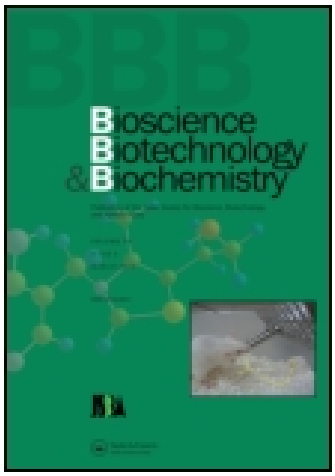

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Stereoselective Synthesis of Regioisomers of Aldobiouronic Acid

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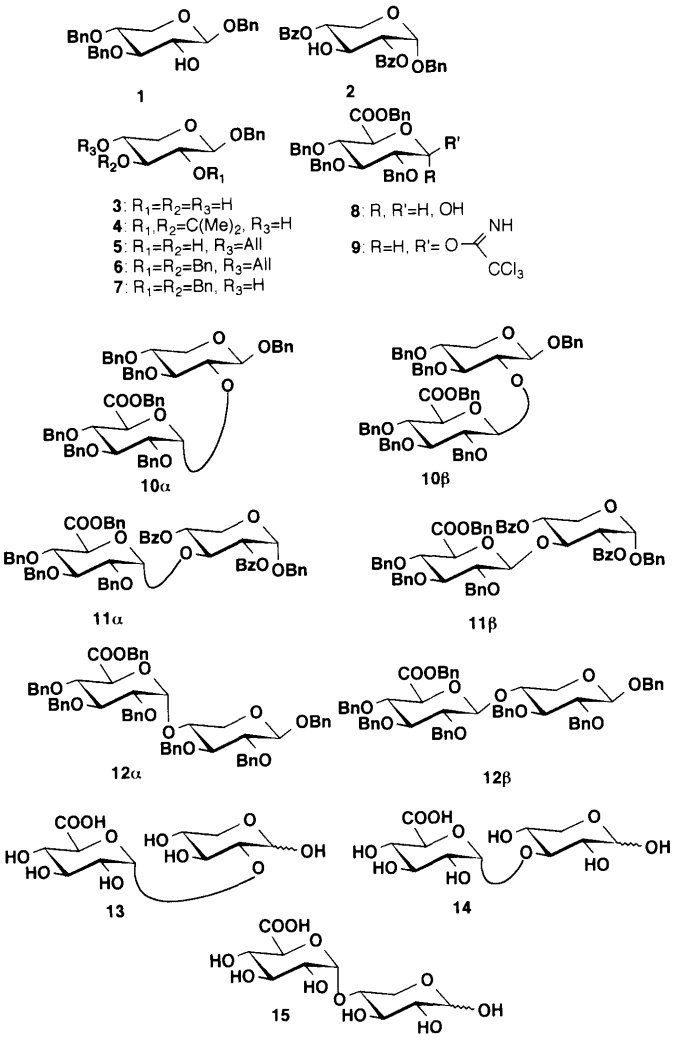
α-Glucuronidase is a very important enzyme for the complete hydrolysis of plant hemicellulose, but the substrate specificity of the enzyme has not previously been reported. In this connection, the three regioisomers of *O*-(α-D-glucopyranosyluronic acid)-D-xylose (aldobiouronic acid), 2-*O*-(α-D-glucopyranosyluronic acid)-D-xylose (13), 3-*O*-(α-D-glucopyranosyluronic acid)-D-xylose (14), and 4-*O*-(α-D-glucopyranosyluronic acid)-D-xylose (15), were stereoselectively synthesized to clarify the substrate specificity.

Glucuronoxylans are important components of plant hemicellulose. The (1→4)-β-D-xylopyranan backbone of these polymers carries occasional α-D-glucopyranosyluronic acid branches at the C-2 or C-3 position of the D-xylose residues.[1] Some of the α-D-glucopyranosyluronic acid residues are also methyl etherified at the C-4 position.[1] 2-*O*-(4-*O*-Methyl-α-D-glucopyranosyluronic acid)-D-xylose (MeGA-2X), 2-*O*-(α-D-glucopyranosyluronic acid)-D-xylose (GA-2X), and 3-*O*-(α-D-glucopyranosyluronic acid)-D-xylose have been isolated from the acid hydrolysate of glucuronoxylan.[1,2] MeGA-2X has been synthesized by Kováč *et al.*,[3] although the chemical synthesis of other aldobiouronic acids has not been described.

