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SYNTHESIS OF A BRANCHED D-GLUCOTETRAOSE, THE REPEATING UNIT OF THE EXTRACELLULAR POLYSACCHARIDES OF *Grifola umbellate*, *Sclerotinia libertiana*, *Porodisculus pendulus*, AND *Schizophyllum commune* FRIES*

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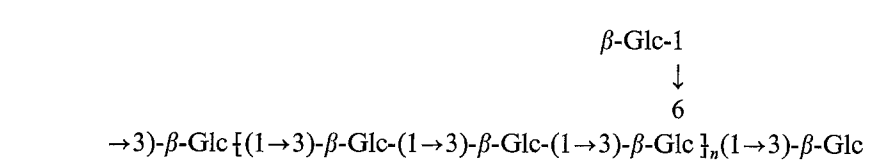
ABSTRACT

The synthesis of D-glucotetraose, 3-*O*-[3-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl]-6-*O*-*β*-D-glucopyranosyl-α (and *β*)-D-glucopyranose, the repeating unit of the extracellular polysaccharides of *Grifora umbellata*, *Sclerotinia libertiana*, *Porodisculus pendulus*, and *Schizophyllum commune* Fries, is described.

INTRODUCTION

Recently, it was proposed that the structure of the extracellular polysaccharides of *Grifola umbellata*[2], *Sclerotinia libertiana*[3], and *Porodisculas pendulus*[4] is **1**. Water-soluble D-glucan **1** from *Grifora umbellata* had been reported[5] to inhibit the growth of subcutaneously implanted Sarcoma 180 in mice. Quite recently, schizophyllan, a water-soluble *β*-D-glucan elaborated by *Schizophyllum commune* Fries, was partially depolymerized by ultrasonic irradiation to a low-molecular-weight polysaccharide[6] that exhibited the same antitumor activity against Sarcoma-180 as the native schizophyllan, and the structure of which was confirmed to be **1**, as proposed earlier for the structure of the native D-glucan[7].



1

A similar (1→3)-*β*-D-glucan structure was also observed for the extracellular polysaccharides of *Rhizobium japonicum*[8], which may play a role in the specificity of symbioses between Rhizobium species and their plant hosts[9]. A family of *β*-D-

*Glucan Synthesis, Part II. For Part I, see ref. 1.
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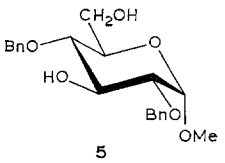
glucans of very similar structure had been isolated from the soybean pathogen *Phytophthora megasperma* var. *sojae*, and they were demonstrated to elicit the formation of phytoalexins from soybeans and potatoes[10].

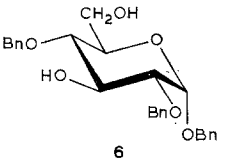
β-Glc	β-Glc	β-Glc
1	1	1
↓	↓	↓
6	6	6
α-Glc-(1→OMe	α (and β)-Glc-(1→OH)	α (and β)-Glc-(1→OH)
3	3	3
↑	↑	↑
1	β-Glc-1	1
β-Glc		β-Glc-(1→3)-β-Glc
2	**3**	**4**

In view of the biological significance, the repeating unit (**4**) of the β-D-glucan structure **1**, as well as simpler structures **2** and **3**, were chosen as the synthetic targets for the present study. Retrosynthetic examination of these target molecules demonstrated the necessary synthons **5** and **6** as two glycosyl acceptors, and **7** and **8** as two glycosyl donors.



5

Bn = $PhCH_2$



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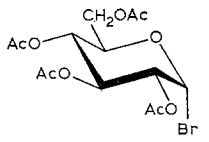

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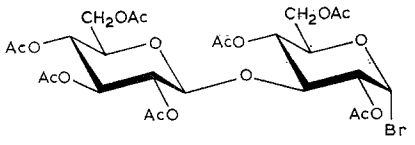


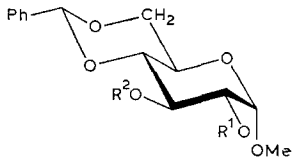
8

RESULTS AND DISCUSSION

*Synthesis of methyl 3,6-di-*O*-β-D-glucopyranosyl-β-D-glucopyranoside* (**2**). — Until 1979, 3,6-di-*O*-β-D-glucopyranosyl-D-glucopyranose derivatives had, to the best of our knowledge, been synthesized[11] in low yield as byproducts of the Koenigs–Knorr reactions between 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (7)

and alkyl 4,6-*O*-benzylidene-β-D-glucopyranosides. In 1980, Eby and Schuerch[12] reported a regioselective synthesis of 2-(4-aminophenyl)ethyl 3,6-di-*O*-β-D-glucopyranosyl-α-D-glucopyranoside that employed, as the key glycosyl acceptor, 2-[4-(*p*-toluenesulfonamido)phenyl]ethyl 2,4-di-*O*-benzyl-α-D-glucopyranoside, which, in turn, was prepared *via* 1,6-anhydro-2,4-di-*O*-benzyl-β-D-glucopyranose[13]. Quite recently, Koto *et al.*[14] reported a regioselective synthesis of 3,6-di-*O*-β-D-glucopyranosyl-β-D-glucopyranose (**3**) that used, as the glycosyl acceptor, benzyl 3-*O*-acetyl-2,4-di-*O*-benzyl-α-D-glucopyranoside, which, in turn, was prepared *via* partial benzylation of benzyl 6-*O*-trityl-α-D-glucopyranoside.

