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Chemical transformation of lactose into 4-*O*-β-D-galactopyranosyl-D-glucuronic acid (pseudolactobiouronic acid) and some derivatives thereof☆

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Abstract

The selective oxidation of the primary alcoholic function of the reducing unit of lactose was achieved in good overall yield (67%) starting from 2′,6′-di-*O*-benzyl-2,3:3′,4′-di-*O*-isopropylidenelactose dimethyl acetal (**1**) through a simple multi-step procedure based on the selective acetylation of OH-5 of **1** (methoxyisopropylation, acetylation, de-methoxyisopropylation) followed by a two-step oxidation at C-6 (TPAP-NMO then TEMPO-NaOCl) and finally, complete removal of the protecting groups.

Keywords: Lactose; Pseudoaldobiouronic acids; Pseudolactobiouronic acid; Oxidations

1. Introduction

Uronic acids are an important class of monosaccharide constituents of various types of animal, bacterial, and plant polysaccharides.[2] Two different types of disaccharides could be formed from an uronic acid and a neutral aldose. Those containing the neutral aldose unit at the reducing end (aldobiouronic acids) are by far the best known ones, because they are obtained as the main products during the acid hydrolysis of natural polysaccharides, owing to the higher stability of the uronic glycosidic bond under these hydrolytic conditions.[2] The other type of disaccharides, having a reducing uronate unit (pseudoaldobiouronic acids), is generally obtained by synthetic procedures, as for instance, 4-*O*-β-D-galactopyranosyl-D-glucuronic acid (**17**, pseudolactobiouronic acid), prepared in low yield from lactose by Chiba et al.[3] or its 3-*O*-β-D-galactopyranosyl isomer, obtained by enzymatic means, and proposed as a food additive[4] with equilibrating properties on the intestinal microflora.[5] We present here, a new and more efficient preparation of **17** and some of its derivatives starting from the diol **1**, easily obtained from lactose through a simple and short procedure recently described by us.[6]

2. Results and discussion

The selective protection of the hydroxyl group at C-5 of **1**, in order to realize a selective oxidation at C-6, was performed using a mixed 1-methoxy-1-methylethyl (MIP) acetal as an easily removable temporary protecting group of the primary alcoholic function. The methoxyisopropylation reaction of **1** with pyridinium tosylate (10% mol) as a catalyst and an excess (1.6 equiv) of 2-methoxypropene in dichloromethane at 0 °C gave, in high yield ($\cong$90%), a 4:1 mixture of the two isomeric mixed acetals **2** and **3**. This reaction, however, proved to be not completely reproducible, variable amounts of the known[6] 5,6-*O*-isopropylidene acetal (**15**) being obtained in some runs, evidently because of a subsequent intramolecular transacetalation of either **2** or **3**.

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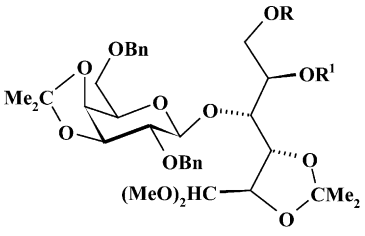
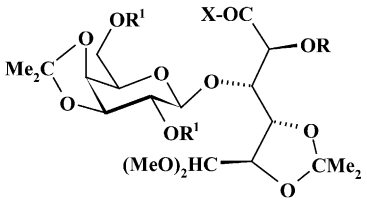


1: R = R^1 = H
2: R = MIP, R^1 = H
3: R = H, R^1 = MIP
4: R = MIP, R^1 = Ac
5: R = Ac, R^1 = MIP
6: R = H, R^1 = Ac
7: R = Ac, R^1 = H

MIP = -C(Me)$_2$OMe

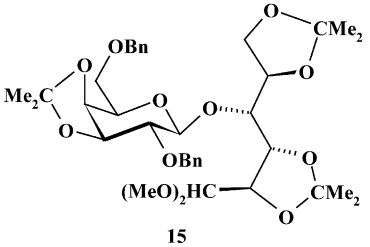


8: X = H, R = Ac, R^1 = Bn
9: X = OH, R = Ac, R^1 = Bn
10: X = OH, R = H, R^1 = Bn
11: X = OH, R = R^1 = H
12: X = OMe, R = Ac, R^1 = Bn
13: X = OMe, R = H, R^1 = Bn
14: X = OMe, R = R^1 = H

This side-reaction was completely avoided with the weaker acid-catalyst, triethylammonium chloride (50% mol) and a small excess (1.2 equiv) of 2-methoxypropene in dichloromethane at room temperature. Under these conditions, we obtained with high yield (93%) and in a completely reproducible manner the isomeric mixed acetal mixture (**2** + **3**) in the same (4:1) ratio.