We have recently reported the substrate specificity of α-glucuronidase from *Aspergillus niger*.[4] This α-glucuronidase has the ability to hydrolyze the α(1→2) glycosidic bond between the D-glucuronic acid and D-xylose residues in MeGA-2X and GA-2X. However, we have not shown whether the enzyme can hydrolyze the α(1→3) and α(1→4) glycosidic bonds between the D-glucuronic acid and D-xylose residues. α-Glucuronidase is a very important enzyme for the complete hydrolysis of plant hemicellulose, and it is necessary to clarify the substrate specificity of this enzyme. The regioisomers of aldobiouronic acid are needed for further studies on the substrate specificity of α-glucuronidase. Moreover, the synthesis of the regioisomers is also important for investigating the structure-function relationships of these uronides for biological activity. In this present study, we investigated the stereoselective synthesis of three *O*-(α-D-glucopyranosyluronic acid)-D-xylose regioisomers.

The D-xylose acceptor unsubstituted at the C-2 position, benzyl 3,4-di-*O*-benzyl-β-D-xylopyranoside (**1**), was prepared by the method of Kováč and Petráková.[5] The D-xylose acceptor unsubstituted at the C-3 position, benzyl 2,4-di-*O*-benzoyl-α-D-xylopyranoside (**2**), was synthesized as described by Helm *et al.*[6] The D-xylose acceptor unsubstituted at the C-4 position was prepared according to the procedure of Takeo *et al.*[7] with slight modification. They used methyl β-D-xylopyranoside as the starting material, while we used benzyl β-D-xylopyranoside (**3**) instead, because the benzyl group at the reducing end can be deprotected more easily than the methyl group. Compound **3** was acetonized with 2-methoxypropene in *N*,*N*-dimethylformamide (DMF) in the presence of a catalytic amount of methanolic hydrogen chloride.[7] Without isolating 2,3-isopropylidene derivative **4**, the resulting com-



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Abbreviations: DMF, *N*,*N*-dimethylformamide; GA-2X, 2-*O*-(α-D-glucopyranosyluronic acid)-D-xylose; MeGA-2X, 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose; TMSOTf, trimethylsilyl trifluoromethanesulfonate.

pound was alkylated with allyl bromide–sodium hydride in DMF, and treated with dilute acid in acetone. Fractional crystallization of the product provided a 58% yield of pure benzyl 4-*O*-allyl-β-D-xylopyranoside (**5**). Benzylation of **5** gave 4-*O*-allyl-2,3-di-*O*-benzyl-β-D-xylopyranoside (**6**, 84%). The isomerization of the allyl group in **6** to the 1-propenyl ether with tris(triphenylphosphine)rhodium(I) chloride[8] and 1,4-diazabicyclo[2.2.2]octane, and subsequent hydrolysis with dilute acid in aqueous acetone[9] gave benzyl 2,3-di-*O*-benzyl-β-D-xylopyranoside (**7**) in a 70% yield.

As a glucuronosyl donor, we chose *O*-(benzyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranosyluronate) trichloroacetimidate (**9**), because **9** is more stable than the halogenated D-glucuronic acid derivatives and can be easily activated with a catalytic amount of various Lewis acids. The trichloroacetimidate was prepared by the reaction of benzyl 2,3,4-tri-*O*-benzyl-D-glucopyranuronate (**8**)[10] with trichloroacetonitrile and potassium carbonate,[11] and β-isomer **9** was isolated by fractional crystallization in a 70% yield. The stereochemistry of **9** was confirmed to be of β-form by the ^{1}H-NMR spectrum, which showed a doublet for the H-1 proton at δ 5.89 ppm ($J_{1,2}=7.0$ Hz).

