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Stereoselective total synthesis of dodecagalacturonic acid, a phytoalexin elicitor of soybean*†

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ABSTRACT

O-(α-D-Galactopyranosyluronic acid)-[(1→4)-*O*-(α-D-galactopyranosyluronic acid)]$_{10}$-D-galactopyranuronic acid (**1**), an endogenous elicitor of the phytoalexin of soybean, was synthesized by way of highly stereoselective glycosylations that involved glycosyl fluorides as the donors and oxidation of the twelve primary hydroxyl groups in the α-(1→4)-linked galactododecaoside derivative.

INTRODUCTION

Linear α-(1→4)-linked oligogalacturonic acids, released from plant cell-wall homogalacturonan by partial hydrolysis with endo-polygalacturonases, the pathogenic fungal enzyme, or an acid catalyst, induce plant defence responses such as the accumulation of phytoalexin[2–4], lignification[2], and the synthesis of a proteinase inhibitor[5]. The oligomers with d.p. 10–13 are active as elicitors of phytoalexin in soybean, and dodecagalacturonic acid **1** (d.p.12) is the most active. The molecular mechanisms of the biological processes induced by the oligogalacturonides remain to be clarified[6]. Homogeneous samples of the oligomers necessary for the biological studies are not extracted easily from natural sources, so that syntheses of oligogalacturonides of defined structure are required.

We have reported[7] a stereocontrolled synthesis of the propyl glycoside (**2**) of decagalacturonic acid as a model compound for the phytoalexin elicitor-active oligogalacturonic acids, where the galactobiosyl donors **3** and **4**, and the acceptor **5** were the intermediates in sequential stereoselective glycosylation reactions, and the resulting galactodecaose derivative **7** was oxidised to give the decacarboxylic acid **8**. However, removal of the allyl group from **8** was not carried out because of undesired oxidation to the 2-oxopropyl group when the Wilkinson catalyst [$(Ph_3P)_3RhCl$ (ref. 8) was used. Therefore, the more-easily removable *tert*-butyldiphenylsilyl group was used as an alternative to the allyl group and this made possible a synthesis[1] of the α-(1→4)-linked trigalacturonic acid. We now describe the synthesis of the dodecagalacturonic acid **1**.

* Dedicated to Professor Leslie Hough in the year of his 65th birthday.
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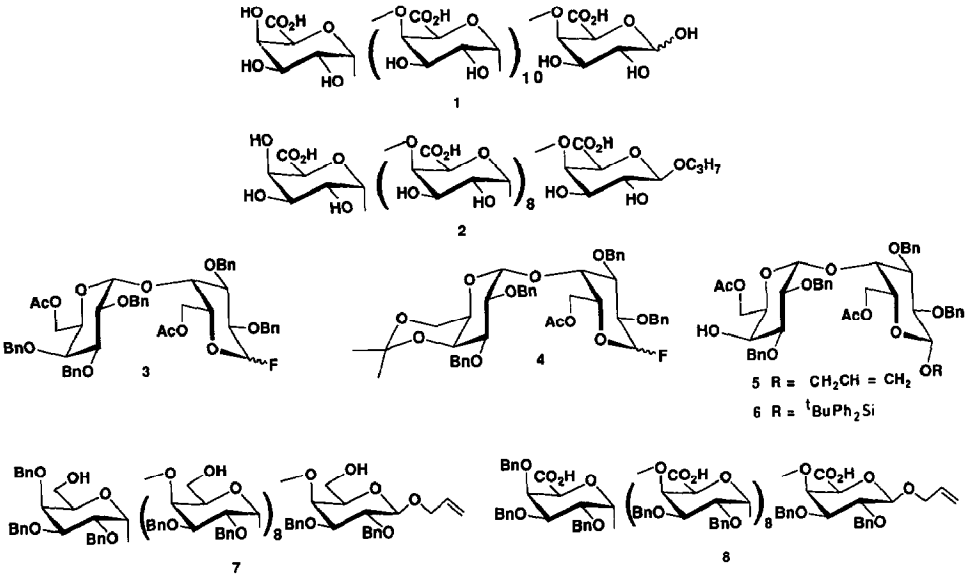
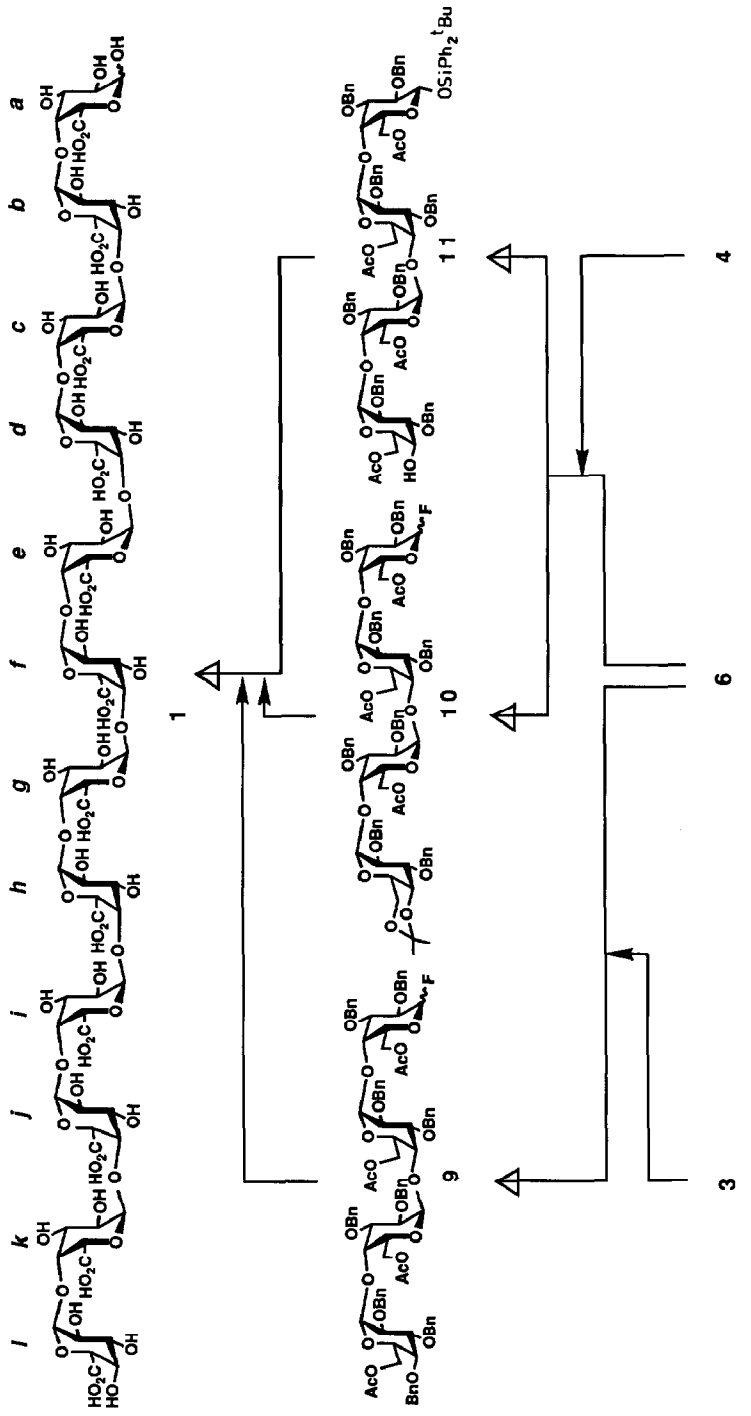