Our synthetic approach to the synthesis of glycosyl acceptors **5** and **6** depends on the regioselective hydrogenolysis of the benzylidene group of **10** and **17** according to the method of Lipták *et al.*[15]. In order to synthesize monobenzyl ether **10**, regioselective benzylation of diol **9** was studied by using three different methods. First, diol **9** was converted into the stannylidene derivative[16] by treatment with Bu_2SnO, and subsequent reaction with benzyl bromide in $HCONMe_2$ gave 2-benzyl ether[17,18] **10** and 3-benzyl ether[17–19] **11** in 69.8 and 20.2% yield, respectively. Second, tributylstannylation[20] of **9**, and subsequent alkylation with benzyl bromide, gave monobenzyl ethers **10** and **11** in 59.1 and 31.9% yield, respectively. Finally, monobenzylation of **9**, using a phase-transfer catalyst according to Garegg *et al.*[18], gave **10** and **11** in 40 and 20% yield, respectively. Therefore, in our hands, the stannylidene approach was found to give the highest yield of the desired monobenzyl ether **10**.

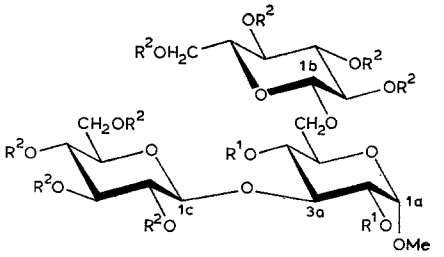


9 $R^1 = R^2 = H$
10 $R^1 = Bn, R^2 = H$
11 $R^1 = H, R^2 = Bn$

Reductive cleavage[15] of the benzylidene group in **10** was achieved with $LiAlH_4$–$AlCl_3$, to give crystalline dibenzyl ether[17] **5** in 71% yield. The assigned structure **5** was confirmed by ^{13}C-n.m.r. data, which showed two deshielded signals for C-2 and C-4 at δ 79.6 and 77.1, due to benzylation at O-2 and O-4, and a shielded signal for C-1 at δ 97.5, due to the β-effect of alkylation at O-2. Absence of the signal[1] at δ ~82.5–82.8, and the presence of a signal at δ 61.9 for C-6, proved that the two benzyl groups were linked neither to O-3 nor O-6. Furthermore, signals for methylene carbon atoms of 2-*O*-benzyl and 4-*O*-benzyl groups appeared at δ 73.1 and 74.5, in agreement with a previous observation[1].

Glycosylation of **5** with 3 equivalents of the D-glucosyl donor **7**, in the presence[21] of $HgBr_2$–powdered molecular sieve 4 A at 45°, gave protected trisaccharide **12** in 59.3% yield. Zemplén deacetylation of **12**, to give **13** in 63% yield, and hydrogenolysis

of **13**, afforded D-glucotrioside **2** in 97% yield. The configurations of the newly introduced anomeric centers were assignable as β from the ^{1}H- and ^{13}C-n.m.r. data. Three anomeric protons were observed as three doublets at δ 4.71, with J 4.0 Hz for H-1a, and δ 4.54 and 4.38, both with J 8.0 Hz, for H-1c and H-1b, respectively.

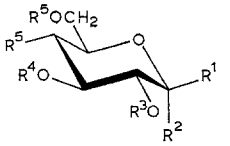


12 R^1 = Bn, R^2 = Ac
13 R^1 = Bn, R^2 = H
2 R^1 = R^2 = H

Two signals at δ 103.15, with $^1J_{CH}$ 160.2 Hz, and δ 99.64, with $^1J_{CH}$ 169.9 Hz, were observed in the ^{13}C-n.m.r. spectrum in the ratio of 2:1, and were assigned to C-1b and C-1c, and C-1a, respectively, from the value[22] of $^1J_{CH}$.

Synthesis of glucotrioside **3** *and glucotetraoside* **4**. — The key glycosyl acceptor **6** was prepared from D-glucose in 4 steps, as follows. D-Glucose was successively treated with (*i*) benzyl alcohol–HCl, and (*ii*) $PhCH(OMe)_2$–$TsOH \cdot H_2O$, to give crystalline benzyl 4,6-*O*-benzylidene-α-D-glucopyranoside[23] (**15**) in 10.6% yield without use of chromatography. Selective monobenzylation of **15** by the stannylidene method afforded a 77% yield of crystalline 2-benzyl ether **16**. The site of benzylation was proved to be O-2 by the transformation of **16** into monoacetate **17**, whose ^{1}H-n.m.r. data revealed a deshielded signal for H-3 as a triplet at δ 5.60 with J 10 Hz, along with a signal for the methyl group of acetate as a singlet at δ 2.03.

Selective cleavage[15] of the benzylidene group of **16** afforded the desired glycosyl acceptor **6** in 53% yield. The substitution pattern of **6** was expected to be at O-2 and O-4 (from the synthetic sequence), and this was confirmed by the ^{13}C-n.m.r.