The routine acetylation with acetic anhydride in pyridine of this mixture led, with about quantitative yield, to a mixture of compounds **4** and **5** that were de-*O*-methoxyisopropylated to the acetates **6** and **7**, by treatment with pyridinium tosylate in methanol. Whereas we were not able to find satisfactory conditions for the separation of compounds **4** and **5** over silica gel, the latter two acetates have different R_f values. Their chromatographic separation, however, caused an extensive isomerization of **6** to **7**, evidently arising from a silica gel promoted migration of the acetyl group from a secondary position to the primary one. For this reason, the crude mixture containing **6** + **7** in 4:1 ratio was directly used for the subsequent oxidation step. Initial attempts to perform the direct oxidation of **6** to **9** were made through tetramethylpyrrolidine-*N*-oxide (TEMPO) mediated reactions (TEMPO/NaOCl/Bu_4NBr[7] or TEMPO/PhI(OAc)$_2$[8]), but in both cases, an about quantitative formation of the 5-ulose derivative **16** was observed. Migration of the acetyl group to the primary position is, evidently, faster than oxidation of the primary alcoholic function, also under the mild conditions employing hypervalent iodine as the co-oxidant.

Compound **16** is an interesting representative of the very little explored class of glycosylated 1,5-dicarbonyl hexoses that we recently used for the stereoselective synthesis of 4-*O*-β-D-galactosylated 1-deoxynojirimycin derivatives.[9] The acetyl migration was, however, completely avoided using a two-step oxidation procedure through the intermediate formation of the aldehyde **8**. The first step was achieved with tetrapropylammonium perruthenate (TPAP) and 4-methylmorpholine-*N*-oxide (NMO) giving a 4:1 mixture of **8** and **16**, which was directly transformed with TEMPO and NaOCl–Bu_4NBr into a mixture of **16** and the expected uronic acid derivative **9**. An easy chromatographic separation of the above mixture gave pure **9** in high yield (70%) from **1**, through an overall procedure that, although rather long (five steps), requires only one chromatographic purification. The acid **9** was further quantitatively transformed into its methyl ester **12** by treatment with an ethereal solution of CH_2N_2. Both **9** and **12** were finally subjected to a standard sequence of deprotection procedures. Their 5-*O*-deacetylation under Zemplen conditions gave, in almost quantitative yield, crystalline **10** and **13**, respectively, that in turn, were debenzylated by catalytic hydrogenolysis with $Pd(OH)_2$ on charcoal to afford, in quantitative yields, the crystalline triols **11** and **14**. Final treatment of **11** and **14** with 90% aqueous trifluoroacetic acid allowed the removal of the two isopropylidene protecting groups, the exposition of the aldehyde function, and the concomitant formation of the six-membered ring at the reducing uronate moiety.



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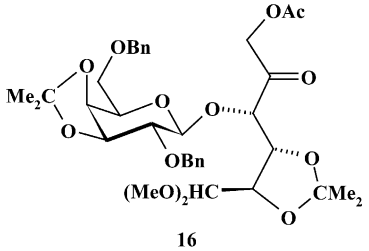

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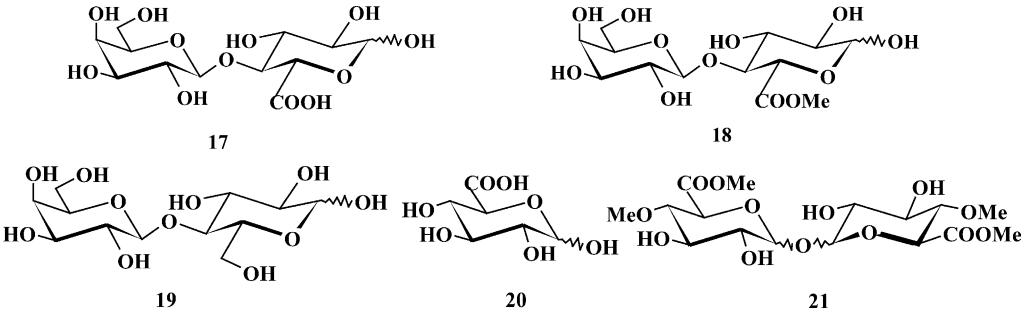

carbon signals of the D-glucuronic moiety are different for the two anomers α- and β-**17**, with a general deshielding for the β form, mainly for C-2 ($\Delta\delta$ 4.2), C-1 ($\Delta\delta$ 4.0), and C-3 and C-5 ($\Delta\delta$ 2.3). We have not found in literature any reference data for an anomeric couple of 4-*O*-substituted D-glucopyranosyluronic acid; however the ^{13}C NMR data reported for the α and β pyranose forms of D-glucuronic acid (**20**)[11] and the α and β pyranosides of methyl 4-*O*-methyl-D-glucuronate (**21**)[12†] show a similar trend (Table 1), thus suggesting the correctness of our assignments.

The previously unreported methyl ester **18** was obtained as a colorless syrup, consisting (^{13}C NMR, D_2O) of an about 1:1 mixture of anomers, showing carbon signal patterns very close to those of **17** (Table 1).