RESULTS AND DISCUSSION

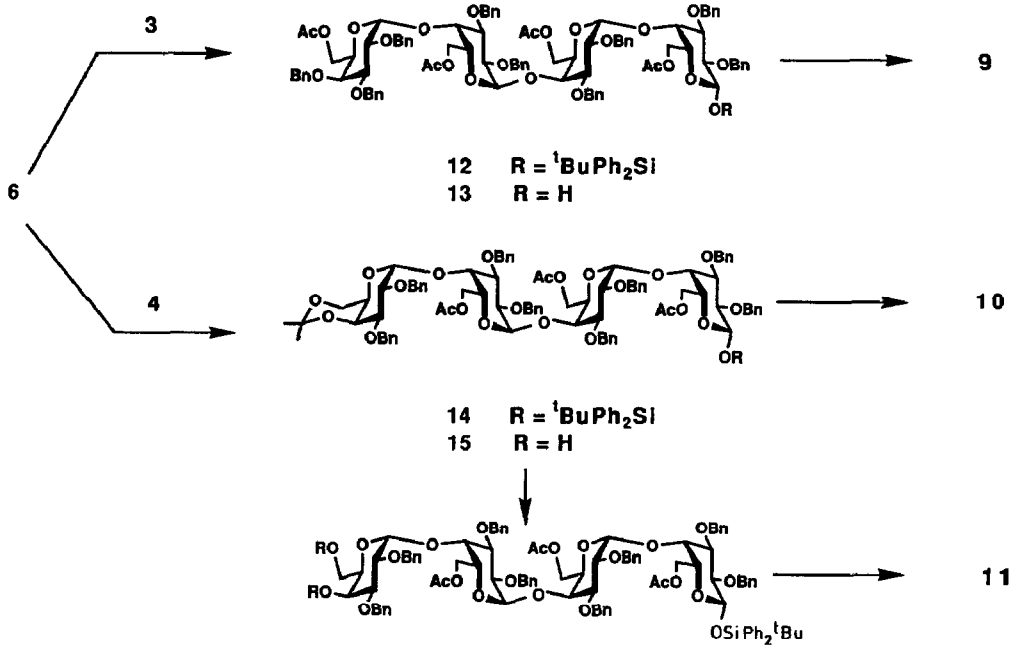
Based on previous knowledge[1,7], a convergent route to **1** was planned that involved the galactotetraosyl intermediates **9**–**11**, each of which would be prepared from the readily obtainable galactobiosyl acceptor **6** and either donor **3** or **4** (Scheme 1).

The synthon **9** was prepared as follows. A $SnCl_2$–$AgClO_4$-promoted glycosylation[9] of **6** with **3** gave **12** (92%) as the sole product. The ^{13}C-n.m.r. spectrum of **12** contained signals for anomeric carbons at 98.0 ($^1J_{C,H}$ 161 Hz, C-1a), 99.4, and 99.7 p.p.m. ($^1J_{C,H}$ 167 and 169 Hz, for C-1bcd) which indicated[10] the newly formed linkage at C-1c to be α. Desilylation of **12** with *n*-Bu_4NF–AcOH[11] in tetrahydrofuran gave **13** (90%), treatment of which with diethylaminosulfur trifluoride[12] in tetrahydrofuran afforded 97% of a 7:18 αβ-mixture of **9**. The synthons **10** and **11** were derived from the common intermediate **14** that was obtained (77%) by the coupling of **4** and **6** with $SnCl_2$–$AgClO_4$ in ether. Synthon **10** was obtained by desilylation of **14** to give **15** (96%), as described for **9**, then fluorination to give an 8:17 αβ-mixture (97%) of the fluoride **10**. Deisopropylidenation of **14** with aqueous 80% AcOH at 60° gave the diol **16** (92%), which was selectively mono-*O*-acetylated with AcCl in pyridine to afford the synthon **11** (93%) and the by-product **17** (4%).

The coupling of **10** and **11** in the presence of $SnCl_2$ and $AgClO_4$ in ether gave the expected product **18** that was deisopropylidenated with aqueous 80% AcOH to yield the galacto-octaoside **22** (62% overall yield). Alternatively, **22** could be obtained by glycosylation of **11** with **4** to give **19** which was deisopropylidenated to afford **20** (77%). Mono-*O*-acetylation of **20** afforded **21** (83%), and glycosylation with **4** followed by hydrolysis of the isopropylidene group afforded **22** (70%). The structure of **22** was confirmed by the ^1H- and ^{13}C-n.m.r. spectral data. Mono-*O*-acetylation of **22** with AcCl–pyridine at −5° gave the galacto-octaosyl acceptor **23** (82%).



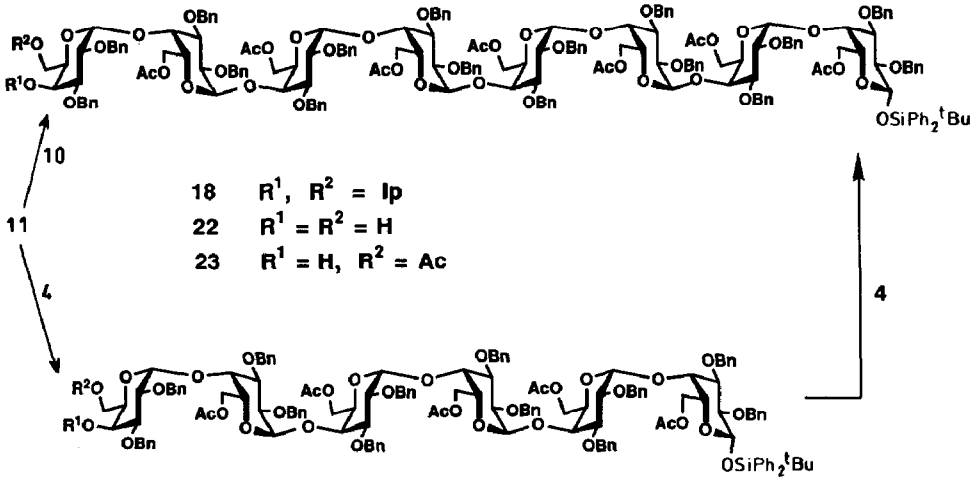
Scheme 1. Synthetic plan for 1.