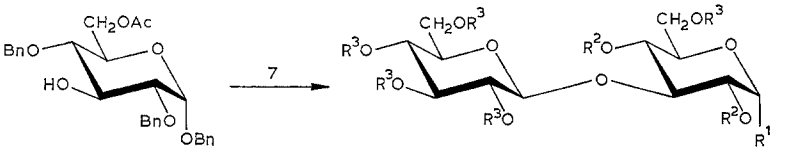


14 R^1 (or R^2) = OBn, R^2 (or R^1) = H
R^3 = R^4 = R^5 = H
15 R^1 = R^3 = R^4 = H, R^2 = OBn, R^5, R^5 = CHPh
16 R^1 = R^4 = H, R^2 = OBn, R^3 = Bn, R^5, R^5 = CHPh
17 R^1 = H, R^2 = OBn, R^3 = Bn, R^4 = Ac, R^5, R^5 = CHPh

spectrum, which contained two deshielded signals, for C-2 and C-4, at δ 79.53 and 77.15, respectively, and the signal for C-6 remained shielded at δ 61.83.

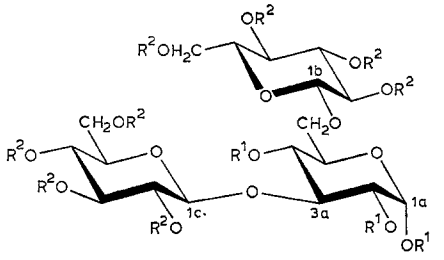
The practical synthesis of laminarabiosyl donor **8** was recently reported, either by use[24] of a partially protected α-D-glucopyranoside, or by acetolysis[25] of laminaran. We have developed an alternative route to **8** by use of partially benzylated α-D-glucopyranoside **6**.

Selective acetylation of the primary hydroxyl group of **6** gave an 82% yield of monoacetate **18**. Reaction of **18** with the D-glucosyl donor **7**, in the presence of $HgBr_2$ and powdered molecular sieve 4 A, led to the isolation of protected laminarabioside **19** in 73% yield. Hydrogenolysis of **19**, to give **20** in 57.5% yield, and acetylation of **20**, afforded the octaacetate **21** as a mixture of the α and β anomer in the ratio of 1:1. Oily **21** was converted into crystalline **8** in 81% yield. The ^{1}H-n.m.r. data for **8** confirmed the structure assigned.



18

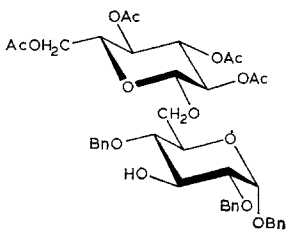
19 R^1 = OBn, R^2 = Bn, R^3 = Ac
20 R^1 = OH, R^2 = H, R^3 = Ac
21 R^1 = OAc, R^2 = R^3 = Ac



22 R^1 = Bn, R^2 = Ac
23 R^1 = Bn, R^2 = H
3 R^1 = R^2 = H

Having prepared the key glycosyl acceptor **6**, and the two glycosyl donors **7** and **8**, syntheses of D-glucotriose (**3**) and D-glucotetraose (**4**) were examined. Glycosylation of **6** with 1.2 equivalents of the D-glucosyl donor **7** according to Sinaÿ *et al.*[21] led to the isolation of properly protected glucobioside **24** in 26% yield, and a 50% recovery of starting diol **6**. On the other hand, glycosylation of **6** with 3.6 equivalents of **7**, using the same Lewis acid and acid captor, afforded a 47% yield of protected D-glucotrioside **22**. Deacetylation of **22**, to give **23**, and hydrogenolysis of **23**, gave

the desired D-glucotriose **3**. The ^{13}C-n.m.r. data confirmed the regio- and stereo-structure of **3**, which showed a signal for C-1b and C-1c at δ 103.04, with $^1J_{CH}$ 162.1 Hz, and two signals, for C-1aα and C-1aβ, in the ratio of 1:1, at δ 96.02 with $^1J_{CH}$ 161.1 Hz, and at δ 92.36 with $^1J_{CH}$ 169.9 Hz, respectively. Two deshielded signals for C-3aβ and C-3aα were also observed, at δ 84.72 and 82.46.



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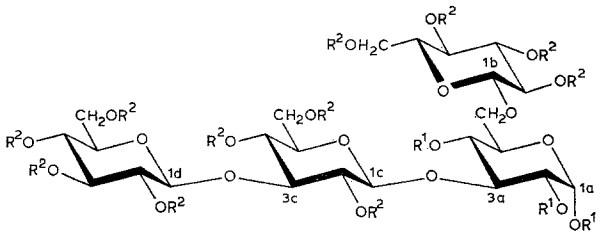

25 R^1 = Bn, R^2 = Ac
26 R^1 = Bn, R^2 = H
4 R^1 = R^2 = H

Properly protected D-glucobioside **24** was now submitted to the glycosylation reaction with **8**, in the presence of $AgOSO_2CF_3$ and powdered molecular sieve 4 A, to give an 81% yield of the protected D-glucotetraoside **25**. Deacylation of **25** to give tribenzyl ether **26** in 76% yield, and hydrogenolysis of **26**, afforded the target D-glucotetraose **4**. The regiochemistry of **4** was evident from the synthetic sequence, and the configurations of the anomeric centers newly introduced were determined to be all β by ^{13}C-n.m.r. data, which contained a signal for C-1b, C-1c, and C-1d at δ 103.08, with $^1J_{CH}$ 163.1 Hz, and two signals, for C-1aβ and C-1aα in the ratio of 1:1, at δ 96.06 with $^1J_{CH}$ 157.6 Hz, and at δ 92.36, with $^1J_{CH}$ 169.9 Hz, respectively. Two deshielded signals for C-3c and C-3aβ, and for C-3aα, were observed at δ 84.56 and 82.30, respectively, due to the α-effect of glucosylation.