In conclusion, we have proposed here, a new efficient synthetic access to pseudolactobiouronic acid (**17**). Although this method requires a rather long sequence of reactions, the simplicity of the methodologies, the high yield of the overall sequence (67%), and the easy availability of the starting material (**1**) appear very attractive in view of the potential use of pseudolactobiouronic acid (**17**) as a food additive.

3. Experimental

General methods.—Compound **1** was prepared according to the published procedure.[6] General methods are those reported in a previous paper of this series.[13] ^{13}C NMR assignments were made, whenever possible, through DEPT experiments, by comparison with previously published data of analogous compounds,[6,13] and in the case of mixtures **2** + **3**, **4** + **5**, and **6** + **7**, on the basis of their relative signal intensities.

5-O-Acetyl-4-O-(2,6-di-O-benzyl-3,4-O-isopropylidene-β-D-galactopyranosyl)-2,3-O-isopropylidene-aldehydo-D-glucuronic acid dimethyl acetal (**9**) *and 6-O-acetyl-4-O-(2,6-di-O-benzyl-3,4-O-isopropylidene-β-D-galactopyranosyl)-2,3-O-isopropylidene-aldehydo-D-xylo-aldohexos-5-ulose dimethyl acetal* (**16**).

Methoxyisopropylation of **1**:. A solution of **1** (4.20 g, 6.50 mmol) and triethylammonium chloride (464 mg, 3.5 mmol) in dry CH_2Cl_2 (90 mL) was stirred under an Ar atmosphere for 10 min at rt. The solution was cooled at 0 °C and a 1:10 (v/v) solution of freshly distilled 2-methoxypropene in dry CH_2Cl_2 (9 mL, 8.10 mmol) was slowly added. After 1 h and 15 min stirring

Table 1
Selected ^{13}C NMR signals in D_2O (chemical shifts, δ) of derivatives **17**–**21**

	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′	C-1	C-2	C-3	C-4	C-5	C-6	COO*Me*
α-**17**	103.4	71.6	73.4	69.3	76.2	61.8	92.9	70.0	71.9	80.0 [a]	71.9	171.2	
β-**17**	103.4	71.6	73.4	69.3	76.2	61.8	96.9	74.2	74.2	80.2 [a]	74.8	173.3	
α-**18**	103.7	71.4	73.2	69.3	76.1	61.8	93.0	70.0	71.4	80.4 [a]	71.9	171.2	54.0
β-**18**	103.7	71.4	73.2	69.3	76.1	61.8	97.0	74.1	74.2	80.6 [a]	74.7	172.1	54.0
α-**19**	103.0	71.1	72.6	68.6	75.4	61.1	91.9	70.2	71.2	78.4	71.5	60.2	
β-**19**	103.0	71.1	72.6	68.6	75.4	61.1	95.8	73.9	74.5	78.4	74.9	60.2	
α-**20**							93.7	73.1	74.1	73.5	72.8	177.7	
β-**20**							97.4	75.5	77.4	73.3	77.0	176.9	
α,α-**21**							95.6	71.5	72.9	82.4	70.9	172.6	54.5
β,β-**21**							100.0	74.2	75.7	82.1	73.3	171.8	54.4

[a] Assignments may have to be interchanged.

† We thank a referee for having attracted our attention to this helpful reference.

at rt the mixture was neutralized with an excess of satd $NaHCO_3$ solution (20 mL), stirred for 10 min, extracted with CH_2Cl_2 (4 × 50 mL), and the organic layer dried over dry $MgSO_4$. The solution was filtered and concentrated under diminished pressure to give a syrupy residue (5.20 g) showing a single spot in TLC analysis (R_f 0.54, 2:3 hexane–EtOAc). A flash chromatography (3:2 hexane–EtOAc + 0.1% Et_3N) gave 4.35 g (93% yield) of the mixed acetals **2** and **3** in a ratio of about 4:1 roughly estimated on the basis of the relative ^{13}C NMR signal intensities. Selected ^1H NMR signals (C_6D_6): δ 3.08, 3.18, and 3.25 (**2**; 3 s, 3 H each, 3 OMe), 3.13, 3.14, and 3.22 (**3**; 3 s, 3 H each, 3 OMe). Selected ^{13}C NMR signals (C_6D_6): δ 24.7 and 24.8 (**2**; MIP C*Me*$_2$), 25.3 and 25.4 (**3**; MIP C*Me*$_2$), 48.6 (**2**; MIP OMe), 49.4 (**3**; MIP OMe), 62.3 (**3**; C-6), 62.7 (**2**; C-6), 69.6 (**2**; C-6′), 69.9 (**3**; C-6′), 72.6 (**2**; C-5 and C-5′), 73.0 (**3**; C-5, and C-5′), 74.3 (**2**; C-4′), 74.5 (**3**; C-4′), 75.7, 76.3, and 78.7 (**3**; C-2, C-3, and C-4), 76.5, 77.7, and 78.2 (**2**; C-2, C-3, and C-4), 79.8 (**2** and **3**; C-3′), 80.7 (**2**; C-2′), 81.2 (**3**; C-2′), 100.3 (**2**; MIP *C*Me$_2$), 101.6 (**3**; MIP *C*Me$_2$), 102.1 (**3**; C-1′), 102.9 (**2**; C-1′), 106.1 (**2** and **3**; C-1).

