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SYNTHESIS OF 4-METHYLUMBELLIFERYL 1,2-*cis*-GLYCOSIDES*

MARIE-CHRISTINE COURTIN-DUCHATEAU AND ALAIN VEYRIÈRES
Laboratoire de Chimie Organique Multifonctionnelle, Bât. 420, Université de Paris-Sud, 91405 Orsay (France)
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ABSTRACT

Condensation of 4-methylumbelliferone (7-hydroxy-4-methylcoumarin) with 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranose (**1**) and 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranose (**9**) in the presence of zinc chloride gave, respectively, a mixture of α- (**2**) and β-D-glucopyranosides (**3**) in the ratio of 2:3, and a mixture of α-D-galactopyranoside (**10**), β-D-galactofuranoside, and β-D-galactopyranoside (**11**) in the ratio of 10:17:23. The proportion of 1,2-*cis*-glycosides could be slightly increased by treating **1** and **9** with the *O*-trimethylsilyl derivative of 4-methylumbelliferone in the presence of stannic chloride. Condensations of the sodium salt of 4-methylumbelliferone with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl chloride, 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl chloride, and 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide in hexamethylphosphoric triamide gave, respectively, pure **2**, pure **10**, and a mixture of α- and β-D-mannopyranosides in the ratio of 1:3, with much better yields. *O*-Deacetylation of **2**, **3**, **10**, and **11**, followed by platinum-catalyzed oxidation, afforded the corresponding 4-methylumbelliferyl D-glycopyranosiduronic acids.

INTRODUCTION

4-Methylumbelliferyl glycosides are commonly used as convenient substrates for the fluorimetric assay of glycoside hydrolase activity[1–3] and as interesting ligands for the study of carbohydrates interacting with proteins[4–5].

The preparation of 4-methylumbelliferyl 1,2-*trans*-glycosides[2,3,6–9] presents no problem, since these glycosides are always available from the corresponding acetylated glycosyl halides by a modification of the Michael method[10], which involves the condensation of halides with 4-methylumbelliferone in alkaline, aqueous acetone, followed by *O*-deacetylation. On the other hand, synthesis of the corresponding 1,2-*cis*-glycosides is more difficult. Only 4-methylumbelliferyl α-D-galactopyranoside (**10**) has been obtained in 1959 by Constantzas and Kocourek[11], who reported a 13% yield by the general Helferich method[10], which involves the condensation of per-

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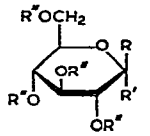
acetylated sugars with 4-methylumbelliferone in boiling xylene in the presence of zinc chloride. Therefore, a general procedure for preparing 4-methylumbelliferyl 1,2-*cis*-glycosides, in good yields and as stereoselectively as possible, was highly desirable. The present publication describes three different methods, the last one being the most satisfactory one.

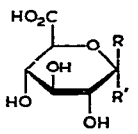
RESULTS AND DISCUSSION

In a first series of experiments, the Helferich method was applied to the condensation of 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranose (**1**) and 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranose (**9**) with 4-methylumbelliferone in the presence of zinc chloride. The high melting point of this phenol[12] (194–195°) does not allow a fusion procedure without extensive decomposition of the carbohydrate derivatives. Therefore, xylene was used as a diluent to allow azeotropic removal of the acetic acid formed by the reaction. High temperatures (145°) were nevertheless necessary to increase the reaction rate and favor the formation of 1,2-*cis*-glycosides. These conditions gave large proportions of resinous material and moderate yields (25–30%) of mixtures of α- and β-D-glycosides. Compound **1** afforded a mixture of α- (**2**) and β-D-glucopyranoside (**3**) in a ratio of 2:3, **3** being identical to the product obtained by the Michael method[2]. Compound **9** gave a mixture of α-D-galactopyranoside (**10**), β-D-galactofuranoside (**15**), and β-D-galactopyranoside (**11**) in a ratio of 10:17:23. Neither the melting point nor the optical rotation observed for **10** agree with the values given by Constantzas and Kocourek[11], and it is probable that the product described by these authors was not stereochemically pure. The 240-MHz n.m.r. spectrum of **15** showed H-1′ as a singlet at δ 5.73, H-4′ as a quartet at δ 4.40 [well shifted upfield when compared to H-4′ in **10** (δ 5.52) or **11** (δ 5.48)], and H-5′ as a sextuplet at δ 5.42 [well shifted downfield when compared to H-5′ in **10** (δ 4.29) or **11** (δ 4.10–4.22)]. These data and the low value of the optical rotation suggest a β-D-furanoside configuration for **15**. To our knowledge, it is the first time that the formation of a furanoside by ring contraction is reported for a Helferich reaction. The presence of some D-galactofuranose pentaacetate in **9** was excluded by examination of its 240-MHz n.m.r. spectrum (no signal[13] at δ 6.2). Moreover, when **9** was treated at 145° with zinc chloride for 2 h, a mixture of **9** and its α-D-pyranose anomer in a ratio of 5:11 was recovered in 28% yield, without any detectable amount of the β-D-furanose anomer. Attempted anomerization of **11** under the conditions of the Helferich reaction failed, only degradation products being observed. The formation of **15** may well occur by migration of one acetyl group from O-4 to O-5 in an open-chain intermediate, such as **25**, where these oxygen atoms are in a *threo* configuration.