Glycosylation of **1**, **2**, or **7** with **9** was carried out in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf)[12] as a catalyst in CH_2Cl_2. Glycosylation of trichloroacetimidate **9** proceeded stereoselectively to afford the corresponding α-glycosides, which were isolated by flash chromatography to give disaccharide derivatives **10α**, **11α**, and **12α** in 60%, 44%, and 53% yields, respectively. The β-isomers of these disaccharides were also isolated to give **11β** and **12β** in 23% and 6% yields, respectively. The purification of **10β** failed, but the ^{1}H-NMR spectrum of the crude disaccharide products containing **10α** and **10β** showed the formation of **10β** in about a 15% yield (**10α**:**10β**=4:1) by comparing the intensities of the newly formed anomeric signals. The structures of the glycosylated products were identified according to their ^{1}H- and ^{13}C-NMR spectra. The configuration at the newly formed anomeric center was assignable from the δ value for C-1′ (α-form: **10α**, 95.5; **11α**, 98.0; **12α**, 99.2 ppm. β-form: **10β**, 101.4; **11β**, 103.9; **12β**, 102.3 ppm). Isolated **10α** and **12α** were deprotected by catalytic hydrogenation in the usual manner to give **13** and **15**, respectively. On the other hand, **11α** was debenzylated by catalytic hydrogenation and then debenzoylated with NaOMe in MeOH to give disaccharide **14**. The structures of **13**, **14**, and **15** were confirmed by their ^{1}H- and ^{13}C-NMR spectra, and by FAB-MS analyses, and were in good agreement with the proposed structure. The ^{1}H-NMR spectra showed that disaccharides **13**, **14**, and **15** were present in the same anomeric ratio of α:β=2:3 in a D_2O solution by comparing the intensities of the H-1′ signals.

In conclusion, the stereoselective synthesis of aldobiouronic acid regioisomers was efficiently achieved by the trichloroacetimidate method for the glycosylation reaction.

We are now studying the substrate specificity of α-glucuronidase toward these regioisomers.

Experimental

General. Melting points are uncorrected. Optical rotation was measured with a JASCO DIP-140 polarimeter as a solution in $CHCl_3$ at 24°C, unless noted otherwise. All solvents were used after being purified in the usual manner. Column chromatography and flash chromatography were carried out in columns of silica gel (Merck, 240–400 mesh). TLC was conducted on silica gel 60 F_{254} plates (Merck), and the products were detected either by UV light or by charring with H_2SO_4. NMR spectra were recorded with a JEOL JNM-EX270 or Bruker AM-500 NMR spectrometer as a solution in $CDCl_3$ or D_2O. The values for δ_H and δ_C are expressed in ppm downfield from tetramethylsilane as an internal standard for solutions in $CDCl_3$, and for solutions in D_2O are expressed downfield from the signal for the 3-(trimethylsilyl)propionic-*2,2,3,3-d*$_4$ acid sodium salt. FAB-MS was performed in the positive-ion mode on a JEOL HX-100 mass spectrometer operated at an accelerating voltage of 10 kV. Samples were dissolved in aq. KCl solution and loaded onto a stainless-steel target with glycerol–thioglycerol as the matrix, argon being used as the bombarding gas.