Acetylation of **2 + 3** *mixtures:* To a solution of the **2 + 3** mixture obtained as reported above (1.79 g, 2.48 mmol) in dry pyridine (10 mL), neat Ac_2O (5.0 mL) was added dropwise. The solution was stirred at rt and left to react for 15 h, the mixture was concentrated under diminished pressure, the residue coevaporated with toluene (5 × 20 mL) to give a crude product showing a single spot in TLC analysis (R_f 0.76, 2:3 hexane–EtOAc). The crude product was chromatographed on silica gel (2:3 hexane–EtOAc + 0.1% Et_3N) to give 1.86 g (98% yield) of a mixture of compounds **4** and **5**, in an approximate ratio of 4:1. Selected ^{13}C NMR signals (C_6D_6): δ 20.8 (**4**; *Me*CO), 21.4 (**5**; *Me*CO), 24.5 and 24.8 (**4**; MIP C*Me*$_2$), 25.4 and 25.8 (**5**; MIP C*Me*$_2$), 48.2 (**4**; MIP OMe), 49.5 (**5**; MIP OMe), 53.5 and 55.3 (**5**; 2 × OMe), 53.6 and 55.7 (**4**; 2 × OMe), 59.3 (**4**; C-6), 65.4 (**5**; C-6), 69.5 (**4**; C-6′), 70.0 (**5**; C-6′), 73.0 (**4**; C-5, and C-5′), 73.2 (**5**; C-5, and C-5′), 74.1 (**4**; C-4′), 74.4 (**5**; C-4′), 75.5, 76.6, and 78.2 (**4**; C-2, C-3, and C-4), 75.0, 75.0, and 76.2 (**5**; C-2, C-3, and C-4), 78.9 and 79.8 (**4** and **5**; C-3′), 80.5 (**4**; C-2′), 80.7 (**5**; C-2′), 100.1 (**4**; MIP *C*Me$_2$), 101.4 (**5**; MIP *C*Me$_2$), 101.7 (**5**; C-1′), 102.4 (**4**; C-1′), 105.9 (**4** and **5**; C-1).

Demethoxyisopropylation of **4 + 5** *mixtures:* A mixture of **4 + 5** (1.86 g, 2.44 mmol), obtained as reported above, was dissolved in MeOH (20 mL) and treated at rt with pyridinium tosylate (16 mg, 0.06 mmol). The mixture was stirred at rt for 30 min until the starting material had disappeared (TLC analysis, 3:2 hexane–EtOAc). The solution was concentrated under diminished pressure, diluted with CH_2Cl_2 (50 mL), and neutralized by addition of satd aq $NaHCO_3$ (20 mL), and extracted with CH_2Cl_2 (4 × 30 mL). The organic phase was dried, filtered, and concentrated under reduce pressure to give a residue (1.60 g, 95% yield) constituted exclusively by **6** and **7**, showing two spots in TLC analysis (R_f 0.40 and 0.33, 9:1 CH_2Cl_2–Me_2CO) in a 77:23 ratio roughly estimated from the relative intensity of the peaks for the analogous carbons. Selected ^{13}C NMR signals ($CDCl_3$): δ 21.4 (**7**; *Me*CO), 21.6 (**6**; *Me*CO), 54.0 and 56.1 (**7**; 2 × OMe), 54.1 and 56.2 (**6**; 2 × OMe), 60.7 (**6**; C-6), 66.3 (**7**; C-6), 69.4 (**6** and **7**; C-6′), 72.0 (**7**; C-5), 72.7 (**6** and **7**; C-5′), 74.2 and 75.2 (**6**; C-5, and C-4′), 74.2 (**7**; C-4′), 76.0, 76.5, and 78.3 (**6**; C-2, C-3, and C-4), 77.0, 77.6, and 77.6 (**7**; C-2, C-3, and C-4), 78.8 (**7**; C-3′), 79.6 (**6**; C-3′), 80.1 (**6**; C-2′), 80.3 (**7**; C-2′), 102.0 (**6**; C-1′), 103.2 (**7**; C-1′), 105.4 (**7**; C-1), 105.6 (**6**; C-1). Anal. Calcd for $C_{36}H_{50}O_{13}$: C, 62.54; H, 7.26. Found: C, 62.50; H, 7.43.