In conclusion, the first synthesis of D-glucotetraose **4**, the repeating unit of the extracellular D-glucans of *Grifola umbellata*, *Sclerotinia libertiana*, *Porodisculus pendulus*, and *Schizophyllum commune* Fries was achieved in a regio- and stereo-controlled way by using the properly protected D-glucobioside **24** as the key intermediate.

EXPERIMENTAL

General. — Melting points were determined with a Yanagimoto micro melting-point apparatus and are uncorrected. Optical rotations were determined with a Perkin–Elmer Model 141 polarimeter for solutions in $CHCl_3$ at 25°, unless otherwise noted. I.r. spectra were recorded with an EPI-G2 Hitachi Spectrophotometer, using KBr discs for the crystalline samples, and neat films for the liquid samples. ^{1}H-N.m.r. spectra were recorded with a Varian HA-100 n.m.r. spectrometer, using tetramethylsilane as the internal standard. ^{13}C-N.m.r. spectra were recorded with a JNM-FX 100FT n.m.r. spectrometer operated at 25.05 MHz. The values of δ_C and

δ_H are expressed in p.p.m. downwards from the internal standard, for solutions in $CDCl_3$, unless otherwise noted. Flash column-chromatography[26] was performed on columns of Wakogel C-300 (Wako Pure Chemical Industries, Ltd.). Thin-layer chromatography was performed on precoated plates (layer thickness, 0.25 mm) of Silica Gel 60 F_{254} (E. Merck, Darmstadt, Germany).

*Methyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-*D*-glucopyranoside* (**10**) *and methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-*D*-glucopyranoside* (**11**). — (*A*) A mixture of **9** (843 mg, 3 mmol) and Bu_2SnO (903 mg, 3.6 mmol) in 10:1 benzene–MeOH (20 mL) was boiled under reflux for 2 h, cooled, and evaporated *in vacuo*. A mixture of the residue with DMF (5 mL)–benzyl bromide (5 mL) was stirred for 2 h at 90–110°, cooled, evaporated *in vacuo*, and the residual oil chromatographed on SiO_2 (100 g) with 7:2 toluene–EtOAc, to give **10** (776 mg, 69.8%), m.p. 126–127° (EtOH), $[\alpha]_D$ +34.7° (*c* 0.95); R_F 0.40 in 7:2 toluene–EtOAc; δ_H: 5.48 (s, 1 H, C*H*Ph), 4.59 (d, 1 H, *J* 4 Hz, H-1), and 3.35 (s, 3 H, OMe).

Anal. Calc. for $C_{21}H_{24}O_6$: C, 67.73; H, 6.50. Found: C, 67.85; H, 6.51.

Further elution gave **11** (225 mg, 20.2%), m.p. 188–189° (EtOH), $[\alpha]_D$ +59.3° (*c* 1.00); R_F 0.29 in 7:2 toluene–EtOAc; δ_H: 5.54 (s, 1 H, C*H*Ph), 4.78 (d, 1 H, *J* 4 Hz, H-1), and 3.42 (s, 3 H, OMe).

Anal. Calc. for $C_{21}H_{24}O_6$: C, 67.73; H, 6.50. Found: C, 67.51; H, 6.46.

(*B*) A mixture of **9** (11.28 g, 40 mmol) and $(Bu_3Sn)_2O$ (17.88 g, 30 mmol) in toluene (100 mL) was boiled under reflux for 15 h, with continuous azeotropic removal of water, and evaporated *in vacuo*. A mixture of the residual oil with benzyl bromide (100 mL) was stirred for 3 days at 80–83° under argon, cooled, and evaporated *in vacuo*; the residue was mixed with EtOAc (100 mL), the mixture treated with[27] aq. KF, and the suspension filtered. The filtrate was washed with H_2O, dried ($MgSO_4$), and evaporated, and the residue was chromatographed on SiO_2 (1 kg) with 3:1 toluene–EtOAc, to give **10** (7.03 g, 59.1%) and **11** (3.75 g, 31.9%).

*Methyl 2,4-di-*O*-benzyl-α-*D*-glucopyranoside* (**5**). — To a stirred solution of **10** (500 mg, 1.34 mmol) in 1:1 Et_2O–CH_2Cl_2 (10 mL) was added, portionwise, $LiAlH_4$ (170 mg, 4.48 mmol). To this stirred mixture was added, dropwise, a solution of $AlCl_3$ (500 mg, 3.75 mmol) in Et_2O (5 mL) during 30 min under reflux. After refluxing for a further 1 h, the excess of $LiAlH_4$ was decomposed by adding EtOAc and H_2O. The mixture was diluted with EtOAc (100 mL), washed with H_2O, dried ($MgSO_4$), and evaporated *in vacuo*. The residue was chromatographed on SiO_2 (45 g) with 1:1 toluene–EtOAc, to give **5** (355 mg, 71%), m.p. 71–72° (EtOH–pentane), $[\alpha]_D$ +98.3° (*c* 1.00); R_F 0.51 in 1:1 toluene–EtOAc; δ_H: 7.4–7.2 (m, 10 H, aromatic), 4.90 and 4.66 (ABq, 2 H, *J* 11 Hz, CH_2-Ph), 4.65 (s, 2 H, CH_2Ph), 4.58 (d, 1 H, *J* 4 Hz, H-1), 4.08 (t, 1 H, *J* 9 Hz, H-3), and 3.29 (s, 3 H, OMe); δ_C: 97.5 ($^1J_{CH}$ 167.0 Hz, C-1), 79.6 (C-2), 77.1 (C-4), 74.5 (4-*O*-CH_2Ph), 73.4 (C-3), 73.1 (2-*O*-CH_2Ph), 70.2 (C-5), 61.9 (C-6), and 55.1 (OMe).