Benzyl 4-O-allyl-β-D-xylopyranoside (**5**). To a stirred suspension of benzyl β-D-xylopyranoside (**3**)[13] (3.20 g, 13.3 mmol) in dry DMF (10 ml) containing 10% HCl in MeOH (60 μl), 2-methoxypropene (3.20 ml, 33.4 mmol) was slowly added dropwise. The mixture was stirred for 1 h at room temperature, before the reaction mixture was diluted with $CHCl_3$ (100 ml) and washed with water (100 ml). The aqueous layer was extracted twice with $CHCl_3$ (50 ml), and the combined $CHCl_3$ solution was made slightly acidic by adding 10% HCl in MeOH (10 μl). After 10 min, the solution was successively washed with aq. $NaHCO_3$, water and brine, dried ($MgSO_4$), and concentrated to dryness. To a stirred solution of the residue in DMF (50 ml), washed NaH (0.64 g, 27.8 mmol) was added portionwise at 0°C, and the mixture was stirred for 30 min at 0°C. To the mixture, allyl bromide (3.50 g, 27.1 mmol) was added dropwise, before stirring for 2 h at room temperature. When the reaction had been completed, the excess amount of NaH was decomposed by adding MeOH. After evaporating to remove the bulk solvent, the residue was dissolved in acetone (30 ml). To this mixture, 1 N aq. HCl (2.0 ml) was added, and the solution was boiled under reflux for 20 min. The resulting solution was neutralized with solid $NaHCO_3$, filtered through a Celite pad, and evaporated. The residue was crystallized from hexane–EtOH to give **5** (2.18 g, 58%), mp 54.5–55.5°C, $[\alpha]_D$ −104.5° (*c* 0.98, $CHCl_3$), R_f 0.17 (hexane–EtOAc 2:1). NMR ($CDCl_3$): δ_H 3.45–3.57 (3H, m, H-5ax., H-2 and H-4), 3.77 (1H, t, *J*=5.8 Hz, H-3), 4.04–4.15 (3H, m, H-5eq. and $OC\underline{H}_2CH=CH_2$), 4.62 (1H, d, *J*=4.6 Hz, H-1), 5.26 (2H, m, $OCH_2CH=C\underline{H}_2$), 5.91 (1H, m, $OCH_2C\underline{H}=CH_2$); δ_C 60.5 (C-5), 70.7 ($O\underline{C}H_2CH=CH_2$), 70.9 (C-2 and C-3), 76.5 (C-4), 101.2 (C-1), 118.1 ($OCH_2CH=\underline{C}H_2$), 134.3 ($OCH_2\underline{C}H=CH_2$). *Anal.* Found: C, 64.11; H, 7.11. Calcd. for $C_{15}H_{20}O_5$: C, 64.27; H, 7.15%.

Benzyl 4-O-allyl-2,3-di-O-benzyl-β-D-xylopyranoside (**6**). To a solution of **5** (2.00 g, 7.11 mmol) in dry DMF, washed NaH (0.50 g, 21.7 mmol) was added portionwise at 0°C. Benzyl bromide (3.60 g, 21.0 mmol) was added dropwise to the mixture, which was then stirred at room temperature for 20 h. After decomposing the excess amount of NaH by adding MeOH, most of the solvent was evaporated off, and the residue was partitioned with EtOAc-water. The EtOAc layer was washed with water and brine, dried ($MgSO_4$), filtered and concentrated. The residue was purified by chromatography (40 g of silica gel, hexane–EtOAc 10:1), and crystallized from hot hexane to give **6** (2.73 g, 84%), mp 61.0–61.2°C, $[\alpha]_D$ −12.6° (*c* 1.00, $CHCl_3$), R_f 0.37 (hexane–EtOAc 10:1). NMR ($CDCl_3$): δ_H 3.20 (1H, dd, *J*=11.5 and 9.9 Hz, H-5ax.), 3.42 (1H, dd, *J*=9.2 and 7.3 Hz, H-2), 3.47–3.59 (2H, m, H-3 and H-4), 4.02 (1H, dd, *J*=11.5 and 5.1 Hz, H-5eq.), 4.16 (2H, m, $OC\underline{H}_2CH=CH_2$), 4.47 (1H, d, *J*=7.3 Hz, H-1), 5.23 (2H, m, $OCH_2CH=C\underline{H}_2$), 5.9 (1H, m, $OCH_2C\underline{H}=CH_2$); δ_C 63.9 (C-5), 71.1 ($O\underline{C}H_2CH=CH_2$), 77.4 (C-4), 81.9 (C-2), 83.7 (C-3), 103.2 (C-1), 117.3 ($OCH_2CH=\underline{C}H_2$), 134.7 ($OCH_2\underline{C}H=CH_2$). *Anal.* Found: C, 75.89; H, 6.99. Calcd. for $C_{29}H_{32}O_5$: C, 75.63; H, 7.00%.