Two-step oxidation of **6 + 7** *mixtures:* To a solution of a **6 + 7** mixture (1.20 g, 1.74 mmol) in dry CH_2Cl_2 (35 mL), activated crushed 4 Å molecular sieves (1.20 g) and NMO (380 mg, 2.85 mmol) were added. After 30 min stirring at rt under Ar, TPAP (73 mg, 0.21 mmol) was added and the mixture was stirred for 2 h and then filtered over two alternate paths of Celite and silica gel, extensively washed first with CH_2Cl_2 and then with EtOAc. The combined organic solutions were concentrated under diminished pressure to give a crude syrup (1.20 g) containing of a mixture of **8** and **16**, in an ≅4:1 ratio, estimated on the basis of relative ^{13}C NMR signal intensities. Selected ^1H NMR signals of **8** ($CDCl_3$): δ 1.30 (s, 6 H, C*Me*$_2$), 1.42 and 1.43 (2 s, 3 H each, C*Me*$_2$), 2.10 (s, 3 H, *Me*CO), 3.37 (s, 6 H, 2 × OMe), 5.46 (d, 1 H, $J_{4,5}$ 3.4 Hz, H-5), 9.83 (s, 1 H, CHO). Selected ^{13}C NMR signals of **8** ($CDCl_3$): δ 20.3 (*Me*CO), 25.9, 26.5, 27.1, and 27.3 (2 × C*Me*$_2$), 53.6 and 55.5 (2 × OMe), 68.8 (C-6′), 71.9 (C-5′), 72.9 and 73.2 (2 × benzylic CH_2), 73.22, 75.3, and 75.5 (C-4, C-5, and C-4′), 77.8, 78.3, 78.9, and 79.7 (C-2, C-3, C-2′, and C-3′), 101.5 (C-1′), 104.7 (C-1), 195.8 (C-6).

The crude product (1.20 g) was dissolved in CH_2Cl_2 (10 mL), TEMPO (43 mg, 0.27 mmol) was added and treated with satd aq $NaHCO_3$ (2.0 mL) containing KBr (14 mg) and Bu_4NCl (32 mg). To the cooled (0 °C) mixture, was slowly added, under vigorous stirring during 45 min, a mixture of commercial aq NaClO solution (3 M, 2.4 mL), satd aq NaCl (2.4 mL) and satd aq $NaHCO_3$ (1.2 mL). The mixture was warmed slowly to rt and stirred until the starting material disappeared (TLC analysis, 1:1 hexane–EtOAc). Dichloromethane (50 mL) was added and the separated organic phase washed with water (20 mL), dried ($MgSO_4$), filtered and concentrated under diminished pressure to give a residue (1.10 g). Flash chromatography of the crude product eluting first with 1:1 hexane–EtOAc, then with 10:1 EtOAc–MeOH, gave pure samples of **16** (218 mg, 18% yield) and **9** (998 mg, 80% yield).

Compound **16**: syrup, R_f 0.41 (1:1 hexane–EtOAc); $[\alpha]_D$ −37.2° (*c* 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.32, 1.35, 1.38, and 1.42 (4 s, 3 H each, 2 × C*Me*$_2$), 2.10 (s, 3 H, *Me*CO), 3.31 and 3.35 (2 s, 3 H each, 2 × OMe); 3.46 (m, 1 H, H-2′), 3.65–3.70 (m, 2 H, H-6′a, and H-6′b), 3.85 (m, 1 H, H-5′), 4.13–4.39 (m, 7 H, H-3, H-3′, H-4, H-4′, H-2, H-1, and H-1′), 4.49 and 4.58 (AB system, 2 H, $J_{A,B}$ 12.1 Hz, benzylic CH_2), 4.80 and 4.88 (AB system, 2 H, $J_{A,B}$ 11.9 Hz, benzylic CH_2), 4.80 and 5.40 (AB system, 2 H, $J_{A,B}$ 18.3 Hz, H-6a, and H-6b), 7.24–7.41 (m, 10 H, aromatic H). ^{13}C NMR ($CDCl_3$): δ 20.2 (*Me*CO), 26.0, 26.5, 27.0, and 27.5 (2 × C*Me*$_2$), 53.4 and 55.3 (2 × OMe), 68.1 and 68.7 (C-6, and C-6′), 72.1 (C-5′), 72.8 and 73.2 (2 × benzylic CH_2), 73.4 and 74.9 (C-4, and C-4′), 78.4, 78.8, and 79.0 (C-3, C-2, and C-3′), 80.8 (C-2′), 101.1 (C-1′), 104.8 (C-1), 109.7 and 110.5 (2 × *C*Me$_2$), 127.4–128.1 (aromatic CH), 137.6 and 137.8 (aromatic C), 169.9 (Me*C*O), 204.1 (C-5). Anal. Calcd for $C_{36}H_{48}O_{13}$: C, 62.78; H, 7.02. Found: C, 62.60; H, 7.10.