12 R = ^{t}BuPh$_2$Si
13 R = H

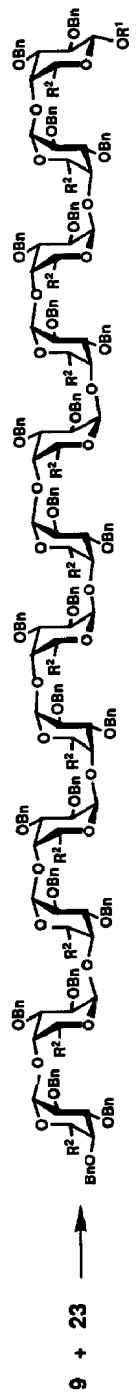
14 R = ^{t}BuPh$_2$Si
15 R = H

16 R = H
17 R = Ac



18 R^1, R^2 = Ip
22 R^1 = R^2 = H
23 R^1 = H, R^2 = Ac

19 R^1, R^2 = Ip
20 R^1 = R^2 = H
21 R^1 = H, R^2 = Ac



9 + 23 ⟶

24 $R^1 = {}^tBuPh_2Si$, $R^2 = CH_2OAc$

25 $R^1 = {}^tBuPh_2Si$, $R^2 = CH_2OH$

26 $R^1 = {}^tBuPh_2Si$, $R^2 = CHO$

27 $R^1 = {}^tBuPh_2Si$, $R^2 = COOH$

28 $R^1 = {}^tBuPh_2Si$, $R^2 = COOMe$

29 $R^1 = H$, $R^2 = COOH$

The glycosylation of **23** with **9** proceeded smoothly in the presence of $SnCl_2$–$AgClO_4$ in 7:3 ether–toluene overnight at $-10°$ to room temperature to afford 64% of the galactododecaoside **24**. The ^{13}C-n.m.r. spectrum of **24** contained signals for anomeric carbons at 98.1 (C-1a) and 99.3 and 99.6 p.p.m. (C-1bcdefghijkl). No signals for C-1β were observed at $\sim$103 p.p.m.. The α and β isomers in this series of oligogalacturonides can be distinguished easily on the basis of the chemical shifts of the C-1 resonances[1].

The dodeca-acetate **24** was *O*-deacetylated with methanolic NaOMe to give **25** (81%) that was oxidized in two steps with Me_2SO–$(COCl)_2$–$^iPr_2EtN$[13] then with $NaClO_2$–2-methyl-2-butene[14] to give crude **27** (50% overall yield). For ease of isolation[1,7], **27** was esterified with ethereal CH_2N_2 to give **28** (80% after preparative t.l.c.). Heating a pyridine solution of **28** with excess of LiI[15] for 30 h, followed by preparative t.l.c., gave a homogeneous sample of **27** (67%). Desilylation of **27** with *n*-Bu_4NF–AcOH in tetrahydrofuran yielded **29** (75%). Finally, **29** was hydrogenolysed (10% Pd–C, aqueous 80% MeOH, 4 days) and the product was purified by anion-exchange chromatography[16] to afford 75% of the pure dodecagalacturonic acid **1**. The details of the biological activity will be reported elsewhere[17], but it may be noted here that **1** was active as an elicitor of soybean phytoalexin and was synergistic with the hepta-β-glucoside elicitor[18].

EXPERIMENTAL

General. — Optical rotations were determined with a Perkin–Elmer Model 241 MC polarimeter, for solutions in $CHCl_3$ at 25°, unless noted otherwise. Column chromatography was performed on silica gel (Merck 70–230 mesh). Flash chromatography was performed on Wako Gel C-300 (200–300 mesh). T.l.c. and h.p.t.l.c. were performed on Silica Gel 60 F_{254} (Merck). N.m.r. spectra were recorded with a JEOL GX500 [^1H (500 MHz)] or FX90Q [^{13}C (22.50 MHz)] spectrometer. Chemical shifts are expressed in p.p.m. downfield from the signal for internal Me_4Si, for solutions in $CDCl_3$, unless noted otherwise, and for solutions in D_2O, in p.p.m. downfield from the signal for Me_4Si, by reference to internal Me_3COH (1.230).

tert-*Butyldiphenylsilyl*O-(*6*-O-*acetyl-2,3,4-tri*-O-*benzyl*-α-D-*galactopyranosyl*)-[(*1*→*4*)-O-(*6*-O-*acetyl-2,3-di*-O-*benzyl*-α-D-*galactopyranosyl*)]$_2$-(*1*→*4*)-*6*-O-*acetyl-2,3-di*-O-*benzyl*-β-D-*galactopyranoside* (**12**). — A mixture of **3** (225 mg, 0.26 mmol) and **6** (200 mg, 0.20 mmol) in dry ether (17 mL) was added under argon with stirring and cooling ($\sim -10°$) to a mixture of $SnCl_2$ (155 mg, 0.82 mmol), $AgClO_4$ (185 mg, 0.89 mmol), and dry powdered molecular sieves 4 Å (1.0 g). After stirring for 3 h at -10–$+10°$, pyridine (2 mL) was added to the mixture, which was diluted with ether–EtOAc (1:1, 50 mL), filtered through Celite, washed with water and brine, dried (Na_2SO_4), and concentrated *in vacuo*. Column chromatography (toluene–EtOAc, 4:1) of the residue on silica gel (70 g) gave **12** (337 mg, 91.7%), $[\alpha]_D^{25}$ +48° (*c* 0.8), R_F 0.67 (7:3 toluene–EtOAc). N.m.r. data: ^1H, δ 1.12 (s, 9 H, CMe_3), 1.78 (s, 3 H, Ac), 1.89 (s, 3 H, Ac), 1.90 (s, 3 H, Ac), 1.93 (s, 3 H, Ac), 3.22 (bt, 1 H, H-5a), 3.25 (dd, 1 H, *J* 2.8 and 9.8 Hz, H-3a), 3.64 (dd, 1 H, *J* 7.3 and 9.8 Hz, H-2a), 7.20–7.46 and 7.68–7.72 (2 m, 51 and 4

H, 11 Ph); ^{13}C, δ 19.2 (CMe_3), 20.6 ($COCH_3$), 20.8 ($COCH_3$), 27.0 [$C(CH_3)_3$], 98.0 ($^1J_{C,H}$ 161.1 Hz, C-1a), 99.4 ($^1J_{C,H}$ 167.2 Hz) and 99.7 ($^1J_{C,H}$ 168.5 Hz) (2:1, C-1b,1c,1d), 169.8, 169.9, and 170.2 (2:1:1, C=O).

Anal. Calc. for $C_{111}H_{122}O_{25}Si$: C, 70.76; H, 6.53. Found: C, 70.78; H, 6.54.

O-(6-O-*Acetyl-2,3,4-tri*-O-*benzyl*-α-D-*galactopyranosyl*)-[(1→4)-O-(6-O-*acetyl-2,3-di*-O-*benzyl*-α-D-*galactopyranosyl*)]$_2$-(1→4)-6-O-*acetyl-2,3-di*-O-*benzyl*-D-*galactopyranose* (**13**). — To a mixture of **12** (101 mg, 0.05 mmol) and AcOH (28 μL, 0.50 mmol) in dry tetrahydrofuran (1.8 mL) was added M *n*-Bu_4NF in tetrahydrofuran (250 μL, 0.25 mmol). The mixture was stirred for 6 days at room temperature, then diluted with ether–EtOAc (1:1, 50 mL), washed with water and brine, dried (Na_2SO_4), and concentrated *in vacuo*. Column chromatography (toluene–EtOAc, 7:3) of the crude product on silica gel (10 g) afforded **13** (79 mg, 89.5%) as an αβ mixture. N.m.r. data: ^1H, δ 1.776 (1.780) (s, 3 H, Ac), 1.883 (1.901) (s, 3 H, Ac), 1.937 (1.940) (s, 3 H, Ac), 2.033 (2.031) (s, 3 H, Ac), 5.26 (H-1aα); ^{13}C, δ 20.6 and 20.7 ($COCH_3$), 91.4 (C-1aα), 97.6 (C-1aβ), 99.3 and 99.6 (C-1b,1c,1d), 169.8, 169.9, 170.1, and 170.4 (C=O).