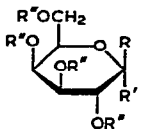
Audichya *et al.*[14] reported that the D-glucosylation of various phenols, catalyzed by stannic chloride at 30–35° in the minimum amount of benzene, favored the formation of α-D-glucopyranosides. In order to obtain a homogeneous reaction mixture, 4-methyl-7-trimethylsilyloxycoumarin, obtained by *O*-trimethylsilylation of 4-methylumbelliferone, was used in this type of condensation; this crystalline



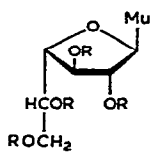
1 R = OAc, R′ = H, R″ = Ac
2 R = H, R′ = Mu, R″ = Ac
3 R = Mu, R′ = H, R″ = Ac
4 R = Cl, R′ = H, R″ = Ac
5 R = R″ = H, R′ = Mu
6 R = Mu, R′ = R″ = H



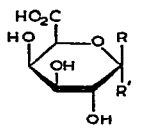
7 R = H, R′ = Mu
8 R = Mu, R′ = H



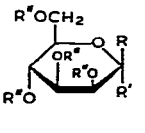
9 R = OAc, R′ = H, R″ = Ac
10 R = H, R′ = Mu, R″ = Ac
11 R = Mu, R′ = H, R″ = Ac
12 R = Cl, R′ = H, R″ = Ac
13 R = R″ = H, R′ = Mu
14 R = Mu, R′ = R″ = H



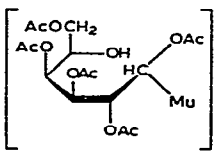
15 R = Ac
16 R = H



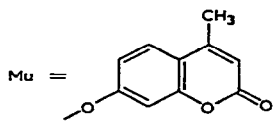
17 R = H, R′ = Mu
18 R = Mu, R′ = H



19 R = H, R′ = Br, R″ = Ac
20 R = H, R′ = Cl, R″ = Ac
21 R = H, R′ = Mu, R″ = Ac
22 R = Mu, R′ = H, R″ = Ac
23 R = R″ = H, R′ = Mu
24 R = Mu, R′ = R″ = H



25



derivative has a low melting point (54–56°), a good solubility in benzene, and is quite appropriate for acid-catalyzed condensations. At room temperature, the reaction with **1** or **9** afforded exclusively β-D-glycopyranosides, whereas in benzene under reflux, formation of mixtures of α- and β-D-glycopyranosides could be observed. Prolonged heating increased slightly the proportion of α-D anomer, not by anomerization of the originally formed β-D anomer but rather by its selective degradation. Here again, attempted anomerizations of β-D-glycopyranosides failed, and, as has already been pointed out[15], "there is no evidence that the first stage of such reactions is the formation of a β-D anomer that anomerizes in a subsequent step". When compared to the zinc chloride-catalyzed condensation described earlier, this procedure gave similar yields of glycosides (18–19%), but a higher proportion of the 1,2-*cis* anomer (83% in the D-*gluco* series, and 79% in the D-*galacto* series, where no furanoside could be detected).

Recently, Blanc-Muesser *et al.*[16] described a stereoselective synthesis of 1,2-*cis*-thioglycosides, based on an SN2 type reaction at the anomeric carbon atom of a 1,2-*trans*-halide with a nucleophile in a dipolar aprotic solvent. The sodium salt of 4-methylumbelliferone had been already used by Delmotte *et al.*[4] in condensations with 1,2-*cis*-chlorides in *N*,*N*-dimethylformamide to give good yields of 1,2-*trans*-glycosides. When this condensation was performed with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl chloride[17] (**4**) in dry hexamethylphosphoric triamide, the reaction was absolutely stereoselective and gave the α-D-glucopyranoside **2** in 50% yield. A major by-product was 2,3,4,6-tetra-*O*-acetyl-1,5-anhydro-D-*arabino*-hex-1-enitol, which might be formed by anomerization of the β-D-chloride, catalyzed by chloride

ions released in the reaction mixture and followed by *trans* elimination of hydrogen chloride. Similar results were obtained with 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl chloride (**12**); thus, the α-D-galactopyranoside **10** was prepared in 47% yield, only traces of the β-D-galactopyranoside **11** being detected in the reaction mixture. These results therefore confirm the SN2 character of the reaction and allow the easy preparation of **2** and **10** in gram amounts.

In the D-*manno* series, only the α-D-mannopyranoside **21** had been prepared by Vervoort and de Bruyne[19] according to the Helferich procedure. When 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide[20] (**19**) was treated in hexamethylphosphoric triamide with the sodium salt of 4-methylumbelliferone, a mixture of α- (**21**) and β-D-mannopyranosides (**22**) in the ratio of 1:3 was obtained in 43% yield. When the α-D-mannopyranosyl chloride[20] **20** was used instead of **19**, the reaction was found to proceed very sluggishly and only the α-D-mannopyranoside **21** was obtained in 30% yield. A possible explanation for this loss of stereoselectivity in the D-*manno* series could be a stronger tendency to give an acetoxonium ion, thus preventing a SN2 type reaction by nucleophilic attack from the β side. With a stronger nucleophile, like a thiophenate ion[16], formation of the acetoxonium ion is no more competitive, and pure SN2 type reactions are also observed in the D-*manno* series. The C–Cl bond being less easily polarized than the C–Br bond, it is not unexpected that the α-D-chloride **20** reacts with complete retention of configuration through an acetoxonium ion.

Conventional *O*-deacetylation of **2**, **3**, **10**, **11**, **15**, **21**, and **22** afforded free 4-methylumbelliferyl glycosides **5**, **6**, **13**, **14**, **16**, **23**, and **24**, respectively.

4-Methylumbelliferyl β-D-glucopyranosiduronic acid (**8**) has been prepared by Marsh and Levvy[21] by catalytic oxidation of **3**, and 4-methylumbelliferyl β-D-galactopyranosiduronic acid (**18**) has been obtained by Anderson[22] in a Michael condensation of methyl (2,3,4-tri-*O*-acetyl-α-D-galactopyranosyl bromide)uronate with 4-methylumbelliferone, followed by *O*-deacetylation and alkaline hydrolysis of the ester group. As substrates for various glycuronidases, four 4-methylumbelliferyl D-glycopyranosiduronic acids **7**, **8**, **17**, and **18** were prepared (in 24–50% yield) from **5**, **6**, **13**, and **14**, respectively, by catalytic oxydation of the primary hydroxyl group with oxygen in the presence of platinum, according to the method of Heyns and Paulsen[23].