Compound **9**: white solid foam, R_f 0.25 (7:3 EtOAc–MeOH); mp 86–88 °C; $[\alpha]_D$ +4.0° (*c* 1.0, $CHCl_3$); ^1H NMR (CD_3OD): δ 1.34, 1.40, 1.49, and 1.54 (4 s, 3 H each, 2 × C*Me*$_2$), 2.11 (s, 3 H, *Me*CO), 3.42 (s, 6 H, 2 × OMe); 3.56–4.80 (m, 11 H, pyranose H), 4.86–5.02 (m, 4 H, 2 × benzylic CH_2), 5.35 (d, 1 H, $J_{4,5}$ 4.4 Hz, H-5), 7.37–7.43 (m, 10 H, aromatic H). ^{13}C NMR (CD_3OD): δ 21.1 (*Me*CO), 26.6, 27.4, 27.7, and 28.1 (2 × C*Me*$_2$), 54.4 and 56.2 (2 × OMe), 70.1 (C-6′), 73.0 (C-5′), 74.3 and 74.3 (2 × benzylic CH_2), 75.3 (C-4′), 76.0 and 76.0 (C-4, and C-5), 77.3, 78.3, and 79.9 (C-2, C-3, and C-3′), 80.5 (C-2′), 102.1 (C-1′), 106.5 (C-1), 110.7 and 111.5 (2 *C*Me$_2$), 128.4–129.3 (aromatic CH), 139.7 and 139.7 (aromatic C), 172.6 (Me*C*O), 176.2 (C-6). Anal. Calcd for $C_{36}H_{48}O_{14}$: C, 61.35; H, 6.86. Found: C, 61.05; H, 6.99.

*Methyl 5-*O*-acetyl-4-*O*-(2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-β-*D*-galactopyranosyl)-2,3-*O*-isopropylidene-aldehydo-*D*-glucuronate dimethyl acetal (**12**).*—A solution of **9** (800 mg, 1.13 mmol) in EtOAc (30 mL) was treated with an ethereal solution of CH_2N_2, until the yellow color persisted. Excess CH_2N_2 was destroyed by dropwise addition of glacial AcOH, and then the slightly acid solution was immediately washed with satd aq $NaHCO_3$, and extracted with EtOAc (3 × 30 mL). The organic phase was dried ($MgSO_4$), filtered, and concentrated under diminished pressure to give **12** (826 mg) as a crude product. After a chromatography on silica gel eluting with 1:1 hexane–EtOAc, a pure sample of **12** (770 mg, 95% yield) was obtained as a syrup, R_f 0.43 (1:1 hexane–EtOAc); $[\alpha]_D$ +9.6° (*c* 1.8, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.28 and 1.32 (2 s, 3 H each, C*Me*$_2$), 1.42 (s, 6 H, C*Me*$_2$), 2.11 (s, 3 H, *Me*CO), 3.34 (m, 1 H, H-2′), 3.34 and 3.35 (2 s, 3 H each, 2 × OMe); 3.62 (s, 3 H, COOMe), 3.66–3.70 (m, 2 H, H-6′a, and H-6′b), 4.11 (m, 1 H, H-5′), 4.19–4.36 (m, 5 H, H-2, H-3, H-4, H-3′, and H-4′), 4.28 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1′), 4.50 (d, 1 H, $J_{1,2}$ 7.0 Hz, H-1), 4.55 (s, 2 H, benzylic CH_2), 4.68 and 4.85 (AB system, 2 H, $J_{A,B}$ 12.0 Hz, benzylic CH_2), 5.22 (d, 1 H, $J_{4,5}$ 7.8 Hz, H-5), 7.26–7.42 (m, 10 H, aromatic H). ^{13}C NMR ($CDCl_3$): δ 20.5 (*Me*CO), 26.2, 26.7, 27.4, and 27.6 (2 × C*Me*$_2$), 52.6 (COO*Me*), 53.4 and 55.8 (2 × OMe), 68.6 (C-6′), 71.9 (C-5′), 73.4 (2 × benzylic CH_2), 71.9, 74.4, 75.2, 76.6, and 76.6 (C-2, C-3, C-4, C-5, and C-4′), 78.7 and 79.3 (C-2′ and C-3′), 102.0 (C-1′), 105.0 (C-1), 109.7 and 110.3 (2 *C*Me$_2$), 127.4–128.3 (aromatic CH), 138.0 and 138.3 (aromatic C), 169.7 and 169.9 (Me*C*O and C-6). Anal. Calcd for $C_{37}H_{50}O_{14}$: C, 61.83; H, 7.01. Found: C, 61.68; H, 7.16.