Anal. Calc. for $C_{95}H_{104}O_{25}$: C, 69.33; H, 6.37. Found: C, 69.26; H, 6.34.

O-(6-O-*Acetyl-2,3,4-tri*-O-*benzyl*-α-D-*galactopyranosyl*)-[(1→4)-O-(6-O-*acetyl-2,3-di*-O-*benzyl*-α-D-*galactopyranosyl*)]$_2$-(1→4)-6-O-*acetyl-2,3-di*-O-*benzyl*-D-*galactopyranosyl fluoride* (**9**). — To a solution of **13** (130 mg, 79 μmol) in dry tetrahydrofuran (1 mL) in an ice–MeOH bath was added diethylaminosulfur trifluoride (DAST; 17 μL, 130 μmol) with stirring. Stirring was continued at room temperature for 15 min, then MeOH (0.5 mL) was added, and the mixture was concentrated *in vacuo*. The residue was extracted with ether–EtOAc (1:1), and the extract was washed with water and brine, dried (Na_2SO_4), and concentrated *in vacuo*. Column chromatography (toluene–EtOAc, 3:1) of the crude product on silica gel (20 g) gave **9** (126 mg, 96.8%) as an αβ-mixture (αβ-ratio 7:18), R_F 0.51 and 0.46 (7:3 toluene–EtOAc). ^1H-N.m.r. data: δ 1.782 (s, 3 H, Ac), 1.903 (1.885) (s, 3 H, Ac), 1.924 (1.948) (s, 3 H, Ac), 2.036 (s, 3 H, Ac), 5.180 (dd, *J* 5.8 and 52.8 Hz, H-1aβ), 5.581 (d, *J* 56.2 Hz, H-1aα).

Anal. Calc. for $C_{95}H_{103}FO_{24}$: C, 69.24; H, 6.30; F, 1.15. Found: C, 69.21; H, 6.29; F, 1.13.

tert-*Butyldiphenylsilyl* O-(*2,3-di*-O-*benzyl-4,6*-O-*isopropylidene*-α-D-*galactopyranosyl*)-[(1→4)-O-(6-O-*acetyl-2,3-di*-O-*benzyl*-α-D-*galactopyranosyl*)]$_2$-(1→4)-6-O-*acetyl-2,3-di*-O-*benzyl*-β-D-*galactopyranoside* (**14**). — Reaction of **4** (578 mg, 0.73 mmol) and **6** (680 mg, 0.66 mmol) was carried out with $SnCl_2$ (290 mg, 1.53 mmol), $AgClO_4$ (315 mg, 1.52 mmol), and dry powdered molecular sieves 4 Å (3 g) in dry ether (35 mL) for 1.5 h at −10–+10°. Work-up, as described for **12**, followed by column chromatography (*n*-hexane–EtOAc–pyridine, 70:30:1) on silica gel (150 g) gave **14** (920 mg, 77.4%), $[\alpha]_D^{24}$ +64° (*c* 1), R_F 0.60 (1:1 *n*-hexane–EtOAc). N.m.r. data: ^1H, δ 1.13 (s, 9 H, CMe_3), 1.33 (s, 3 H, $=CMe_2$), 1.41 (s, 3 H, $=CMe_2$), 1.89 (s, 3 H, Ac), 1.90 (s, 3 H, Ac), 2.01 (s, 3 H, Ac), 3.03 (d, 1 H, *J* 11.9 Hz, H-6d), 3.21 (t, 1 H, *J* 6.7 Hz, H-5a), 3.24 (dd, 1 H, *J* 2.7 and 9.8 Hz, H-3a), 3.31 (d, 1 H, *J* 11.3 Hz, H-6d), 3.63 (dd, 1 H, *J* 7.3 and 9.8 Hz, H-2a), 7.16–7.49 and 7.69–7.73 (2 m, 46 and 4 H, 10 Ph); ^{13}C, δ 18.3 [$=C(CH_3)_2$], 19.2 (CMe_3), 20.7 and 20.9 ($COCH_3$), 27.1 [$C(CH_3)_3$], 29.5 [$=C(CH_3)_2$], 98.0 (C-1a), 98.3 ($=CMe_2$), 99.2, 99.4, and 100.2 (C-1b,1c,1d), 169.7, 169.9, and 170.2 (C=O).

Anal. Calc. for $C_{105}H_{118}O_{24}Si$: C, 70.37; H, 6.64. Found: C, 69.91; H, 6.60.

O-*(2,3-Di*-O-*benzyl-4,6*-O-*isopropylidene*-α-D-*galactopyranosyl)*-*[(1→4)*-O-*(6*-O-*acetyl-2,3-di*-O-*benzyl*-α-D-*galactopyranosyl)]$_2$*-*(1→4)*-*6*-O-*acetyl-2,3-di*-O-*benzyl*-D-*galactopyranose* (**15**). — Desilylation of **14** (390 mg, 0.22 mmol), as described above for **13**, followed by column chromatography (toluene–EtOAc–pyridine, 65:35:1) on silica gel (40 g) gave **15** (325 mg, 96.1%) as an αβ-mixture. ^{1}H-N.m.r. data: δ 1.323 (s, 3 H, $=CMe_2$), 1.403 (s, 3 H, $=CMe_2$), 1.935 (s, 3 H, Ac), 1.963 (1.978) (s, 3 H, Ac), 2.033 (2.031) (s, 3 H, Ac), 5.266 (H-1aα).

Anal. Calc. for $C_{89}H_{100}O_{24}$: C, 68.80; H, 6.49. Found: C, 68.31; H, 6.50.

O-*(2,3-Di*-O-*benzyl-4,6*-O-*isopropylidene*-α-D-*galactopyranosyl)*-*[(1→4)*-O-*(6*-O-*acetyl-2,3-di*-O-*benzyl*-α-D-*galactopyranosyl)]$_2$*-*(1→4)*-*6*-*O*-*acetyl-2,3-di*-O-*benzyl*-D-*galactopyranosyl fluoride* (**10**). — Treatment of **15** (300 mg, 0.19 mmol) with DAST (42 μL, 0.32 mmol) in dry tetrahydrofuran (1.5 mL), as described for **9**, followed by column chromatography (toluene–EtOAc–pyridine, 70:30:1) on silica gel (25 g) gave **10** (290 mg, 96.5%) as an αβ-mixture (αβ-ratio 8:17), R_F 0.51 and 0.46 (1:1 *n*-hexane–EtOAc). ^{1}H-N.m.r. data: δ 1.325 (s, 3 H, $=CMe_2$), 1.408 (s, 3 H, $=CMe_2$), 1.923 (1.946) (s, 3 H, Ac), 1.982 (1.965) (s, 3 H, Ac), 2.036 (s, 6 H, Ac), 5.177 (dd, *J* 6.1 and 52.8 Hz, H-1a, β anomer), 5.585 (d, *J* 55.5 Hz, H-1a, α anomer).