EXPERIMENTAL

General methods. — Melting points are uncorrected. Optical rotations were measured with a Roussel–Jouan electronic, digital micropolarimeter. I.r. spectra were recorded with a Unicam model SP 1100 spectrometer, n.m.r. spectra with a spectrometer constructed in this University[24] at 240 MHz, with chloroform-*d* as solvent and tetramethylsilane as internal standard, and u.v. spectra with a Shimadzu model MPS-50 L spectrometer. T.l.c. was performed on plates of silica gel (with fluorescence indicator; layer thickness 0.25 mm; E. Merck, Darmstadt, Germany). The compounds were detected by spraying the plates with 1:19 (v/v) conc. sulfuric

acid–ethanol. Silica gel Merck (70–325 mesh; E. Merck) was used for column chromatography. Microanalyses were performed by the Laboratoire Central de Micro-Analyse du C.N.R.S.

4-Methyl-7-trimethylsilyloxycoumarin. — A stirred suspension of 4-methylumbelliferone (10 g, 57 mmol) in hexamethyldisilazane (24 ml) was heated under reflux for 15 h. The clear solution was evaporated and the residue crystallized after distillation (11.5 g, 82%), b.p.$_{0.01\,mm}$ 130°, m.p. 54–56°; ν_{max}^{KBr} 2980 (CH), 1730 (C=O, δ-lactone), 1625 (C=C), and 1515 cm^{-1} (Ph).

Anal. Calc. for $C_{13}H_{16}O_3Si$: C, 62.87; H, 6.49; Si, 11.30. Found: C, 62.88; H, 6.40; Si, 11.21.

*4-Methylumbelliferyl 2,3,4,6-tetra-*O*-acetyl-α-* (**2**) *and -β-*D*-glucopyranoside* (**3**). — *Method A.* A stirred suspension of 4-methylumbelliferone (15 g, 85 mmol) and 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranose[25] (**1**, 10 g, 26 mmol) in xylene (100 ml) was gently boiled at 145° until a few ml of the solvent had distilled. A solution of zinc chloride (2.5 g, 18 mmol) in 19:1 (v/v) acetic acid–acetic anhydride (10 ml) was added, and the solvent was distilled at 145° for 1 h at atmospheric pressure, then for 30 min at 15 torr. After cooling, the dark residue was extracted with chloroform (200 ml). The extract was successively washed twice with a saturated solution of potassium hydrogencarbonate and four times with water, dried, and evaporated. The partially *O*-deacetylated residue (10.6 g) was treated overnight at room temperature with 1:1 (v/v) acetic anhydride–pyridine (100 ml); t.l.c. (19:1, v/v, chloroform–acetone) then showed, besides the acetate of 4-methylumbelliferone (R_F 0.48) and a mixture of **1** and its α-D anomer (R_F 0.34), two new compounds having R_F 0.27 and 0.18, respectively. Evaporation of the solvents gave a residue that was chromatographed twice on silica gel, first with 97:3 (v/v) chloroform–ethanol, then with 19:1 (v/v) chloroform–acetone to afford **2** (1.25 g, 10%, R_F 0.27) and **3** (1.95 g, 15%, R_F 0.18).

Compound **2** crystallized from ethanol, m.p. 131–133°, $[\alpha]_D^{20}$ +200° (*c* 0.5, chloroform); ν_{max}^{KBr} 1740 (OAc and C=O, δ-lactone), 1622 (C=C), 1225 (OAc) and 860 cm^{-1} (Ph); n.m.r.: δ 2.04, 2.05, 2.06, and 2.07 (4 s, 12 H, 4 OAc), 2.42 (d, 3 H, *J* 1 Hz, Me), 4.03 (q, 1 H, $J_{5',6'_a}$ 2.2 Hz, $J_{6'_a,6'_b}$ 12.5 Hz, H-6'a), 4.07 (oct, 1 H, $J_{4',5'}$ 10.1 Hz, $J_{5',6'_a}$ 2.2 Hz, $J_{5',6'_b}$ 4.4 Hz, H-5'), 4.27 (q, 1 H, $J_{5',6'_b}$ 4.4 Hz, $J_{6'_a,6'_b}$ 12.5 Hz, H-6'b), 5.07 (q, 1 H, $J_{1',2'}$ 3.5 Hz, $J_{2',3'}$ 10.1 Hz, H-2'), 5.17 (t, 1 H, $J_{3',4'} = J_{4',5'}$ 10.1 Hz, H-4'), 5.69 (t, 1 H, $J_{2',3'} = J_{3',4'}$ 10.1 Hz, H-3'), 5.81 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1'), 6.21 (d, 1 H, *J* 1 Hz, H-3), 7.05 (q, 1 H, $J_{5,6}$ 8.5 Hz, $J_{6,8}$ 2.4 Hz, H-6), 7.10 (d, 1 H, $J_{6,8}$ 2.4 Hz, H-8), and 7.55 (d, 1 H, $J_{5,6}$ 8.5 Hz, H-5); λ_{max}^{MeOH} 315 (ε 12 900), 285 (ε 8 950), and 245.5 nm (ε 2 390).

Anal. Calc. for $C_{24}H_{26}O_{12}$: C, 56.91; H, 5.17; O, 37.91. Found: C, 57.09; H, 5.10; O, 37.78.