*4-*O*-(2,6-Di-*O*-benzyl-3,4-*O*-isopropylidene-β-*D*-galactopyranosyl)-2,3-*O*-isopropylidene-aldehydo-*D*-glucuronic acid dimethyl acetal* (**10**).*—*A solution of **9** (800 mg, 1.13 mmol) in MeOH (7 mL) was treated with 1 M methanolic NaOMe (1.5 mL) and stirred at rt for 1 h. The solution was neutralized with solid CO_2 and concentrated under diminished pressure. The residue was poured into water (5 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The organic extracts were dried, filtered, and concentrated to give pure (^{13}C NMR) **10** (750 mg, quantitative) as a white amorphous solid; R_f 0.21 (7:3 EtOAc–MeOH); mp 65–67 °C; $[\alpha]_D$ −3.9° (*c* 1.0, $CHCl_3$); ^1H NMR (CD_3OD): δ 1.27 and 1.30 (2 s, 3 H each, C*Me*$_2$), 1.42 (s, 6 H, C*Me*$_2$), 3.28 and 3.29 (2 s, 3 H each, 2 × OMe); 3.16–3.90 (m, 18 H), 7.22–7.43 (m, 10 H, aromatic H). ^{13}C NMR (CD_3OD): δ 26.5, 27.1, 27.8, and 28.2 (2 × C*Me*$_2$), 54.2 and 56.4 (2 × OMe), 70.0 (C-6′), 73.0 and 74.0 (C-4′ and C-5′), 74.3 (2 × benzylic CH_2), 75.2, 77.1, 77.3, and 78.7 (C-2, C-3, C-4, and C-5), 80.1 and 80.4 (C-2′ and C-3′), 101.5 (C-1′), 106.2 (C-1), 110.8 and 111.8 (2 × *C*Me$_2$), 128.6–129.5 (aromatic CH), 139.2 and 139.5 (aromatic C), 177.9 (C-6). Anal. Calcd for $C_{34}H_{46}O_{13}$: C, 61.62; H, 7.00. Found: C, 61.87; H, 7.13.

*Methyl 4-*O*-(2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-β-*D*-galactopyranosyl)-2,3-*O*-isopropylidene-aldehydo-*D*-glucuronate dimethyl acetal* (**13**).*—*A solution of **12** (800 mg, 1.11 mmol) was treated as described above for the preparation of **10**, giving in quantitative yield **13** (756 mg) as a crystalline white solid, R_f 0.33 (1:1 hexane–EtOAc); mp 117 °C (from Et_2O–hexane); $[\alpha]_D$ +10.1° (*c* 0.9, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.32, 1.35, 1.40, and 1.41 (4 s, 3 H each, 2 × C*Me*$_2$), 3.33 and 3.34 (2 s, 3 H each, 2 × OMe), 3.40 (m, 2 H, H-2′, and H-5), 3.70 (s, 3 H, COO*Me*), 3.67–3.91 (m, 3 H, H-5′, H-6′a, and H-6′b), 4.07–4.17 and 4.43–4.50 (2 m, 5 H, H-2, H-3, H-4, H-3′, and H-4′), 4.31 (d, 1 H, $J_{1,2}$ 6.0 Hz, H-1), 4.46 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1′), 4.54 and 4.56 (AB system, 2 H, $J_{A,B}$ 12.1 Hz, benzylic CH_2), 4.75 and 4.90 (AB system, 2 H, $J_{A,B}$ 11.5 Hz, benzylic CH_2), 7.23–7.46 (m, 10 H, aromatic H). ^{13}C NMR ($CDCl_3$): δ 26.3, 26.5, 27.3, and 27.8 (2 × C*Me*$_2$), 52.2 (COO*Me*), 53.4

and 55.6 (2 × OMe), 69.0 (C-6′), 72.1, 72.2, 73.5, and 75.3 (C-4, C-5, C-4′, and C-5′), 73.5 (2 × benzylic CH_2), 77.0 and 77.1 (C-2, and C-3), 79.0 and 79.5 (C-2′, and C-3′), 102.2 (C-1′), 105.0 (C-1), 109.8 and 110.7 (2 × CMe_2), 127.6–128.5 (aromatic CH), 137.9 and 138.0 (aromatic C), 172.7 (C-6). Anal. Calcd for $C_{35}H_{48}O_{13}$: C, 62.12; H, 7.15. Found: C, 62.38; H, 7.21.