Anal. Calc. for $C_{89}H_{99}FO_{23}$: C, 68.71; H, 6.41; F, 1.22. Found: C, 68.99; H, 6.45; F, 1.22.

tert-*Butyldiphenylsilyl* O-*(2,3-di*-O-*benzyl*-α-D-*galactopyranosyl)*-*[(1→4)*-O-*(6*-O-*acetyl-2,3-di*-O-*benzyl*-α-D-*galactopyranosyl)]$_2$*-*(1→4)*-*6*-O-*acetyl-2,3-di*-O-*benzyl*-β-D-*galactopyranoside* (**16**). — A solution of **14** (511 mg, 0.29 mmol) in aqueous 80% AcOH (8 mL) was heated for 30 min at 60°, then concentrated *in vacuo*. Column chromatography (*n*-hexane–EtOAc, 1:1) of the residue on silica gel (75 g) gave **16** (457 mg, 91.5%), $[\alpha]_D^{26}$ +63° (*c* 1), R_F 0.23 (7:3 toluene–EtOAc). N.m.r. data: ^{1}H, δ 1.13 (s, 9 H, CMe_3), 1.90 (s, 6 H, 2 Ac), 1.95 (s, 3 H, Ac), 4.55 (d, 1 H, *J* 7.0 Hz, H-1a), 4.96 (d, 2 H, *J* 3.4 Hz) and 4.98 (d, 1 H, *J* 3.4 Hz) (H-1b,1c,1d); ^{13}C, δ 19.2 (CMe_3), 20.6 and 20.8 ($COCH_3$), 27.0 [$C(CH_3)_3$], 98.0 ($^1J_{C,H}$ 158.7 Hz, C-1a), 99.2, 99.4, and 99.8 ($^1J_{C,H}$ 173.3 Hz, C-1b,1c,1d), 169.8, 169.9, and 170.2 (C=O).

Anal. Calc. for $C_{102}H_{114}O_{24}Si \cdot H_2O$: C, 69.21; H, 6.49. Found: C, 69.16; H, 6.54.

tert-*Butyldiphenylsilyl* O-*(6*-O-*acetyl-2,3-di*-O-*benzyl*-α-D-*galactopyranosyl)*-*[(1→4)*-O-*(6*-O-*acetyl-2,3-di*-O-*benzyl*-α-D-*galactopyranosyl)]$_2$*-*(1→4)*-*6*-O-*acetyl-2,3-di*-O-*benzyl*-β-D-*galactopyranoside* (**11**). — To a solution of **16** (415 mg, 0.24 mmol) in dry pyridine (2.5 mL) was added acetyl chloride (26 μL, 0.37 mmol) with stirring and cooling (ice–water bath). The mixture was allowed to warm to 5–15°, stirred for 4 h, diluted with water (1 mL), and then concentrated *in vacuo*. The residue was extracted with ether–EtOAc (1:1), and the extract was washed with water and brine, dried (Na_2SO_4), and concentrated *in vacuo*. Column chromatography (toluene–EtOAc, 7:3) of the residue on silica gel (70 g) gave the penta-acetate **17** (19 mg, 4.4%), then **11** (395 mg, 93.0%), $[\alpha]_D^{26}$ +57°, R_F 0.43 (7:3 toluene–EtOAc). N.m.r. data: ^{1}H, δ 1.12 (s, 9 H, CMe_3), 1.89 (s, 3 H, Ac), 1.90 (s, 3 H, Ac), 1.91 (s, 3 H, Ac), 1.94 (s, 3 H, Ac), 3.22 (t, 1 H, *J* 6.7 Hz, H-5a), 3.25 (dd, 1 H, *J* 2.7 and 10.1 Hz, H-3a), 3.64 (dd, 1 H, *J* 7.3 and 10.1 Hz,

H-2a), 4.55 (d, 1 H, *J* 7.3 Hz, H-1a), 4.95 (m, 3 H, H-1b,1c,1d); ^{13}C, δ 19.2 (CMe_3), 20.8 ($COCH_3$), 27.0 [$C(CH_3)_3$], 98.0 (C-1a), 99.3 and 99.6 (C-1b,1c,1d), 169.8, 169.9, and 170.2 (C=O).

Anal. Calc. for $C_{104}H_{116}O_{25}Si\cdot H_2O$: C, 68.93; H, 6.45. Found: C, 68.81; H, 6.48.

tert-*Butyldiphenylsilyl* O-*(2,3-di*-O-*benzyl-4,6*-O-*isopropylidene*-α-D-*galactopyranosyl)*-*[(1→4)*-O-*(6*-O-*acetyl-2,3-di*-O-*benzyl*-α-D-*galactopyranosyl)]$_4$*-*(1→4)*-*6*-O-*acetyl-2,3-di*-O-*benzyl*-β-D-*galactopyranoside* (**19**). — Reaction of **4** (90 mg, 114 μmol) and **11** (155 mg, 86 μmol) was carried out with $SnCl_2$ (45 mg, 237 μmol), $AgClO_4$ (50 mg, 241 μmol), and powdered molecular sieves 4 Å (500 mg) in dry ether (5.5 mL) for 3 h at −10–+15°, and the mixture was worked-up as described for **12**. Column chromatography (toluene–EtOAc–pyridine, 80:40:1) of the crude product on silica gel (40 g) gave slightly impure **19** (155 mg), R_F 0.57 (7:3 toluene–EtOAc), and further elution with toluene–EtOAc (1:1) gave a more polar fraction that contained **20** (33 mg), R_F 0.20. The products were combined and used for the next reaction.

Compound **19**. ^{1}H-N.m.r. data: δ 1.12 (s, 9 H, CMe_3), 1.32 (s, 3 H, $=CMe_2$), 1.40 (s, 3 H, $=CMe_2$), 1.89 (bs, 6 H, 2 Ac), 1.92 (s, 3 H, Ac), 1.94 (s, 3 H, Ac), 1.96 (s, 3 H, Ac).

tert-*Butyldiphenylsilyl* O-*(2,3-di*-O-*benzyl*-α-D-*galactopyranosyl)*-*[(1→4)*-O-*(6*-O-*acetyl-2,3-di*-O-*benzyl*-α-D-*galactopyranosyl)]$_4$*-*(1→4)*-*6*-O-*acetyl-2,3-di*-O-*benzyl*-β-D-*galactopyranoside* (**20**). — The above mixture of **19** and **20** was treated with aqueous 80% AcOH (4 mL) for 30 min at 60°, and concentrated *in vacuo*. Column chromatography (toluene–EtOAc, 7:3) of the residue on silica gel (35 g) afforded **20** (162 mg, 77.4% from **11**), $[\alpha]_D^{23}$ +59° (*c* 0.5). N.m.r. data: ^{1}H, δ 1.12 (s, 9 H, CMe_3), 1.88 (bs, 9 H, 3 Ac), 1.91 (s, 3 H, Ac), 1.96 (s, 3 H, Ac), 3.22 (bt, 1 H, H-5a), 3.25 (dd, 1 H, *J* 2.8 and 9.8 Hz, H-3a), 3.39 (m, 1 H, H-6f), 3.44 (dd, 1 H, *J* 3.7 and 11.6 Hz, H-6f), 3.63 (dd, 1 H, *J* 7.3 and 9.8 Hz, H-2a), 4.55 (d, 1 H, *J* 7.3 Hz, H-1a); ^{13}C, δ 19.2 (CMe_3), 20.8 ($COCH_3$), 27.0 [$C(CH_3)_3$], 97.9 ($^1J_{C,H}$ 159.9 Hz, C-1a), 99.2 and 99.7 ($^1J_{C,H}$ 170.9 Hz, C-1b,1c,1d,1e,1f), 169.6, 169.7, 169.9, and 170.2 (C=O).