Compound **3** crystallized from ethanol, m.p. 143–144°, $[\alpha]_D^{20}$ −39° (*c* 0.635, chloroform) {lit.[2] m.p. 144°, $[\alpha]_D^{20}$ −40° (*c* 0.5, chloroform)}; ν_{max}^{KBr} 1750 (OAc), 1720 (C=O, δ-lactone), 1615 (C=C), 1230 (OAc), and 840 cm^{-1} (Ph); n.m.r.: δ 2.05 (s, 3 H, OAc), 2.07 (s, 6 H, 2 OAc), 2.13 (s, 3 H, OAc), 2.42 (d, 3 H, *J* 1 Hz, Me), 3.93 (oct, 1 H, $J_{4',5'}$ 9.8 Hz, $J_{5',6'_a}$ 2.4 Hz, $J_{5',6'_b}$ 5.8 Hz, H-5'), 4.18 (q, 1 H,

$J_{5',6'_a}$ 2.4 Hz, $J_{6'_a,6'_b}$ 12.7 Hz, H-6'a), 4.31 (q, 1 H, $J_{5',6'_b}$ 5.8 Hz, $J_{6'_a,6'_b}$ 12.7 Hz, H-6'b), 5.13–5.34 (m, 4 H, H-1', H-2', H-3', H-4'), 6.20 (d, 1 H, J 1 Hz, H-3), 6.93 (q, 1 H, $J_{5,6}$ 8.7 Hz, $J_{6,8}$ 2.2 Hz, H-6), 6.96 (d, 1 H, $J_{6,8}$ 2.2 Hz, H-8), and 7.53 (d, 1 H, $J_{5,6}$ 8.7 Hz, H-5); λ_{max}^{MeOH} 317 (ε 13 000), 287 (ε 8 980), and 246 nm (ε 2 600).

Method B. A solution of **1** (1.0 g, 2.6 mmol) in benzene (10 ml) was boiled until 9 ml of the solvent had distilled. 4-Methyl-7-trimethylsilyloxycoumarin (1.3 g, 5.2 mmol) and stannic chloride (0.6 ml, 5.5 mmol) were added, and the mixture was stirred under reflux for 2 h. After being cooled, the solution was diluted with chloroform (20 ml), washed twice with a saturated solution of potassium hydrogencarbonate and four times with water, dried, and evaporated. The residue was chromatographed twice on silica gel with 23:2 (v/v) dichloromethane–acetone to afford the α-D-glycoside **2** (192 mg, 15%) and its β-D anomer **3** (38 mg, 3%). One of the faster-moving compounds in the reaction mixture was identical to 7-acetoxy-4-methylcoumarin (23 mg, 2%), m.p. 152–153° (lit.[12] m.p. 153–154°).

Method C. To a solution of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl chloride[17] (**4**, 370 mg, 1 mmol) in dry hexamethylphosphoric triamide (4 ml) was added 4-methylumbelliferone sodium salt[4] (400 mg, 2 mmol). The reaction mixture was stirred for 36 h at room temperature, whereupon t.l.c. in 23:2 (v/v) dichloromethane–acetone revealed only a trace of **4** (R_F 0.66), an unsaturated compound detected with alkaline potassium permanganate (R_F 0.63), a major product (R_F 0.56) and some 4-methylumbelliferone (R_F 0.44). The mixture was poured into ice–water (150 ml) and stirred for 3 h at 0°. The solid material was collected, washed with water, dried, and chromatographed on silica gel with 23:2 (v/v) dichloromethane–acetone to afford pure **2** (192 mg, 38%). The combined filtrate and washings were extracted three times with ethyl acetate; the extract was washed once with 0.1M hydrochloric acid and then three times with water, dried, and evaporated. The residue was chromatographed with 23:2 (v/v) dichloromethane–acetone to afford additional **2** (60 mg; total yield, 50%). No trace of the β-D anomer **3** could be found in the reaction mixture.

4-Methylumbelliferyl α-D-glucopyranoside (**5**). — Compound **2** (573 mg, 1.3 mmol) was *O*-deacetylated overnight at room temperature with 10:1:1 (v/v) methanol–triethylamine–water (120 ml). The solution was evaporated, and the residue was dried by repeated distillations with ethanol and crystallized from ethanol (**5**, 373 mg, 96%), m.p. 209–210°, $[\alpha]_D^{20}$ +162° (*c* 0.5, pyridine); ν_{max}^{KBr} 3370 (OH), 1708 (C=O, δ-lactone), 1612 (C=C), and 845 cm^{-1} (Ph); λ_{max}^{MeOH} 318 (ε 13 400), 289 (ε 8 290) and 247.5 nm (ε 2 150).

Anal. Calc. for $C_{16}H_{18}O_8 \cdot 0.75H_2O$: C, 54.61; H, 5.59; O, 39.79. Found: C, 54.62; H, 5.58; O, 39.85.

4-Methylumbelliferyl β-D-glucopyranoside (**6**). — Compound **3** (2.0 g, 3.9 mmol) was *O*-deacetylated for 3 h at room temperature with 8mM sodium methoxide in methanol (300 ml). Concentration of the solution afforded a crystalline material that was filtered off, washed with cold methanol, and recrystallized from ethanol to give **6** (970 mg, 72%), m.p. 211–213°, $[\alpha]_D^{20}$ −68° (*c* 0.5, pyridine) {lit.[2] m.p. 211°, $[\alpha]_D^{20}$

$-89.5°$ (*c* 0.5, water); ν_{max}^{KBr} 3440 (OH), 1728 (C=O, δ-lactone), 1630 (C=C), 862 and 850 cm^{-1} (Ph); λ_{max}^{MeOH} 317.5 (ε 13 900), 289 (ε 8 770), and 247 nm (ε 2 270).

4-Methylumbelliferyl α-D-glucopyranosiduronic acid (**7**). — To a stirred solution of **5** (400 mg, 1.1 mmol) in water (20 ml) at 90° were added freshly reduced platinum catalyst (130 mg) and sodium hydrogencarbonate (96 mg, 1.1 mmol). Oxygen was passed into the mixture through a sintered-glass disk. After 1 h, more catalyst (70 mg) was added, and the reaction was complete after 1 h as shown by t.l.c. in 4:1:5 (v/v) butanol–ethanol–water. The hot mixture was filtered through a bed of Celite and 0.48M hydrochloric acid (2.5 ml) was added to the cooled filtrate. The slightly acidic solution was concentrated under a stream of nitrogen to give a crystalline material (125 mg, 31%).