Benzyl 2,3-di-O-benzyl-β-D-xylopyranoside (**7**). A mixture of **6** (2.32 g, 5.34 mmol), tris(triphenylphosphine)rhodium(I) chloride (0.95 g, 1.03 mmol), and 1,4-diazabicyclo[2.2.2]octane (1.70 g, 15.2 mmol) in 10:3:1 EtOH–toluene–water (90 ml) was boiled for 9 h under reflux. The reaction mixture was concentrated to dryness, and the residue was extracted with $CHCl_3$ (100 ml). The extract was successively washed with cold dil. HCl, aq. $NaHCO_3$, water and brine, dried ($MgSO_4$) and concentrated. To a solution of the residue in 9:1 acetone–water (20 ml), 1 N aq. HCl (0.20 ml) was added, and the solution was boiled for 10 min under reflux. After neutralizing the resulting solution with solid $NaHCO_3$, the mixture was filtered and concentrated. A solution of the residue in $CHCl_3$ was washed with water and brine, dried and concentrated. The residue was subject to

column chromatography (50 g of silica gel, benzene–EtOAc 10 : 1) to give **7** (1.59 g, 71%), mp 125.5–126.0°C, $[\alpha]_D$ −62.9° (*c* 1.00, $CHCl_3$), R_f 0.24 (hexane–EtOAc 2 : 1). NMR ($CDCl_3$): δ_H 2.44 (1H, s, OH), 3.29 (1H, dd, J=8.6 and 11.6 Hz, H-5ax.), 3.42 (1H, t, J=7.7 Hz, H-3), 3.43 (1H, dd, J=6.2 and 7.7 Hz, H-2), 3.70 (1H, m, H-4), 4.04 (1H, dd, J=4.7 and 11.6 Hz, H-5eq.), 4.57 (1H, J=6.2 Hz, H-1); δ_C 64.4 (C-5), 69.1 (C-4), 80.1 (C-2), 82.0 (C-3), 102.7 (C-1). *Anal.* Found: C, 74.11; H, 6.74. Calcd. for $C_{26}H_{28}O_5$: C, 74.26; H, 6.71%.

*O-(Benzyl 2,3,4-tri-O-benzyl-β-*D*-glucopyanosyluronate) trichloroacetimidate* (**9**). To a solution of benzyl 2,3,4-tri-*O*-benzyl-D-glucopyranuronate (**8**;[10] 1.50 g, 2.70 mmol) in dry CH_2Cl_2 (15 ml), K_2CO_3 (1.50 g, 10.8 mmol) and trichloroacetonitrile (1.50 ml, 15.0 mmol) were added, and the mixture was stirred under N_2 at room temperature for 4 h. The resulting solution was filtered through a Celite pad, and the insoluble material was washed with CH_2Cl_2. The filtrate and washings were combined and concentrated, and the residual syrup was crystallized from hexane–EtOAc to give **9** (1.46 g, 70%), mp 96.2–97.6°C, $[\alpha]_D$ +5.66° (*c* 1.12, $CHCl_3$), R_f 0.56 (hexane–EtOAc 2 : 1). NMR ($CDCl_3$): δ_H 3.77 (2H, m, H-2 and H-4), 3.94 (1H, t, J=9.0 Hz, H-3), 4.14 (1H, d, J=9.7 Hz, H-5), 5.89 (1H, d, J=7.0 Hz, H-1), 8.73 (1H, s, NH); δ_C 75.0 (C-5), 78.8 (C-2), 80.4 (C-4), 83.6 (C-3), 90.7 (CCl$_3$), 98.0 (C-1), 160.9 (C(=NH)CCl$_3$), 166.1 (C=O). *Anal.* Found: C, 62.10; H, 5.15; N, 1.88. Calcd. for $C_{36}H_{34}O_7NCl_3$: C, 61.85; H, 4.90; N, 2.00%.

Glycosylation reaction. To a solution of glucuronosyl donor **9** (0.60 mmol) and xylose acceptor **1**, **2**, or **7** (0.50 mmol) in dry CH_2Cl_2 (5.0 ml), 0.4 M TMSOTf in dry CH_2Cl_2 (70 μl) was added under N_2 at −20°C. The solution was stirred at −20°C for 30 min. When the reaction had been completed, Et_3N was added to quench the catalyst, and the resulting solution was diluted with CH_2Cl_2, washed with water and brine, dried and concentrated. Subsequent flash chromatography of the residue gave each pure disaccharide derivative.