Anal. Calc. for $C_{146}H_{162}O_{36}Si$: C, 69.56; H, 6.48. Found: C, 69.92; H, 6.53.

tert-*Butyldiphenylsilyl* O-*(6*-O-*acetyl-2,3-di*-O-*benzyl*-α-D-*galactopyranosyl)*-*[(1→4)*-O-*(6*-O-*acetyl-2,3-di*-O-*benzyl*-α-D-*galactopyranosyl)]$_4$*-*(1→4)*-*6*-O-*acetyl-2,3-di*-O-*benzyl*-β-D-*galactopyranoside* (**21**). — Selective mono-acetylation of **20** (145 mg, 58 μmol), as described for **11**, with acetyl chloride (11 μL, 155 μmol) in dry pyridine (1.5 mL) for 5 h at 0° and column chromatography (toluene–EtOAc, 7:3) of the crude product on silica gel (30 g) gave **21** (123 mg, 83.4%), $[\alpha]_D^{24}$ +54° (*c* 0.5), R_F 0.42 (7:3 toluene–EtOAc). N.m.r. data: ^{1}H, δ 1.116 (s, 9 H, CMe_3), 1.875 (s, 3 H, Ac), 1.881 (s, 3 H, Ac), 1.887 (s, 3 H, Ac), 1.899 (s, 3 H, Ac), 1.917 (s, 3 H, Ac), 1.963 (s, 3 H, Ac), 3.215 (bt, 1 H, H-5a), 3.247 (dd, 1 H, *J* 2.8 and 9.8 Hz, H-3a), 3.633 (dd, 1 H, *J* 7.3 and 9.8 Hz, H-2a), 4.545 (d, 1 H, *J* 7.3 Hz, H-1a); ^{13}C, δ 19.2 (CMe_3), 20.7 ($COCH_3$), 27.0 [$C(CH_3)_3$], 98.0 (C-1a), 99.3 (C-1b,1c,1d,1e,1f), 169.6, 169.8, 169.9, and 170.2 (C=O).

Anal. Calc. for $C_{148}H_{164}O_{37}Si$: C, 69.36; H, 6.45. Found: C, 69.23; H, 6.45.

tert-*Butyldiphenylsilyl* O-*(2,3-di*-O-*benzyl*-α-D-*galactopyranosyl)*-*[(1→4)*-O-*(6*-O-*acetyl-2,3-di*-O-*benzyl*-α-D-*galactopyranosyl)]$_6$*-*(1→4)*-*6*-O-*acetyl-2,3-di*-O-*benzyl*-β-D-*galactopyranoside* (**22**). — *(a) By coupling of* **10** *and* **11**. Reaction of **10** (130 mg,

84 μmol) and **11** (107 mg, 60 μmol) in the presence of $SnCl_2$ (45 mg, 237 μmol), $AgCl_4$ (50 mg, 241 μmol), and powdered molecular sieves 4 Å (400 mg) in dry ether (4 mL) was carried out for 3.5 h at −10 to +10°. The mixture was worked-up as described above for **12**, and column chromatography (toluene–EtOAc–pyridine, 70:30:1) of the crude product on silica gel (40 g) gave slightly impure **18** (135 mg) and a more polar fraction that contained **22** (38 mg). The fractions were combined and treated with aqueous 80% AcOH (3 mL) at 60° for 30 min, and the mixture was concentrated *in vacuo*. Column chromatography (toluene–EtOAc, 7:3) of the residue on silica gel (20 g) gave **22** (122 mg, 62.2% from **11**), $[\alpha]_D^{24}$ +62° (*c* 0.7), R_F 0.53 (3:2 toluene–EtOAc). N.m.r. data: ^1H, δ 1.11 (s, 9 H, CMe_3), 1.88 (s, 9 H, 3 Ac), 1.89 (s, 3 H, Ac), 1.90 (s, 9 H, 3 Ac), 3.21 (bt, 1 H, H-5a), 3.25 (dd, 1 H, *J* 2.8 and 9.8 Hz, H-3a), 3.38 (m, 1 H, H-6h), 3.44 (m, 1 H, H-6h), 3.63 (dd, 1 H, *J* 7.3 and 9.8 Hz, H-2a), 4.53 (d, 1 H, *J* 7.3 Hz, H-1a); ^{13}C, δ 19.2 (CMe_3), 20.8 ($COCH_3$), 27.1 [$C(CH_3)_3$], 98.1 ($^1J_{C,H}$ 159.9 Hz, C-1a), 99.3 and 99.8 ($^1J_{C,H}$ 170.0 Hz, C-1b,1c,1d,1e,1f,1g,1h), 169.8, 170.2, and 170.7 (C=O).

Anal. Calc. for $C_{190}H_{210}O_{48}Si$: C, 69.37; H, 6.43. Found: C, 69.54; H, 6.45.

(b) By coupling of **4** *and* **21**. Reaction of **4** (42 mg, 53 μmol) and **21** (100 mg, 40 μmol) was performed with $SnCl_2$ (22 mg, 116 μmol), $AgClO_4$ (24 mg, 116 μmol), and molecular sieves 4 Å (250 mg) in dry ether (3 mL) for 3 h at −10–+10°, and the mixture was worked-up as described above in (*a*). Column chromatography of the crude product gave two fractions that contained **18** and **22**, respectively. Treatment of the combined fractions with aqueous 80% AcOH, followed by column chromatography of the product, afforded **22** (90 mg, 70% from **21**).

tert-*Butyldiphenylsilyl* O-*(6-*O*-acetyl-2,3-di-*O*-benzyl-α-*D*-galactopyranosyl)-[(1→4)-*O*-(6-*O*-acetyl-2,3-di-*O*-benzyl-α-*D*-galactopyranosyl)]$_6$-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzyl-β-*D*-galactopyranoside* (**23**). — Acetylation of **22** (80 mg, 24 μmol), with acetyl chloride (4.5 μL, 63 μmol) in dry pyridine (0.6 mL) for 4 h at 0–10°, gave **23** (66 mg, 81.5%), $[\alpha]_D^{22}$ +54° (*c* 0.7), R_F 0.41 (7:3 toluene–EtOAc). N.m.r. data: ^1H, δ 1.115 (s, 9 H, CMe_3), 1.868 (s, 3 H, Ac), 1.879 (s, 3 H, Ac), 1.885 (s, 3 H, Ac), 1.896 (s, 3 H, Ac), 1.899 (s, 3 H, Ac), 1.901 (s, 3 H, Ac), 1.904 (s, 3 H, Ac), 1.954 (s, 3 H, Ac), 3.227 (bt, 1 H, H-5a), 3.246 (dd, 1 H, *J* 2.8 and 9.8 Hz, H-3a), 3.631 (dd, 1 H, *J* 7.0 and 9.8 Hz, H-2a), 4.544 (d, 1 H, *J* 7.0 Hz, H-1a); ^{13}C, δ 19.2 (CMe_3), 20.7 ($COCH_3$), 27.0 [$C(CH_3)$], 97.8 (C-1a), 99.2 (C-1b,1c,1d,1e,1f,1g,1h), 169.7, 169.8, and 170.1 (C=O).