A portion of this material (30 mg) was dissolved in 3:1 (v/v) ethanol–water (50 ml) and applied to a column (1 × 8.5 cm) of Dowex 1 (X-8, HCO_2^-, 200–400 mesh) ion-exchange resin. The column was washed with water (50 ml) and eluted with a gradient from water (500 ml) to 4.9M formic acid (500 ml). Elution was followed by absorbance at 254 nm, 6-ml fractions being collected. Fractions 43–73 containing pure **7** were combined and concentrated to a small volume; water was added and repeatedly evaporated to remove most of the formic acid. Compound **7** crystallized from the concentrated aqueous solution (20 mg), m.p. 202–203°, $[\alpha]_D^{20}$ +113° (*c* 0.283, pyridine); ν_{max}^{KBr} 3530 and 3400 (OH), 1715 (CO_2H and C=O, δ-lactone), 1610 (C=C), and 845 cm^{-1} (Ph); λ_{max}^{MeOH} 317 (ε 13 800), 288 (ε 8 800), and 247 nm (ε 2 340).

Anal. Calc. for $C_{16}H_{16}O_9$: C, 54.55; H, 4.58; O, 40.88. Found: C, 54.29; H, 4.65; O, 40.82.

4-Methylumbelliferyl β-D-glucopyranosiduronic acid (**8**). — This compound was prepared from **6** (400 mg), as described for **7**. The product was recrystallized from ethanol–water (**8**, 175 mg, 43%), m.p. 139–145°, $[\alpha]_D^{20}$ −108° (*c* 0.25, pyridine) {lit.[21] m.p. 139–140°, $[\alpha]_D^{22}$ −119° (*c* 0.25, water); lit.[22] m.p. 139–140°, $[\alpha]_D^{20}$ −114° (*c* 0.25, water)}; ν_{max}^{KBr} 3380 (OH), 1700 and 1685 (CO_2H and C=O, δ-lactone), 1610 (C=C), and 848 cm^{-1} (Ph); λ_{max}^{MeOH} 317 (ε 12 900), 290 (ε 8 290), and 246 nm (ε 2 180).

4-Methylumbelliferyl 2,3,4,6-tetra-O-acetyl-α- (**10**) *and -β-D-galactopyranoside* (**11**), *and 2,3,5,6-tetra-O-acetyl-β-D-galactofuranoside* (**15**). — *Method A.* A stirred suspension of 4-methylumbelliferone (19.8 g, 112 mmol) and 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranose[25] (**9**, 13.2 g, 34 mmol) in xylene (90 ml) was distilled for a few minutes at 145° and atmospheric pressure. A pinch of zinc dust and a solution of zinc chloride (3.0 g, 22 mmol) in 19:1 (v/v) acetic acid–acetic anhydride (12 ml) were added, and distillation was continued at 145° for 1 h at atmospheric pressure, then for 1 h at 15 torr. After cooling, the residue was extracted with chloroform (300 ml). T.l.c. (9:1, v/v, chloroform–acetone) showed, besides 4-methylumbelliferone (R_F 0.24) and a mixture of **9** and its α-D anomer (R_F 0.48), three new compounds having R_F 0.43, 0.37, and 0.30. The extract was successively washed twice with a saturated solution of potassium hydrogencarbonate and four times with water, dried, and evaporated. The residue (9.1 g) was chromatographed five times on silica gel with 9:1 (v/v) chloroform–acetone. Fractions showing a single spot at R_F 0.43 were

combined and evaporated to give **10** (1.02 g, 6%), which was crystallized from ethanol, m.p. 186.5–188.5°, $[\alpha]_D^{20}$ +210° (*c* 0.281, chloroform) {lit.[11] m.p. 173–174°, $[\alpha]_D^{20}$ +113.9° (*c* 3.0, chloroform)}; ν_{max}^{KBr} 1735 (OAc and C=O, δ-lactone), 1612 (C=C), 1228 (OAc), and 855 cm^{-1} (Ph); n.m.r.: δ 1.95, 2.03, 2.08, and 2.18 (4 s, 12 H, 4 OAc), 2.41 (d, 3 H, *J* 1 Hz, Me), 4.06–4.10 (m, 2 H, H-6'a and H-6'b), 4.29 (t, 1 H, $J_{5',6'_a} = J_{5',6'_b}$ 6.6 Hz, H-5'), 5.30 (q, 1 H, $J_{2',3'}$ 10.3 Hz, $J_{3',4'}$ 3.5 Hz, H-3'), 5.52 (d, 1 H, $J_{3',4'}$ 3.4 Hz, H-4'), 5.56 (q, 1 H, $J_{1',2'}$ 3.3 Hz, $J_{2',3'}$ 10.3 Hz, H-2'), 5.83 (d, 1 H, $J_{1',2'}$ 3.3 Hz, H-1'), 6.20 (d, 1 H, *J* 1 Hz, H-3), 7.02 (q, 1 H, $J_{5,6}$ 8.6 Hz, $J_{6,8}$ 2.2 Hz, H-6), 7.08 (d, 1 H, $J_{6,8}$ 2.2 Hz, H-8), and 7.54 (d, 1 H, $J_{5,6}$ 8.6 Hz, H-5); λ_{max}^{MeOH} 317 (ε 12 800), 287 (ε 8 740), and 247 nm (ε 2 250).

Anal. Calc. for $C_{24}H_{26}O_{12}$: C, 56.91; H, 5.17; O, 37.91. Found: C, 56.93; H, 5.16; O, 37.73.