*Benzyl O-(benzyl 2,3,4-tri-O-benzyl-α-*D*-glucopyranosyluronate)-(1→2)-3,4-di-O-benzyl-β-*D*-xylopyranoside* (**10α**). The glycosylation product was purified by a flash chromatography (80 g of silica gel, benzene–EtOAc 20 : 1) to give compound **10α**. The formation of **10β** was confirmed by the ^{1}H-NMR spectrum of the crude glycosylated product. The α/β ratio of the disaccharide derivatives was estimated as 4 : 1 by comparing the NMR intensities of the newly formed anomeric signals. However, the isolation of **10β** failed, because complete separation of **10β** was difficult by flash chromatography.

Compound **10α**: 287.1 mg, 60%, $[\alpha]_D$ +3.61° (*c* 1.00, $CHCl_3$), R_f 0.45 (hexane–EtOAc 3 : 1). NMR ($CDCl_3$): δ_H 3.26 (1H, m, H-5ax.), 3.52 (1H, dd, J=3.5 and 9.8 Hz, H-2′), 3.66 (1H, dd, J=9.2 and 9.9 Hz, H-4′), 3.68 (2H, m, H-3 and H-4), 3.81 (1H, dd, J=7.5 and 9.3 Hz, H-2), 3.95 (1H, dd, J=4.5 and 11.4 Hz, H-5eq.), 3.96 (1H, t, J=9.4 Hz, H-3′), 4.61 (1H, d, J=7.5 Hz, H-1), 5.70 (1H, d, J=3.5 Hz, H-1′); δ_C 63.7 (C-5), 70.4 (C-2), 75.3 (C-4′), 78.5 (C-4), 79.0 (C-5′), 80.0 (C-2′), 80.9 (C-3), 81.3 (C-3′), 95.5 (C-1′), 103.4 (C-1), 170.2 (C-6′). *Anal.* Found: C, 74.67; H, 6.30. Calcd. for $C_{60}H_{60}O_{11}$: C, 74.59; H, 6.36%.

*Benzyl O-(benzyl 2,3,4-tri-O-benzyl-α- and β-*D*-glucopyranosyluronate)-(1→3)-2,4-di-O-benzyl-α-*D*-xylopyranoside* (**11α** and **11β**). The glycosylation products were purified by a flash chromatography (80 g of silica gel, benzene–EtOAc 20 : 1) to give compounds **11α** and **11β**.

Compound **11α**: 216.7 mg, 44%, $[\alpha]_D$ +26.8°C (*c* 1.07, $CHCl_3$), R_f 0.74 (benzene–EtOAc 15 : 1). NMR ($CDCl_3$): δ_H 3.37 (1H, dd, J=3.3 and 9.6 Hz, H-2′), 3.53 (1H, t, J=9.6 Hz, H-3′), 3.86 (2H, m, H-5ax. and H-4′), 4.03 (1H, dd, J=5.9 and 10.9 Hz, H-5eq.), 4.43 (1H, d, J=9.9 Hz, H-5′), 5.18 (1H, d, J=3.6 Hz, H-1), 5.23 (1H, d, J=3.3 Hz, H-1′), 5.27 (1H, dd, J=3.6 and 9.6 Hz, H-2), 5.48 (1H, m, H-4); δ_C 58.8 (C-5), 70.9 (C-3), 72.1 (C-4), 72.3 (C-2), 74.4 (C-4′), 78.4 (C-5′), 79.5 (C-2′), 80.5 (C-3′), 95.2 (C-1), 98.0 (C-1′), 165.1 and 165.6 (C=O), 169.6 (C-6′). *Anal.* Found: C, 72.94; H, 5.64. Calcd. for $C_{60}H_{56}O_{13}$: C, 73.16; H, 5.73%.