Anal. Calc. for $C_{192}H_{212}O_{49}Si \cdot H_2O$: C, 68.84; H, 6.44. Found: C, 68.47; H, 6.36.

tert-*Butyldiphenylsilyl* O-*(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-*D*-galactopyranosyl)-[(1→4)-*O*-(6-*O*-acetyl-2,3-di-*O*-benzyl-α-*D*-galactopyranosyl)]$_{10}$-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzyl-β-*D*-galactopyranoside* (**24**). — Glycosylation of **23** (100 mg, 30 μmol) with **9** (75 mg, 46 μmol) was achieved in dry ether (3.5 mL)–dry toluene (1.5 mL), using $SnCl_2$ (23 mg, 121 μmol), $AgClO_4$ (26 mg, 125 μmol), and molecular sieves 4 Å (400 mg) at −10° to room temperature overnight, and the mixture was worked-up as described above. Column chromatography (toluene–EtOAc, 3:1) of the crude product on silica gel (25 g) gave **24** (117 mg) with some contaminants. Preparative t.l.c. gave pure **24** (95 mg, 63.5%), $[\alpha]_D^{22}$ +56° (*c* 0.6), R_F 0.60 (7:3 toluene–EtOAc). N.m.r. data: ^1H, δ 1.115 (s, 9 H, CMe_3), 1.782 (s, 3 H, Ac), 1.853 (s, 3 H, Ac), 1.881 (s, 3 H, Ac), 1.887 (bs, 9

H, 3 Ac), 1.889 (bs, 9 H, 3 Ac), 1.901 (s, 3 H, Ac), 1.908 (s, 3 H, Ac), 1.958 (s, 3 H, Ac), 3.210 (bt, 1 H, H-5a), 3.244 (dd, 1 H, *J* 2.4 and 9.8 Hz, H-3a), 3.629 (dd, 1 H, *J* 7.0 and 9.8 Hz, H-2a), 4.558 (d, 1 H, *J* 7.0 Hz, H-1a); ^{13}C, δ 19.3 (CMe_3), 20.8 ($COCH_3$), 27.1 [$C(CH_3)_3$], 98.1 (C-1a), 99.3 and 99.6 (C-1b,1c,1d,1e,1f,1g,1h,1i,1j,1k,1*l*), 169.8 and 169.9 (C=O).

Anal. Calc. for $C_{287}H_{314}O_{73}Si \cdot H_2O$: C, 69.25; H, 6.36. Found: C, 68.92; H, 6.29.

tert-*Butyldiphenylsilyl* O-*(2,3,4-tri*-O-*benzyl*-α-D-*galactopyranosyl)*-*[(1→4)*-O-*(2,3-di*-O-*benzyl*-α-D-*galactopyranosyl)]$_{10}$*-*(1→4)*-*2,3-di*-O-*benzyl*-β-D-*galactopyranoside* (**25**). — To a solution of **24** (55 mg, 11 μmol) in dry tetrahydrofuran (0.5 mL) was added methanolic 0.1M NaOMe (1 mL). The mixture was stirred overnight at room temperature, diluted with MeOH, treated with excess of Amberlyst 15 (H^+) resin, filtered, and concentrated *in vacuo*. Preparative t.l.c. (toluene–$CHCl_3$–EtOAc, 3:2:5) of the residue afforded **25** (40 mg, 81%), $[\alpha]_D^{22}$ +79° (*c* 0.5), R_F 0.26. ^{1}H-N.m.r. data: δ 1.09 (s, 9 H, CMe_3), 4.55 (d, 1 H, *J* 7.3 Hz, H-1a).

tert-*Butyldiphenylsilyl* O-*(2,3,4-tri*-O-*benzyl*-α-D-*galactopyranosyluronic acid)*-*[(1→4)*-O-*(2,3-di*-O-*benzyl*-α-D-*galactopyranosyluronic acid)]$_{10}$*-*(1→4)*-*2,3-di*-O-*benzyl*-β-D-*galactopyranosiduronic acid* (**27**). — Dry methyl sulfoxide (100 μL, 1.4 mmol) was added to a solution of oxalyl chloride (53 μL, 608 μmol) in dry CH_2Cl_2 (1.3 mL) under argon with stirring and cooling (−78°). After 15 min, a solution of **25** (40 mg, 9.0 μmol) in dry CH_2Cl_2 (1.5 mL) was added, stirring was continued for 15 min, then *N*,*N*-di-isopropylethylamine (450 μL, 2.6 mmol) was added. After stirring for 5 min, the mixture was allowed to warm to room temperature, stirred for 15 min, diluted with $CHCl_3$ (30 mL), washed successively with dilute HCl, water, and brine, dried (Na_2SO_4), and concentrated *in vacuo*. A solution of the resulting crude aldehyde **26** in ^{t}BuOH (2.5 mL) and 2-methyl-2-butene (0.6 mL, 5.7 mmol) was stirred overnight with a solution of $NaClO_2$ (105 mg, 1.2 mmol) and $NaH_2PO_4 \cdot 2H_2O$ (105 mg, 0.67 mmol) in water (1.1 mL). The mixture was concentrated *in vacuo*, diluted with water, and extracted with *n*-hexane, and the aqueous layer was acidified with dilute HCl and extracted with EtOAc. The extract was washed with water and brine, dried (Na_2SO_4), and concentrated *in vacuo*. Preparative t.l.c. ($CHCl_3$–acetone–AcOH, 9:1:1) of the residue gave a fraction that contained **27**, which was extracted with $CHCl_3$–MeOH–AcOH (8:1:1) and concentrated *in vacuo*. A solution in EtOAc was washed with dilute HCl, water, and brine, dried (Na_2SO_4), and concentrated *in vacuo* to give **27** (21 mg).

A solution of **27** (12 mg, 2.6 μmol) in EtOAc (0.5 mL) was treated with freshly distilled ethereal diazomethane (large excess), then concentrated *in vacuo*. Preparative t.l.c. (toluene–EtOAc, 7:3) of the residue gave **28** (10 mg), R_F 0.56 (7:3 toluene–EtOAc). ^{1}H-N.m.r. data: δ 1.170 (s, 9 H, CMe_3), 3.087 (s, 3 H, OMe), 3.194 (s, 3 H, OMe), 3.199 (bs, 6 H, 2 OMe), 3.204 (bs, 6 H, 2 OMe), 3.208 (s, 3 H, OMe), 3.237 (s, 3 H, OMe), 3.253 (s, 3 H, OMe), 3.365 (s, 3 H, OMe), 3.443 (s, 3 H, OMe), 3.449 (s, 3 H, OMe).

