyl ether afforded the analytical sample, m.p. 102–104°, $[\alpha]_D$ −22.5° (chloroform) (Found: C, 71.49; H, 6.77. $C_{70}H_{76}O_{16}$ calc.: C, 71.65; H, 6.53%).

*Methyl 3-*O*-benzyl-4-*O*-[2-*O*-benzyl-3,4-di-*O*-(2,3,4-tri-*O*-benzyl-β-*D*-xylopyranosyl)-β-*D*-xylopyranosyl]-β-*D*-xylopyranoside* (**3**). — Sodium hydride (2.4 g) was added portionwise and with stirring to benzyl alcohol (120 mL) and, when a clear solution had been formed (~15 min), compound **2** (11.5 g) was added to the resultant solution of benzyl alcoholate anion in benzyl alcohol. After 5 h at 80–90°, the orange solution was diluted with ethanol (150 mL), deionized with Dowex 50W (H^+ form) resin, and organic solvents were removed, finally at 140°/133 Pa. T.l.c. (solvent *C*)

of the residue showed the presence mainly of **3** (R_F 0.4, compare 0.5 for the starting material), which was isolated by chromatography. The compound could not be crystallized and, after drying at 40°/2 kPa for 5 h, it was obtained as a glassy solid that had $[\alpha]_D$ −42° (chloroform) (Found: C, 72.08; H, 6.80. $C_{77}H_{84}O_{17}$ calc.: C, 72.17; H, 6.61%).

*Methyl 3-*O*-benzyl-2-*O*-(methyl 2,3-di-*O*-benzyl-α-* (**4a**) *and -β-*D*-glucopyranosyluronate)-4-*O*-[2-*O*-benzyl-3,4-di-*O*-(2,3,4-tri-*O*-benzyl-β-*D*-xylopyranosyl)-β-*D*-xylopyranosyl-β-*D*-xylopyranoside* (**4b**). — Compound **7** (0.2M solution in dichloromethane, 15 mL, 3 mmol) was added at −10° to a stirred mixture of **3** (1.28 g, 1 mmol), triethylamine (0.45 mL, 3.2 mmol, or a corresponding amount of *sym*-collidine or diisopropylethylamine), and silver perchlorate (0.62 g, 3 mmol). The solution was allowed to warm to ~20° and, after 0.5 h, t.l.c. (solvent *A*) showed the absence of starting materials (R_F 0.2 and 0.75) and that two main products had been formed (R_F 0.3 and 0.35). The mixture was diluted with chloroform (30 mL), filtered, and the filtrate washed successively with M hydrochloric acid, saturated aqueous sodium hydrogencarbonate, and water. The colorless solution was dried, evaporated, and the crude product chromatographed with solvent *D*. T.l.c. of fractions that appeared homogeneous in t.l.c. (solvents *A* and *D*) showed that they contained several minor impurities. Re-chromatography of combined fractions containing **4a** and **4b** with solvent *E* then gave the bulk of each component chromatographically pure. A small amount of unresolved material was rechromatographed to give, ultimately, **4a** (0.95 g, 57.2%) and **4b** (0.35 g, 21%) as amorphous solids.

Compound **4a** had $[\alpha]_D$ −10° (*c* 1.2, chloroform) (Found: C, 71.60; H, 6.72. $C_{99}H_{108}O_{23}$ calc.: C, 71.37: H, 6.53%).

Compound **4b** had $[\alpha]_D$ −29° (*c* 1.9, chloroform) (Found: C, 71.22; H, 6.74%).

*Methyl 2-*O*-(methyl 4-*O*-methyl-α-* (**5a**) *and -β-*D*-glucopyranosyluronate)-4-*O*-[3,4-di-*O*-(β-*D*-xylopyranosyl)-β-*D*-xylopyranosyl]-β-*D*-xylopyranoside* (**5b**). — Water was added almost to turbidity to a solution of **4a** (0.7 g) in acetone (20 mL), followed by a suspension of 5% palladium-on-charcoal catalyst (0.2 g) in a little acetone, and the mixture was stirred at room temperature in an atmosphere of hydrogen until t.l.c. (solvents *B* and *F*) showed that the reaction was complete. After conventional isolation, chromatographically pure **5a** was obtained in theoretical yield. It was dissolved in hot methanol, and on cooling the compound separated as a gel (when **4a** was used slightly impure in the hydrogenation, chromatographically pure **5a** could be obtained, as the impurities remained in the supernatant solution). After one more precipitation in the aforementioned manner, and drying at 40°/2 kPa, compound **5a** formed a glassy solid having $[\alpha]_D$ −24.6° (water) (Found: C, 45.36; H, 6.30. $C_{29}H_{48}O_{23}$ calc.: C, 45.55; H, 6.33).

Hydrogenolysis of **4b** and conventional isolation afforded amorphous **5b** in theoretical yield, $[\alpha]_D$ −82° (water) (Found: C, 45.38; H, 6.18%).

*Methyl 3-*O*-methyl-2-*O*-(methyl 2,3,4-tri-*O*-methyl-α-* (**6a**) *and -β-*D*-glucopyranosyluronate)-4-*O*-[2-*O*-methyl-3,4-di-*O*-(2,3,4-tri-*O*-methyl-β-*D*-xylopyranosyl)-β-*D*-xylopyranosyl]-β-*D*-xylopyranoside* (**6b**). — A mixture of **5a** (0.1 g), silver oxide

(0.5 g), and methyl iodide (1 mL) in *N*,*N*-dimethylformamide (3 mL) was stirred in the dark for 8 h. The same amount of each of the reagents was again added and stirring was continued for a further 16 h. The mixture was processed conventionally and the crude product eluted from a column of silica gel to remove some undermethylated material (R_F <0.2, solvent *G*). The main product (R_F 0.3), isolated as a solid foam (0.1 g, ~90%), had $[\alpha]_D$ −27° (chloroform) (Found: C, 51.90; H, 7.71. $C_{39}H_{68}O_{23}$ calc.: C, 51.76; H, 7.57%).

Methylation of **5b** and isolation in the manner just described gave amorphous **6b**, $[\alpha]_D$ −82° (chloroform) (Found: C, 51.74; H, 7.53%).

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