Fractions showing a single spot at R_F 0.37 were combined and evaporated to give **15** (1.77 g, 10%) as a foam, $[\alpha]_D^{20}$ −85° (*c* 0.637, chloroform); ν_{max}^{KBr} 1755 (OAc and C=O, δ-lactone), 1620 (C=C), and 1235 cm^{-1} (OAc); n.m.r.: δ 2.02 and 2.14 (2 s, 6 H, 2 OAc), 2.15 (s, 6 H, 2 OAc), 2.41 (d, 3 H, *J* 1.3 Hz, Me), 4.19 (q, 1 H, $J_{5',6'_a}$ 7.1 Hz, $J_{6'_a,6'_b}$ 11.8 Hz, H-6'a), 4.33 (q, 1 H, $J_{5',6'_b}$ 4.3 Hz, $J_{6'_a,6'_b}$ 11.8 Hz, H-6'b), 4.40 (q, 1 H, $J_{3',4'}$ 5.4 Hz, $J_{4',5'}$ 3.9 Hz, H-4'), 5.13 (q, 1 H, $J_{2',3'}$ 1.9 Hz, $J_{3',4'}$ 5.4 Hz, H-3'), 5.36 (d, 1 H, $J_{2',3'}$ 1.9 Hz, H-2'), 5.42 (sext, 1 H, $J_{4',5'}$ 3.9 Hz, $J_{5',6'_a}$ 7.1 Hz, $J_{5',6'_b}$ 4.3 Hz, H-5'), 5.75 (s, 1 H, H-1'), 6.19 (d, 1 H, *J* 1.3 Hz, H-3), 6.96 (q, 1 H, $J_{5,6}$ 8.8 Hz, $J_{6,8}$ 2.3 Hz, H-6), 6.99 (d, 1 H, $J_{6,8}$ 2.3 Hz, H-8), and 7.53 (d, 1 H, $J_{5,6}$ 8.8 Hz, H-5); λ_{max}^{MeOH} 316 (ε 12 500), 287 (ε 8 150), and 247 nm (ε 2 220).

Anal. Calc. for $C_{24}H_{26}O_{12}$: C, 56.91; H, 5.17; O, 37.91. Found: C, 56.85; H, 5.20; O, 37.77.

Fractions showing a single spot at R_F 0.30 were combined and evaporated to give **11** (2.46 g, 14%), which was crystallized from ethanol, m.p. 142–144°, $[\alpha]_D^{20}$ −6.7° (*c* 1.65, chloroform) {lit.[11] m.p. 141–142°, $[\alpha]_D^{20}$ −7.9° (*c* 1.1, chloroform); {lit.[6] m.p. 143–145.5°, $[\alpha]_D^{25}$ −6.5° (*c* 1.54, chloroform); lit. [7]m.p. 144°, $[\alpha]_D^{20}$ −8° (*c* 1, chloroform); lit.[8] m.p. 142°, $[\alpha]_D^{20}$ −7.5° (*c* 1, chloroform)}; ν_{max}^{KBr} 1755 and 1725 (OAc and C=O, δ-lactone), 1610 (C=C), 1220 (OAc), and 848 cm^{-1} (Ph); n.m.r.: δ 2.02, 2.07, 2.11, and 2.18 (4 s, 12 H, 4 OAc), 2.41 (d, 3 H, *J* 1 Hz, Me), 4.10–4.22 (m, 3 H, H-5', H-6'a and H-6'b), 5.13 (d, 1 H, $J_{1',2'}$ 8.2 Hz, H-1'), 5.14 (q, 1 H, $J_{2',3'}$ 10.5 Hz, $J_{3',4'}$ 3.1 Hz, H-3'), 5.48 (d, 1 H, $J_{3',4'}$ 3.1 Hz, H-4'), 5.51 (q, 1 H, $J_{1',2'}$ 8.2 Hz, $J_{2',3'}$ 10.5 Hz, H-2'), 6.20 (d, 1 H, *J* 1 Hz, H-3), 6.90 (q, 1 H, $J_{5,6}$ 8.6 Hz, $J_{6,8}$ 2.3 Hz, H-6), 6.97 (d, 1 H, $J_{6,8}$ 2.3 Hz, H-8), and 7.53 (d, 1 H, $J_{5,6}$ 8.6 Hz, H-5); λ_{max}^{MeOH} 317 (ε 13 100), 287 (ε 9 000), and 247 nm (ε 2 590).

Method B. Compound **9** (5.0 g, 13 mmol) and 4-methyl-7-trimethylsilyloxycoumarin (6.4 g, 26 mmol) were treated in the presence of stannic chloride (2.8 ml, 26 mmol), as described for **1**. After processing, the residue was chromatographed twice on silica gel with 23:2 (v/v) dichloromethane–acetone to afford the α-D-galactopyranoside **10** (990 mg, 15%) and the β-D-galactopyranoside **11** (262 mg, 4%). No trace of the β-D-galactofuranoside **15** could be found in the reaction mixture.

Method C. 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl chloride[18] **(12)**, (370 mg,

1 mmol) was treated with 4-methylumbelliferone (sodium salt) (400 mg, 2 mmol) in dry hexamethylphosphoric triamide (4 ml), as described for **4**. After processing, the crude product was chromatographed on silica gel with 23:2 (v/v) dichloromethane–acetone to afford the α-D-galactopyranoside **10** (237 mg, 47%). Only traces of the β-D-galactopyranoside **11** could be detected in the reaction mixture.

4-Methylumbelliferyl α-D-galactopyranoside (**13**). — Compound **10** (800 mg, 1.6 mmol) was *O*-deacetylated for 15 min at room temperature with 3mM sodium methoxide in 1:2 (v/v) *p*-dioxane–methanol (150 ml). The solution was neutralized with Dowex 50 (H^+) ion-exchange resin and evaporated. The residue was crystallized from ethanol to give **13** (416 mg, 78%), m.p. 212–217°, $[\alpha]_D^{20}$ +135° (*c* 1.37, pyridine) {lit.[11] m.p. 221–222°, $[\alpha]_D^{20}$ +237.0° (*c* 0.30, water)}; ν_{max}^{KBr} 3440 (OH), 1735 (C=O, δ-lactone), 1620 (C=C), and 845 cm^{-1} (Ph); λ_{max}^{MeOH} 317 (ε 13 300), 289 (ε 8 660), and 248 nm (ε 2 160).