Anal. Calc. for $C_{21}H_{26}O_6$: C, 67.36; H, 7.00. Found: C, 67.37; H, 7.00.

*Methyl 2,4-di-*O*-benzyl-3,6-di-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl)-α-*D*-glucopyranoside* (**12**). — To a stirred mixture of powdered molecular sieve 4 A

(1 g), $HgBr_2$ (108 mg, 0.3 mmol), and **5** (190 mg, 0.5 mmol) in $Cl(CH_2)_2Cl$ (5 mL) was added dropwise a solution of **7** (617 mg, 1.5 mmol) in $Cl(CH_2)_2Cl$ (5 mL). The mixture was stirred for 4 h at 15–20° and then for 4 h at 45°, Et_3N (2 mL) was added, the mixture was evaporated *in vacuo*, and the residue was chromatographed on SiO_2 C-300 (70 g) with 2:1 toluene–EtOAc, to give **12** (313 mg, 59.3%); R_F 0.23 in 2:1 toluene–EtOAc.

*Methyl 2,4-di-*O*-benzyl-3,6-di-*O*-β-*D*-glucopyranosyl-α-*D*-glucopyranoside* (**13**). — A solution of **12** (313 mg, 0.30 mmol) in MeOH (5 mL)–M NaOMe (0.3 mL) was stirred for 15 h at 15–20°, the base neutralized with Amberlist 15 (H^+), and the suspension filtered. The filtrate was evaporated *in vacuo*, and the residue was chromatographed on SiO_2 C-300 (10 g) with 15:2 CH_2Cl_2–MeOH, to give **13** (134 mg, 63.0%); R_F 0.18 in 15:2 CH_2Cl_2–MeOH.

*Methyl 3,6-di-*O*-β-*D*-glucopyranosyl-α-*D*-glucopyranoside* (**2**). — A mixture of **13** (134 mg, 0.19 mmol) and 10% Pd–C (50 mg) in AcOH (2 mL) was stirred under H_2 for 24 h at 15–20°. Processing afforded an oily product that still carried some benzyl groups (as judged by its ^{1}H-n.m.r. spectrum). A mixture of this oil and 10% Pd–C (100 mg) in 1:1 EtOH–H_2O (2 mL) was stirred under H_2 for 2 days; processing then afforded amorphous **2** (98.6 mg, 97%) $[\alpha]_D$ +28.5° (*c* 0.50, MeOH); R_F 0.19 in 10:5:4 1-BuOH–i-PrOH–H_2O; δ_H (D_2O, 75°, 400 MHz): 4.708 (d, 1 H, *J* 4.0 Hz, H-1a), 4.538 (d, 1 H, *J* 8.0 Hz, H-1c), and 4.381 (d, 1 H, *J* 8.0 Hz, H-1b); δ_C (D_2O, 75°): 103.15 ($^1J_{CH}$ 160.2 Hz, C-1b,1c), 99.64 ($^1J_{CH}$ 169.9 Hz, C-1a), 82.53 (C-3a), 76.25 (C-3b*,3c*), 75.94 (C-5b*,5c*), 73.80 (C-2b**), 73.48 (C-2c**), 71.07 (C-5a), 70.83 (C-2a), 69.94 (C-4b,4c), 68.85 (C-6a), 68.14 (C-4a), 61.09 (C-6b,6c), and 55.63 (OMe).

Anal. Calc. for $C_{19}H_{34}O_{16} \cdot H_2O$: C, 42.53; H, 6.76. Found: C, 42.62; H, 6.87.

*Benzyl 4,6-*O*-benzylidene-α-*D*-glucopyranoside*[23] (**15**). — To benzyl alcohol (500 mL) was added AcCl (50 mL) dropwise, and the mixture was stirred for 15 min at 20°. To the solution was added D-glucose (100 g), and the mixture was stirred for 3 days at 15–20° and for 1 day at 50°, the acid neutralized with Amberlist **21** (OH^-), and the suspension filtered. The filtrate was evaporated *in vacuo*, the residual oil dissolved in DMF (100 mL), $PhCH(OMe)_2$ (244 mL) and $TsOH \cdot H_2O$ (13 g) were added, the mixture was stirred for 2 days at 40–45°, and the acid was neutralized with Et_3N. The solution was evaporated *in vacuo*, the residue was partitioned between EtOAc and H_2O, the organic layer was dried ($MgSO_4$), and evaporated *in vacuo*, and the residue was triturated with EtOH, to give crystalline **15** (38.1 g, 10.6%); m.p. 158–159° (CH_2Cl_2–Et_2O), $[\alpha]_D$ +131.3° (*c* 1.22); R_F 0.28 in 1:1 toluene–EtOAc; δ_H: 5.50 (s, 1 H, C*H*Ph), 4.97 (d, 1 H, *J* 4 Hz, H-1), 4.77 (d, 1 H, *J* 11 Hz, CH_2Ph), and 4.53 (d, 1 H, *J* 11 Hz, CH_2Ph).