A mixture of **28** (14.5 mg, 3.0 μmol) and anhydrous LiI (45 mg, 336 μmol) in dry pyridine (6 mL) was heated under reflux under argon for 30 h, then concentrated *in vacuo*. A solution of the residue in water was acidified with dilute HCl and extracted with EtOAc, and the extract was washed with water and brine, dried (Na_2CO_3), and

concentrated *in vacuo*. Preparative t.l.c. ($CHCl_3$–acetone–AcOH, 9:1:1) of the residue followed by work-up, as described above, afforded **27** (9.3 mg), $[\alpha]_D^{25}$ +100° (*c* 0.9). ^{1}H-N.m.r. data: δ 1.17 (s, 9 H, CMe_3).

O-*(2,3,4-Tri-O-benzyl-α-D-galactopyranosyluronic acid)-[(1→4)-O-(2,3-di-O-benzyl-α-D-galactopyranosyluronic acid)]$_{10}$-(1→4)-2,3-di-O-benzyl-D-galactopyranuronic acid* (**29**). — To a mixture of **28** (9.3 mg, 2.0 μmol) and AcOH (10 μL, 175 μmol) in dry tetrahydrofuran (0.8 mL) was added M *n*-Bu_4NF in tetrahydrofuran (100 μL, 100 μmol). The mixture was stirred at room temperature for 5 days, diluted with EtOAc, washed with dilute HCl (pH 2), water, and brine, dried (Na_2SO_4), and concentrated *in vacuo*. Preparative t.l.c. ($CHCl_3$–MeOH–AcOH, 18:2:1) of the residue gave a product, a solution of which in EtOAc was washed with dilute HCl, water, and brine, dried (Na_2SO_4), and concentrated *in vacuo* to give **29** (6.6 mg, 74.8%). ^{1}H-N.m.r. data: δ 5.07 (d, 1 H, *J* 2.8 Hz) and 5.14 (bs, 10 H) (H-1b,1c,1d,1e,1f,1g,1h,1i,1j,1k,1*l*), 5.41 (d, *J* 3.1 Hz, H-1aα).

O-*(α-D-Galactopyranosyluronic acid)-[(1→4)-O-(α-D-galactopyranosyluronic acid)]$_{10}$-(1→4)-D-galactopyranuronic acid* (**1**). — A mixture of **29** (4.2 mg, 0.96 μmol) and 10% Pd–C (3 mg) in aqueous 80% MeOH was stirred in an atmosphere of hydrogen for 4 days at room temperature, then filtered through Celite, and concentrated *in vacuo*. The residue was washed through a short column of Sephadex LH-20 with water, and the resulting crude product was purified by anion-exchange chromatography on Mono-Q (HR 5/5) with a linear gradient of $NH_4 \cdot HCO_3$ buffer (0.2→1M). Fractions were assayed for uronosyl residue by the *m*-hydroxybiphenyl method[19]. The fractions containing **1** were combined and concentrated *in vacuo*, and $NH_4 \cdot HCO_3$ was removed by evaporation of water several times from the residue, to leave **1** (1.5 mg), $[\alpha]_D^{24}$ +81° (*c* 0.1, water). ^{1}H-N.m.r. data (D_2O, ^{t}BuOH standard, 80°): δ 3.49 (dd, *J* 7.6 and 10.1 Hz, H-2aβ), 3.76 (bd, 12 H, H-2b,2c,2d,2e,2f,2g,2h,2i,2j,2k,2*l*, and H-3a), 3.90 (dd, 1 H, *J* 3.7 and 10.1 Hz, H-3*l*), 3.98 (bd, 1 H, H-3b,3c,3d,3e,3f,3g,3h,3i,3j,3k, and 3*l*), 4.45 (bd, 11 H, H-4b,4c,4e,4f,4g,4h,4i,4j,4k, and 4*l*), 4.58 (d, *J* 7.6 Hz, H-1aβ), 4.77 (bs, 11 H, H-5b,5c,5d,5e,5f,5g,5h,5i,5j,5k, and 5*l*), 5.10 (bs, 11 H, H-1b,1c,1d,1e,1f,1g,1h,1i,1j,1k, and 1*l*), 5.31 (bd, H-1aα).

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REFERENCES

1 Y. Nakahara and T. Ogawa, *Carbohydr. Res.*, 200 (1990) 363–376.
2 E. A. Nothnagel, M. McNeil, P. Albersheim, and A. Dell, *Plant Physiol.*, 71 (1983) 916–926; M. G. Hahn, A. G. Darvill, and P. Albersheim, *ibid.*, 68 (1981) 1161–1169.
3 K. R. Davis, G. D. Lyon, A. G. Darvill, and P. Albersheim, *Plant Physiol.*, 74 (1984) 52–60; K. R. Davis, A. G. Darvill, P. Albersheim, and A. Dell, *ibid.*, 80 (1986) 568–577.
4 D. F. Jin and C. A. West, *Plant Physiol.*, 74 (1984) 989–992.
5 P. Bishop, D. J. Makus, G. Pearce, and C. A. Ryan, *Proc. Natl. Acad. Sci. U.S.A.*, 78 (1981) 3536–3540.
6 A. G. Darvill and P. Albersheim, *Annu. Rev. Plant Physiol.*, 35 (1984) 243–275; M. McNeil, A. G. Darvill, S. C. Fry, and P. Albersheim, *Annu. Rev. Biochem.*, 53 (1984) 625–663.
7 Y. Nakahara and T. Ogawa, *Carbohydr. Res.*, 167 (1987) c1–c7; Y. Nakahara and T. Ogawa, *ibid.*, 194 (1989) 95–114.
8 E. J. Corey and J. W. Suggs, *J. Org. Chem.*, 38 (1973) 3224; P. A. Gent and R. Gigg, *J. Chem. Soc., Chem. Commun.*, (1974) 277–278.
9 T. Mukaiyama, Y. Murai, and S. Shoda, *Chem. Lett.*, (1981) 431–432.
10 K. Bock, I. Lundt, and C. Pedersen, *Tetrahedron Lett.*, (1973) 1037 – 1040; K. Bock and C. Pedersen, *J. Chem. Soc., Perkin Trans. 2* (1974) 293–297.
11 W. Kinzy and R. R. Schmidt, *Liebigs Ann. Chem.*, (1985) 1537–1545.
12 W. Rosenbrook, Jr., D. A. Riley, and P. A. Lartey, *Tetrahedron Lett.*, 26 (1985) 3–4; G. H. Posner and S. R. Haines, *ibid.*, 26 (1985) 5–8.
13 K. Omura and D. Swern, *Tetrahedron*, 34 (1978) 1651–1660.
14 G. A. Kraus and B. Roth, *J. Org. Chem.*, 45 (1980) 4825–4830.
15 J. McMurry, *Org. React.*, 24 (1976) 187–224.
16 F. Cervone, M. G. Hahn, G. D. Lorenzo, A. Darvill, and P. Albersheim, *Plant Physiol.*, 90 (1989) 542–548.
17 M. G. Hahn, personal communication.
18 K. R. Davis, A. G. Darvill, and P. Albersheim, *Plant Mol. Biol.*, 6 (1986) 23–32.
19 N. Blumenkrantz and G. Asboe-Hansen, *Anal. Biochem.*, 54 (1973) 484–489.