Anal. Calc. for $C_{16}H_{18}O_8$: C, 56.80; H, 5.36; O, 37.84. Found: C, 56.40; H, 5.74; O, 37.99.

4-Methylumbelliferyl β-D-galactopyranoside (**14**). — Compound **12** (3.00 g) was *O*-deacetylated as described for **2**. Compound **14** crystallized from ethanol (1.63 g, 81%), m.p. 232–233°, $[\alpha]_D^{20}$ −61° (*c* 0.75, pyridine) {lit.[11] m.p. 230°, $[\alpha]_D^{20}$ −61.2° (*c* 0.74, pyridine); lit[6] m.p. 227.5–230°, $[\alpha]_D^{24}$ −37° (*c* 1.23, *N*,*N*-dimethylformamide); lit.[7] m.p. 242°, $[\alpha]_D^{20}$ −57° (*c* 0.03, water); lit.[8] m.p. 234°, $[\alpha]_D^{20}$ −40° (*c* 0.017, water)}; ν_{max}^{KBr} 3465 and 3230 (OH), 1718 (C=O, δ-lactone), 1620 (C=C), and 860 cm^{-1} (Ph); λ_{max}^{MeOH} 317 (ε 14 500), 289 (ε 8 830) and 248 nm (ε 1 920).

4-Methylumbelliferyl β-D-galactofuranoside (**16**). — Compound **15** (400 mg) was *O*-deacetylated as described for **10**. Compound **16** crystallized from ethanol (205 mg, 77%), m.p. 127° and 160–162°, $[\alpha]_D^{20}$ −180° (*c* 0.522, pyridine); ν_{max}^{KBr} 3400 (OH), 1710 (C=O, δ-lactone), 1628 (C=C), and 850 cm^{-1} (Ph); λ_{max}^{MeOH} 318 (ε 13 500), 290 (ε 8 140), and 247 nm (ε 2 420).

Anal. Calc. for $C_{16}H_{18}O_8 \cdot 0.5H_2O$: C, 55.33; H, 5.51; O, 39.16. Found: C, 55.35; H, 5.50; O, 39.26.

4-Methylumbelliferyl α-D-galactopyranosiduronic acid (**17**). — Compound **13** (75 mg) was oxidized, as described for **5**. T.l.c. in 35:30:3 (v/v) acetic acid–chloroform–water showed the reaction to be completed after 1.25 h. After processing and purification on a column of Dowex 1 (X-8, HCO_2^-, 200–400 mesh) ion-exchange resin, the product crystallized from water to give **17** (39 mg, 50%), m.p. 170–173°, $[\alpha]_D^{20}$ +40° (*c* 0.298, pyridine); ν_{max}^{KBr} 3560 and 3450 (OH), 1735 and 1720 (CO_2H and C=O, δ-lactone), 1620 (C=C), and 850 cm^{-1} (Ph); λ_{max}^{MeOH} 318 (ε 12 800), 289 (ε 7 930), and 248 nm (ε 2 080).

Anal. Calc. for $C_{16}H_{16}O_9 \cdot 0.75H_2O$: C, 52.53; H, 4.82; O, 42.65. Found: C, 52.46; H, 5.04; O, 42.12.

4-Methylumbelliferyl β-D-galactopyranosiduronic acid (**18**). — Compound **14** (500 mg) was oxidized, as described for **5**. The product crystallized from water (**18**, 124 mg, 24%), m.p. 195–197°, $[\alpha]_D^{20}$ −141° (*c* 0.290, pyridine), $[\alpha]_D^{20}$ −160° (*c* 0.269, ethanol) {lit.[22] m.p. 142–144°, $[\alpha]_D^{20}$ −208° (*c* 0.5, ethanol)}; ν_{max}^{KBr} 3495 (OH), 1718

and 1690 (CO_2H and C=O, δ-lactone), 1617 (C=C), and 840 cm^{-1} (Ph); λ_{max}^{MeOH} 318 (ε 13 100), 289 (ε 8 220), and 248 nm (ε 2 230).

Anal. Calc. for $C_{16}H_{16}O_9$: C, 54.55; H, 4.58; O, 40.88. Found: C, 54.42; H, 4.65; O, 40.85.

4-Methylumbelliferyl 2,3,4,6-tetra-O-acetyl-α- (**21**) *and -β-D-mannopyranoside* (**22**). — 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl bromide[20] (**19**, 4.10 g, 10 mmol) was treated with 4-methylumbelliferone (sodium salt) (4.00 g, 20 mmol) in dry hexamethylphosphoric triamide (35 ml), as described for **4**. After 20 h, t.l.c. in 23:2 (v/v) dichloromethane–acetone revealed the absence of **19** (R_F 0.64), and the presence of an unsaturated compound (R_F 0.59), two condensation products (R_F 0.54 and 0.48), and some 4-methylumbelliferone (R_F 0.40). After processing, the residue was chromatographed on silica gel with 23:2 (v/v) dichloromethane–acetone. Fractions showing a single spot at R_F 0.54 were combined and evaporated to give **21** (542 mg, 11%), which crystallized from ethanol, m.p. 161–163°, $[\alpha]_D^{20}$ +106° (*c* 0.321, chloroform) {lit.[19] m.p. 160–161°, $[\alpha]_D^{22}$ +136.0° (*c* 2, chloroform)}; ν_{max}^{KBr} 1750 (OAc and C=O, δ-lactone), 1625 (C=C), 1255 (OAc), and 852 cm^{-1} (Ph); n.m.r.: δ 2.03 (s, 6 H, 2 OAc) 2.05 (s, 3 H, OAc), 2.22 (s, 3 H, OAc), 2.41 (d, 3 H, *J* 1 Hz, Me), 4.04 (q, 1 H, $J_{6'_a,6'_b}$ 12.8 Hz, $J_{5',6'_a}$ 2.3 Hz, H-6'a), 4.29 (q, 1H, $J_{6'_a,6'_b}$ 12.8 Hz, $J_{5',6'_b}$ 5.9 Hz, H-6'b), 5.37 (t, 1 H, $J_{3',4'} = J_{4',5'}$ 9.8 Hz, H-4'), 5.46 (q, 1 H, $J_{1',2'}$ 1.9 Hz, $J_{2',3'}$ 3.5 Hz, H-2'), 5.54 (q, 1 H, $J_{2',3'}$ 3.5 Hz, $J_{3',4'}$ 9.8 Hz, H-3'), 5.57 (d, 1 H, $J_{1',2'}$ 1.9 Hz, H-1'), 6.19 (d, 1 H, *J* 1 Hz, H-3), 7.02 (q, 1 H, $J_{5,6}$ 8.6 Hz, $J_{6,8}$ 2.4 Hz, H-6), 7.10 (d, 1 H, $J_{6,8}$ 2.4 Hz, H-8), and 7.23 (d, 1 H, $J_{5,6}$ 8.6 Hz, H-5); λ_{max}^{MeOH} 315 (ε 13 500), 285 (ε 9 190), and 245 nm (ε 2 400).