4-O-(3,4-O-Isopropylidene-β-D-galactopyranosyl)-2,3-O-isopropylidene-aldehydo-D-glucuronic acid dimethyl acetal (**11**).—A solution of **10** (740 mg, 1.12 mmol) in MeOH (10 mL) containing 5% $Pd(OH)_2$ on charcoal (40 mg) was stirred at rt under an H_2 atmosphere for 24 h. The solution was diluted with MeOH (30 mL), filtered over Celite, concentrated under diminished pressure to give pure (^{13}C NMR) **11** as a white solid (526 mg, 97% yield), R_f 0.31 (2:3 EtOAc–MeOH); mp 163–164 °C (from EtOAc); $[\alpha]_D$ +3.3° (*c* 1.0, $CHCl_3$); ^{1}H NMR (CD_3OD): δ 1.33 and 1.47 (2 s, 3 H each, C*Me*$_2$), 1.42 (s, 6 H, C*Me*$_2$), 3.44 and 3.45 (2 s, 3 H each, 2 × OMe), 3.20–4.93 (m, 16 H). ^{13}C NMR (CD_3OD): δ 26.6, 26.6, 27.6, and 28.5 (2 × C*Me*$_2$), 54.6 and 57.3 (2 × OMe), 62.6 (C-6′), 74.5, 74.9, 74.9, and 75.2 (C-4, C-5, C-2′, and C-5′), 77.2, 77.6, 78.6, and 81.1 (C-2, C-3, C-3′, and C-4′), 102.0 (C-1′), 107.4 (C-1), 111.1 and 111.4 (2 × *C*Me$_2$), 177.8 (C-6). Anal. Calcd for $C_{20}H_{34}O_{13}$: C, 49.79; H, 7.10. Found: C, 49.98; H, 7.24.

Methyl 4-O-(3,4-O-isopropylidene-β-D-galactopyranosyl)-2,3-O-isopropylidene-aldehydo-D-glucuronate dimethyl acetal (**14**).—A solution of **13** (450 mg, 0.66 mmol) was debenzylated in EtOAc (6 mL) as described above for **11** to give **14** as a white solid (328 mg, quantitative), R_f 0.16 (EtOAc); mp 118–120 °C (from EtOAc–hexane); $[\alpha]_D$ +29.7° (*c* 1.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.32 and 1.50 (2 s, 3 H each, C*Me*$_2$), 1.42 (s, 6 H, C*Me*$_2$), 3.50 (s, 6 H, 2 × OMe); 3.53–3.75 (m, 3 H), 3.79 (s, 3 H, COO*Me*), 3.81–4.09 (m, 8 H), 4.25 (d, 1 H, $J_{1',2'}$ 8.1 Hz, H-1′), 4.37 (d, 1 H, $J_{1,2}$ 6.6 Hz, H-1), 4.45 (m, 1 H), 4.56 (dd, 1 H, $J_{2,3}$ 7.7 Hz, H-2). ^{13}C NMR ($CDCl_3$): δ 26.1, 26.1, 27.0, and 28.0 (2 × C*Me*$_2$), 52.5 (COO*Me*), 54.4 and 57.5 (2 × OMe), 62.2 (C-6′), 71.8 (C-5′), 73.4, 73.4, 74.6, and 75.3 (C-4, C-5, C-2′, and C-4′), 76.4 and 76.9 (C-2, and C-3), 79.0 (C-3′), 102.1 (C-1′), 106.9 (C-1), 110.1 and 110.4 (2 × *C*Me$_2$), 173.0 (C-6). Anal. Calcd for $C_{21}H_{36}O_{13}$: C, 50.80; H, 7.31. Found: C, 50.95; H, 7.36.

4-O-β-D-Galactopyranosyl-α,β-D-glucuronic acid (pseudolactobiouronic acid) (**17**).—A solution of **11** (480 mg, 1.0 mmol) in 90% aq CF_3COOH (5 mL) was stirred at rt for 30 min, then concentrated at reduced pressure, coevaporated with toluene (3 × 25 mL), and stored 24 h under diminished pressure over KOH pellets, giving a white solid residue (350 mg, quantitative) constituted (^{13}C NMR, D_2O) exclusively by a 45:55 α/β anomeric mixture of **17**, as established on the basis of the integration of the anomeric carbon signals. An analytical sample of **17** was obtained by crystallization from a 1:5 mixture of 10% aq MeOH and EtOH, R_f 0.16 (2:3 EtOAc–MeOH); mp 177–180 °C; $[\alpha]_D$ +28.9° (*c* 0.8, water); lit.[3] mp 159–160 °C; $[\alpha]_D$ +45.5° (*c* 1.11, water); ^{13}C NMR data see Table 1. Anal. Calcd for $C_{12}H_{20}O_{12}$: C, 40.45; H, 5.66. Found: C, 40.30; H, 5.80.

Methyl 4-O-β-D-galactopyranosyl-α,β-D-glucuronate (**18**).—A solution of **14** (280 mg, 0.56 mmol) was hydrolyzed as reported above for **11** giving pure **18** (208 mg, quantitative) as a syrup containing (^{13}C NMR, D_2O) an about 1:1 α/β anomeric mixture, R_f 0.15 (7:3 EtOAc–MeOH); $[\alpha]_D$ +38.0° (*c* 1.0, MeOH); ^{13}C NMR data see Table 1. Anal Calcd. for $C_{13}H_{22}O_{12}$: C, 42.17; H, 5.99. Found: C, 42.06; H, 5.88.

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