Anal. Calc. for $C_{20}H_{22}O_6$: C, 67.02; H, 6.19. Found: C, 66.99; H, 6.21.

*Benzyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-*D*-glucopyranoside* (**16**). — A mixture of **15** (15 g, 42 mmol) and Bu_2SnO (10.5 g, 42 mmol) in 10:1 benzene–MeOH (132

*The assignments with asterisks may have to be interchanged.

mL) was boiled, and stirred, under reflux for 2.5 h, cooled, and evaporated; the residue was dissolved in DMF (70 mL), benzyl bromide (70 mL) was added, and the mixture was stirred for 3.5 h at 100–120°, cooled, and evaporated *in vacuo*. A solution of the residue in EtOAc (400 mL) was stirred with aq. KF for 3 h, and the suspension was filtered, to remove precipitated Bu_2SnF_2. The filtrate was dried ($MgSO_4$), and evaporated, to afford an oily product which was triturated with EtOH, to give crystalline **16** (14.3 g, 77%), m.p. 133–134° (EtOH), $[\alpha]_D$ +110.6° (*c* 1.1); R_F 0.52 in 3:1 toluene–EtOAc; δ_H: 7.5–7.3 (m, 15 H, aromatic) and 5.54 (s, 1 H, C*H*Ph).

The 3-acetate of **17** was prepared in the usual way; δ_H 5.60 (t, 1 H, *J* 10 Hz, H-3) and 2.03 (s, 3 H, Ac).

*Benzyl 2,4-di-*O*-benzyl-α-*D*-glucopyranoside* (**6**). — To a stirred mixture of **16** (5.0 g, 14.5 mmol) and $LiAlH_4$ (2.2 g, 58.0 mmol) in 1:1 CH_2Cl_2–Et_2O (100 mL), was added, dropwise, a solution of $AlCl_3$ (7.7 g, 58.0 mmol) in Et_2O (50 mL) during 30 min under reflux, and the mixture was stirred for 2 h under reflux. Processing as described for **5**, and purification by flash chromatography on SiO_2 C-300 (300 g) with 3:1 toluene–EtOAc, gave **6** (2.66 g, 53%), m.p. 77–78° (EtOH–pentane), $[\alpha]_D$ +157.6° (*c* 1.3); R_F 0.27 in 3:1 toluene–EtOAc; δ_C: 95.16 ($^1J_{CH}$ 167.0 Hz, C-1), 79.53 (C-2), 77.15 (C-4), 74.58 (4-*O*-CH_2Ph), 73.33 (C-3), 72.63 (2-*O*-CH_2Ph), 70.68 (C-5), 69.31 (1-*O*-CH_2Ph), and 61.83 (C-6).

Anal. Calc. for $C_{27}H_{30}O_6$: C, 72.00; H, 6.71. Found: C, 71.72; H, 6.75.

*Benzyl 6-*O*-acetyl-2,4-di-*O*-benzyl-α-*D*-glucopyranoside* (**18**). — To a stirred solution of **6** (3.0 g, 6.76 mmol) in CH_2Cl_2 (10 mL)–pyridine (1.09 mL, 13.5 mmol) was added AcCl (0.53 mL, 7.44 mmol) dropwise at −10 to −15°; the mixture was stirred for 2 h, further AcCl (0.03 mL) was added (in order to consume **6** completely), and extractive processing then afforded an oily product which was chromatographed on SiO_2 C-300 (300 g) with 10:1 toluene–EtOAc, to give **18** (2.68 g, 82%), $[\alpha]_D$ +157.2° (*c* 1.3); R_F 0.51 in 3:1 toluene–EtOAc; δ_H: 2.0 (s, 3 H, Ac).

Anal. Calc. for $C_{29}H_{32}O_7$: C, 70.71; H, 6.55. Found: C, 70.75; H, 6.60.

*Benzyl 6-*O*-acetyl-2,4-di-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl)-α-*D*-glucopyranoside* (**19**). — To a stirred mixture of **18** (3.5 g, 7.1 mmol), powdered molecular sieve 4 A (5.0 g), and $HgBr_2$ (1.024 g, 2.84 mmol) in $Cl(CH_2)_2Cl$ (5 mL) was added dropwise a solution of **7** (4.5 g, 10.9 mmol) in $Cl(CH_2)_2Cl$ (15 mL). The mixture was stirred for 2.5 h at 20°, filtered through Celite, and the filtrate successively washed with 3% aq. $AgNO_3$ and aq. $NaHCO_3$, dried ($MgSO_4$), and evaporated *in vacuo*. The residue was chromatographed on SiO_2 C-300 (200 g) with 8:1 toluene–EtOAc, to give **19** (4.3 g, 73.0%), $[\alpha]_D$ +65.6° (*c* 1.5); R_F 0.47 in 8:1 toluene–EtOAc; δ_H: 7.38 (bs, 15 H, aromatic), 2.10 (s, 3 H, Ac), 2.04 (s, 9 H, 3 Ac), and 1.98 (s, 3 H, Ac).