Compound **11β**: 113.3 mg, 23%, $[\alpha]_D$ +20.6°C (*c* 0.87, $CHCl_3$), R_f 0.66 (benzene–EtOAc 15 : 1). NMR ($CDCl_3$): δ_H 3.32–3.45 (2H, m, H-2′ and H-4′), 3.74 (1H, dd, J=8.9 and 9.6 Hz, H-3′), 3.80 (1H, t, J=10.9 and 10.6 Hz, H-5ax.), 3.94 (1H, d, J=9.9 Hz, H-5′), 4.10 (1H, dd, J=5.6 and 10.9 Hz, H-5eq.), 4.73 (1H, d, J=6.0 Hz, H-1′), 5.16 (1H, dd, J=4.0 and 9.9 Hz, H-2), 5.19 (1H, d, J=4.0 Hz, H-1); δ_C 59.1 (C-5), 70.3 (C-3), 74.0 (C-4), 74.6 (C-2), 74.7 (C-4′), 79.2 (C-5′), 81.6 (C-2′), 83.7 (C-3′), 95.1 (C-1), 103.9 (C-1′), 165.4 and 166.1 (C=O), 168.3 (C-6′). *Anal.* Found: C, 73.25; H, 5.79. Calcd. for $C_{60}H_{56}O_{13}$: C, 73.16; H, 5.73%.

*Benzyl O-(benzyl 2,3,4-tri-O-benzyl-α- and β-*D*-glucopyranosyluronate)-(1→4)-2,3-di-O-benzyl-β-*D*-xylopyranoside* (**12α** and **12β**). The glycosylation products were purified by a flash chromatography (80 g of silica gel, benzene–EtOAc 20 : 1) to give compounds **12α** and **12β**.

Compound **12α**: 253.6 mg, 53%, $[\alpha]_D$ +1.71° (*c* 0.53, $CHCl_3$), R_f 0.52 (benzene–EtOAc 15 : 1). NMR ($CDCl_3$): δ_H 3.30 (1H, dd, J=9.8 and 11.1 Hz, H-5ax.), 3.45 (1H, dd, J=9.0 and 7.6 Hz, H-2), 3.54 (1H, dd, J=9.6 and 3.6 Hz, H-2′), 3.60 (1H, t, J=9.0 Hz, H-3), 3.97 (1H, t, J=9.6 Hz, H-3′), 4.15 (1H, dd, J=5.5 and 11.1 Hz, H-5eq.), 4.23 (1H, d, J=9.9 Hz, H-5′), 4.44 (1H, d, J=7.6 Hz, H-1), 5.15 (1H, d, J=3.6 Hz, H-1′); δ_C 64.5 (C-5), 71.1 (C-4), 78.8 (C-4′), 78.9 (C-5′), 79.3 (C-3), 80.8 (C-2′), 81.7 (C-2), 82.8 (C-3′), 99.2 (C-1′), 102.9 (C-1), 169.1 (C-6′). *Anal.* Found: C, 74.31; H, 6.21. Calcd. for $C_{60}H_{60}O_{11}\cdot 0.5H_2O$: C, 74.59; H, 6.36%.

Compound **12β**: 28.7 mg, 5.9%, $[\alpha]_D$ −23.8° (*c* 0.82, $CHCl_3$), R_f 0.45 (benzene–EtOAc 15 : 1). NMR ($CDCl_3$): δ_H 3.21 (1H, m, H-4), 3.44 (1H, t, J=8.1 Hz, H-3), 3.44 (1H, t, J=8.6 Hz, H-2′), 3.61 (2H, m, H-2 and H-4′), 3.83–4.06 (3H, m, H-3′, H-5eq. and H-5ax.), 3.85 (1H, d, J=9.9 Hz, H-5′), 4.53 (1H, d, J=7.9 Hz, H-1), 4.86 (1H, d, J=8.6 Hz, H-1′); δ_C 62.9 (C-5), 74.6 (C-4), 77.3 (C-4′), 79.4 (C-5′), 81.2 (C-3), 81.8 (C-2′), 82.0 (C-2), 83.8 (C-3′), 102.3 (C-1′), 102.8 (C-1), 168.1 (C-6′). *Anal.* Found: C, 75.29; H, 6.31. Calcd. for $C_{60}H_{60}O_{11}$: C, 75.30; H, 6.32%.