Anal. Calc. for $C_{24}H_{26}O_{12}$: C, 56.91; H, 5.17; O, 37.91. Found: C, 56.64; H, 5.03; O, 37.73.

Fractions showing a single spot at R_F 0.48 were combined and evaporated to give **22** (1.60 g, 32%), which crystallized from ethanol, m.p. 177–178°, $[\alpha]_D^{20}$ −95° (*c* 0.368, chloroform); ν_{max}^{KBr} 1750 (OAc and C=O, δ-lactone), 1620 (C=C), 1240 (OAc), and 855 cm^{-1} (Ph); n.m.r.: δ 2.03, 2.08, 2.13, and 2.26 (4 s, 12 H, 4 OAc), 2.40 (s, 3 H, Me), 3.87–3.95 (m, 1 H, H-5'), 4.22 (q, 1 H, $J_{6'_a,6'_b}$ 12 Hz, $J_{5',6'_a}$ 2.3 Hz, H-6'a), 4.34 (q, 1 H, $J_{6'_a,6'_b}$ 12 Hz, $J_{5',6'_b}$ 6.2 Hz, H-6'b), 5.17 (q, 1 H, $J_{2',3'}$ 3 Hz, $J_{3',4'}$ 9.6 Hz, H-3'), 5.32 (t, 1 H, $J_{4',5'} = J_{3',4'}$ 9.6 Hz, H-4'), 5.72 (d, 1 H, $J_{1',2'}$ 2.2 Hz, H-1'), 6.18 (s, 1 H, H-3), 6.90 (q, 1 H, $J_{5,6}$ 8.6 Hz, $J_{6,8}$ 2.3 Hz, H-6), 6.96 (d, 1 H, $J_{6,8}$ 2.3 Hz, H-8), and 7.52 (d, 1 H, $J_{5,6}$ 8.6 Hz, H-5); λ_{max}^{MeOH} 315 (ε 13 900), 286 (9 220), and 245 nm (2 560).

Anal. Calc. for $C_{24}H_{26}O_{12}$: C, 56.91; H, 5.17; O, 37.91. Found: C, 56.73; H, 5.13; O, 37.63.

When 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl chloride[20] (**20**, 330 mg, 0.9 mmol) was treated with 4-methylumbelliferone (sodium salt) (360 mg, 1.8 mmol) in dry hexamethylphosphoric triamide (4 ml) at room temperature, only formation of the α-D-mannopyranoside **21** could be detected. After 42 h, large amounts of **20** were still present and an additional amount of 4-methylumbelliferone (sodium salt) (540 mg, 2.7 mmol) was added. Stirring was continued for 16 h, and the reaction

mixture was processed as for **4**. Compound **21** was obtained by column chromatography on silica gel with 23:2 (v/v) dichloromethane–acetone (130 mg, 30%).

4-Methylumbelliferyl α-D-mannopyranoside (**23**). — Compound **21** (93 mg) was *O*-deacetylated, as described for **13**. Compound **23** crystallized from ethanol–water (42 mg, 63%), m.p. 220–224°, $[\alpha]_D^{20}$ +157° (*c* 0.4, water) {lit.[19] m.p. 222–225°, $[\alpha]_D^{22}$ +178.2° (*c* 2, methanol)}; ν_{max}^{KBr} 3400 (OH), 1682 (C=O, δ-lactone), 1620 (C=C), and 850 cm^{-1} (Ph); λ_{max}^{MeOH} 318 (ε 14 200), 288 (ε 8 810), and 247 (ε 2 310).

Anal. Calc. for $C_{16}H_{18}O_8$: C, 56.80; H, 5.36; O, 37.84. Found: C, 56.87; H, 5.68; O, 37.58.

4-Methylumbelliferyl β-D-mannopyranoside (**24**). — Compound **22** (605 mg) was *O*-deacetylated, as described for **13**. Compound **24** crystallized from ethanol–water (247 mg, 61%), m.p. 241–243°, $[\alpha]_D^{20}$ −105° (*c* 0.275, pyridine); ν_{max}^{KBr} 3450 and 3300 (OH), 1725 (C=O, δ-lactone), 1625 (C=C), and 870 cm^{-1} (Ph); λ_{max}^{MeOH} 317 (ε 14 200), 289 (ε 8 690), and 247.5 nm (ε 2 210).

Anal. Calc. for $C_{16}H_{18}O_8$: C, 56.80; H, 5.36; O, 37.84. Found: C, 56.66; H, 5.40; O, 37.79.

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