Anal. Calc. for $C_{43}H_{50}O_{16}$: C, 62.77; H, 6.12. Found: C, 62.30; H, 6.11.

*6-*O*-Acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl)-*D*-glucopyranose* (**20**). — A mixture of **19** (1.84 g, 2.24 mmol) and 10% Pd–C (500 mg) in AcOH (15 mL) was stirred for 2 days at 40° under H_2, and then filtered through Celite. The filtrate was evaporated *in vacuo*, and the residue was chromatographed on SiO_2

C-300 (30 g) with 1:5 toluene–EtOAc, to give **20** (710 mg, 57.5%), m.p. 170–171° (CH_2Cl_2–hexane); R_F 0.24 in 1:5 toluene–EtOAc; δ_H: 2.12 (s, 9 H, 3 Ac), 2.06 (s, 3 H, Ac), and 2.04 (s, 3 H, Ac).

Anal. Calc. for $C_{22}H_{32}O_{16}$: C, 47.83; H, 5.84. Found: C, 47.56; H, 5.80.

*1,2,4,6-Tetra-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl)-α- and -β-*D*-glucopyranose* (**21**). — Compound **20** (540 mg, 0.98 mmol) was acetylated with AcCl and pyridine, to give **21** (as a mixture of the α and β anomers in the ratio of 1:1); R_F 0.5 in 1:3 toluene–EtOAc; δ_H: 6.22 (d, 0.5 H, *J* 4 Hz, H-1α), and 5.61 (d, 0.5 H, *J* 8 Hz, H-1β). Mixture **21** was used directly for the next step.

*2,4,6-Tri-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl)-α-*D*-glucopyranosyl bromide* (**8**). — A solution of **21** (504 mg) in 30% HBr–AcOH (5 mL) was stirred for 1.5 h at 15–20°. The mixture was co-evaporated several times with toluene *in vacuo*, and the residue was chromatographed on SiO_2 C-300 (50 g) with 1:1 toluene–EtOAc, to give **8** (689 mg, 81%), m.p. 182–183° (EtOAc–i-Pr_2O), $[\alpha]_D$ +98.8° (*c* 1.1); δ_H: 6.50 (d, 1 H, *J* 4 Hz, H-1), 2.20 (s, 3 H, Ac), 2.11 (s, 6 H, 2 Ac), 2.08 (s, 3 H, Ac), 2.02 (s, 3 H, Ac), 2.00 (s, 3 H, Ac), and 1.97 (s, 3 H, Ac).

Anal. Calc. for $C_{26}H_{35}BrO_{10}$: C, 44.64; H, 5.04; Br, 11.42. Found: C, 44.62; H, 5.05; Br, 11.51.

*Benzyl 2,4-di-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl)-α-*D*-glucopyranoside* (**24**). — To a stirred mixture of **6** (2.66 g, 5.91 mmol), powdered molecular sieve 4 A (10 g), and $HgBr_2$ (510 mg, 1.42 mmol) in $Cl(CH_2)_2Cl$ (15 mL) was added dropwise a solution of **7** (2.915 g, 7.09 mmol) in $Cl(CH_2)_2Cl$ (15 mL). The mixture was stirred for 3.5 h at 20°, and filtered (Celite). The filtrate was diluted with EtOAc, successively washed with aq. $NaHCO_3$ and H_2O, dried ($MgSO_4$), and evaporated *in vacuo*. The residue was chromatographed on SiO_2 C-300 (200 g) with 2:1 i-Pr_2O–Et_2O, to give recovered **6** (1.3 g, 50%) and **24** (1.2 g, 26%), $[\alpha]_D$ +67.8° (*c* 1.07); R_F 0.23 in 2:1 i-Pr_2O–Et_2O; δ_H: 2.07 (s, 3 H, Ac), 2.04 (s, 3 H, Ac), 2.02 (s, 3 H, Ac), and 1.97 (s, 3 H, Ac).

Anal. Calc. for $C_{41}H_{48}O_{15}$: C, 63.10; H, 6.20. Found: C, 62.77; H, 6.21.

*Benzyl 2,4-di-*O*-benzyl-3,6-di-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl)-α-*D*-glucopyranoside* (**22**). — To a stirred mixture of **6** (374 mg, 0.83 mmol), powdered molecular sieve 4 A (4 g), and $HgBr_2$ (216 mg, 0.6 mmol) in $Cl(CH_2)_2Cl$ (5 mL) was added dropwise a solution of **7** (1.233 g, 3 mmol) in $Cl(CH_2)_2Cl$ (5 mL). The mixture was stirred for 24 h at 45°, diluted with $Cl(CH_2)_2Cl$ (30 mL), and filtered (Celite). The filtrate was successively washed with aq. $NaHCO_3$ and H_2O, dried ($MgSO_4$), and evaporated *in vacuo*. The residue was chromatographed on SiO_2 C-300 (100 g) with 3:1 toluene–EtOAc, to give **22** (440 mg, 47%), $[\alpha]_D$ +12.8° (*c* 0.58); R_F 0.15 in 2:1 i-Pr_2O–Et_2O; δ_H: 7.38 (bs, 15 H, aromatic), 2.09 (s, 3 H, Ac), 2.08 (s, 3 H, Ac), 2.06 (s, 6 H, 2 Ac), 2.04 (s, 9 H, 3 Ac), and 1.94 (s, 3 H, Ac).