*2-O-(α-*D*-Glucopyranosyluronic acid)-*D*-xylose* (**13**). A solution of **10α** (280.0 mg, 0.29 mmol) in acetic acid (5 ml) was hydrogenated in the presence of 10% Pd–C (50 mg) at atmospheric pressure and room temperature for 24 h. The insoluble material was collected on a Celite pad and washed with MeOH, before the combined filtrate and washings were evaporated. The residue was purified by anion-exchange chromatography on DEAE-Sephadex A-25 (30 × 180 mm, Pharmacia) with a linear gradient of an $NH_4\cdot HCO_3$ buffer (0.05→0.3 M). Fractions were assayed for total sugar concentration by the phenol–sulfuric acid method. The fractions containing **13** were combined and concentrated, and $NH_4\cdot HCO_3$ was removed by evaporating water from the residue several times to give **13** (77.3 mg, 81%), $[\alpha]_D$ +98.8° (*c* 0.77, H_2O) [lit.[15] +88–+98°]. NMR (D_2O, 500 MHz): δ_H 4.71 (0.4H, d, J=10.1 Hz, H-5′α), 4.61 (0.6H, d, J=10.2 Hz, H-5′β), 4.72 (0.6H, d, J=7.9 Hz, H-1β), 5.11 (0.4H, d, J=3.7 Hz, H-1α), 5.37 (0.4H, d, J=3.7 Hz, H-1′α), 5.38 (0.6H, d, J=3.6 Hz, H-1′β); δ_C (125 MHz): 63.5 (C-5α), 67.7 (C-5β), 79.3 (C-5′α), 81.5 (C-5′β), 92.4 (C-1α), 99.5 (C-1′α), 99.6 (C-1′α), 100.6 (C-1β). FAB-MS *m/z*: 365, ([M+K]$^+$).

*3-O-(α-*D*-Glucopyranosyluronic acid)-*D*-xylose* (**14**). A solution of **11α** (210.0 mg, 0.21 mmol) in acetic acid (4 ml) was hydrogenated in the presence of 10% Pd–C (50 mg) at atmospheric pressure and room temperature for 24 h. After removing the catalyst by filtration, the filtrate was concentrated. The residue was dissolved in MeOH (5 ml), before 0.1 N NaOMe in MeOH (0.4 ml) was added to the solution. After the reaction had been completed, the reaction mixture was neutralized with solid CO_2, and the solution was concentrated. The residue was purified by anion-exchange chromatography, as described for **13**, to give **14** (54.9 mg, 80%), $[\alpha]_D$ +19.5° (*c* 0.50, H_2O). [lit.[2] +18.5° (*c* 3.28, H_2O)]. NMR (D_2O, 500 MHz): δ_H 4.50 (0.4H, d, J=10.1 Hz, H-5′α), 4.55 (0.6H, d, J=10.2 Hz, H-5′β), 4.57 (0.6H, d, J=8.0 Hz, H-1β), 5.18 (0.4H, d, J=3.6 Hz, H-1α), 5.38 (0.4H, d, J=4.4 Hz, H-1′α), 5.39 (0.6H, d, J=4.0 Hz, H-1′β); δ_C (125 MHz): 63.7 (C-5α), 67.7 (C-5β), 82.1 (C-5′α), 84.4 (C-5′β), 95.1 (C-1α), 99.4 (C-1β), 101.5 (C-1′β), 101.7 (C-1′α). FAB-MS *m/z*: 365, ([M+K]$^+$).

*4-O-(α-*D*-Glucopyranosyluronic acid)-*D*-xylose* (**15**). Debenzylation of **12α** (250.0 mg, 0.26 mmol), in the same manner as that described for **13**, with 10% Pd–C (50 mg) in acetic acid (5 ml) at room temperature for 24 h, and anion-exchange chromatography of the crude product on DEAE-Sephadex A-25, as also described for **13**, gave **15** (72.4 mg, 85%), $[\alpha]_D$ +86.7° (*c* 0.54, H_2O). NMR (D_2O, 500 MHz): δ_H 4.57 (0.6H, d, J=7.9 Hz, H-1β), 5.18 (0.4H, d, J=3.7 Hz, H-1′α), 5.20 (0.6H, d, J=3.7 Hz, H-1′β); δ_C (125 MHz): 62.6 (C-5α), 66.8 (C-5β), 81.0 (C-5′α), 81.1 (C-5′β), 94.6 (C-1α), 99.1 (C-1β), 102.7 (C-1′α), 102.7 (C-1′β). FAB-MS *m/z*: 365, ([M+K]$^+$).

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