Anal. Calc. for $C_{55}H_{66}O_{24}$: C, 59.45; H, 5.99. Found: C, 59.19; H, 6.02.

*Benzyl 2,4-di-*O*-benzyl-3,6-di-*O*-β-*D*-glucopyranosyl-α-*D*-glucopyranoside* (**23**). — A solution of **22** (251 mg) in MeOH (5 mL)–M NaOMe (0.1 mL) was stirred for

15 h at 20°. The usual processing afforded **23** (167 mg, 95%) as a white powder, R_F 0.86 in 5:1 CH_2Cl_2–MeOH, which was used directly for the next step.

3,6-Di-O-β-D-glucopyranosyl-α- and -β-D-glucopyranose (**3**). — A mixture of **23** (167 mg, 0.22 mmol) and 10% Pd–C (100 mg) in 1:1 EtOH–H_2O (10 mL) was stirred under H_2 for 3 days at 20°. Processing gave **3** as a white powder (48 mg, 44%), $[\alpha]_D$ −13.0 (30 min) → +6.8° (3 days) (*c* 0.53, H_2O); R_F 0.17 in 10:5:4 1-BuOH–i-PrOH–H_2O; δ_C (D_2O): 103.04 ($^1J_{CH}$ 162.1 Hz, C-1b,1c), 96.02 ($^1J_{CH}$ 161.1 Hz, C-1αβ), 92.36 ($^1J_{CH}$ 169.9 Hz, C-1aα), 84.72 (C-3aβ), and 82.46 (C-3aα).

Anal. Calc. for $C_{18}H_{32}O_{16} \cdot 1.5\ H_2O$: C, 40.68; H, 6.64. Found: C, 40.94; H, 6.78.

Benzyl 2,4-di-O-benzyl-6-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-3-O-[2,4,6-tri-O-acetyl-3-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-β-D-glucopyranosyl]-α-D-glucopyranoside (**25**). — To a stirred mixture of **24** (359 mg, 0.46 mmol), powdered molecular sieve 4 A (4 g), and $AgOSO_2CF_3$ (150 mg, 0.59 mmol) in $Cl(CH_2)_2Cl$ (5 mL) was added dropwise a solution of **8** (270 mg, 0.39 mmol) in $Cl(CH_2)_2Cl$ (5 mL) at −10 to −15°. The mixture was stirred for 3 h at −10 to −15°, and processed as usual. The crude product was chromatographed on SiO_2 C-300 (30 g) with 3:2 toluene–EtOAc, to give **25** (438 mg, 81%), $[\alpha]_D$ +1.8° (*c* 0.53); R_F 0.23 in 3:2 toluene–EtOAc; δ_H: 7.32 (bs, 15 H, aromatic), 2.08 (s, 3 H, Ac), 2.06 (s, 3 H, Ac), 2.02 (s, 21 H, 3 Ac), 1.97 (s, 3 H, Ac), and 1.92 (s, 3 H, Ac).

Anal. Calc. for $C_{67}H_{82}O_{32}$: C, 57.51; H, 5.91. Found: C, 57.16; H, 5.90.

Benzyl 2,4-di-O-benzyl-6-O-β-D-glucopyranosyl-3-O-(3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)-α-D-glucopyranoside (**26**). — A solution of **25** (552 mg, 0.39 mmol) in MeOH (5 mL)–M NaOMe (0.18 mL) was stirred for 15 h at 15°. Neutralization of the base with Amberlist **15** (H^+), and the usual processing, gave **26** (284 mg, 76%), m.p. 187–188° (MeOH), $[\alpha]_D$ +16.0° (*c* 0.5, MeOH); R_F 0.76 in 3:3:2:2 1-BuOH–i-PrOH–EtOH–H_2O; δ_H (MeSO-d_6): 7.40 (bs, 15 H, aromatic).

Anal. Calc. for $C_{45}H_{60}O_{21} \cdot MeOH$: C, 57.01; H, 6.66. Found: C, 56.87; H, 6.37.

6-O-β-D-Glucopyranosyl-3-O-(3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)-α- and -β-D-glucopyranose (**4**). — A mixture of **26** (219 mg, 0.23 mmol) and 10% Pd–C (200 mg) in 1:1 EtOH–H_2O (10 mL) was stirred under H_2 for 2 days at 30°. Filtration through Celite, and the usual processing, afforded **4** (148 mg, 90%), $[\alpha]_D$ −0.3° (*c* 0.50, H_2O); R_F 0.27 in 3:3:2:2 1-BuOH–i-PrOH–EtOH–H_2O; δ_C: 103.08 ($^1J_{CH}$ 163.1 Hz, H-1b,1c,1d), 96.06 ($^1J_{CH}$ 157.6 Hz, H-1aβ), 92.36 ($^1J_{CH}$ 169.9 Hz, H-1aα), 84.56 (H-3c,3aβ), and 82.30 (H-3aα).

Anal. Calc. for $C_{24}H_{42}O_{21} \cdot 1.5\ H_2O$: C, 41.56; H, 6.54. Found: C, 41.62; H, 6.55.

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