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Note

Synthesis of lactodifucotetraose

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O-α-L-Fucopyranosyl-(1→2)-*O*-β-D-galactopyranosyl-(1→4)-*O*-[α-L-fucopyranosyl-(1→3)]-D-glucose (lactodifucotetraose, **8**) was first isolated from human milk by Kuhn and Gauhe[1] in 1958. The tetrasaccharide **8** has also been detected in the urines of human blood-group O(H) secretors[2] and pregnant and lactating women[3], and found to be the principal neutral carbohydrate of platypus milk[4]. The chemical synthesis of **8** is now described.

The synthetic route to **8** was based on the transformation of lactose into a suitably substituted derivative having HO-3 and HO-2′ free, namely, benzyl 6-*O*-benzoyl-2,3′-di-*O*-benzyl-4′,6′-*O*-benzylidene-β-lactoside (**4**), followed by the condensation of this derivative with 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide[5,6] (**6**) under catalysis by halide ion[6,7], and removal of the protecting groups.

We chose benzyl 4′,6′-*O*-benzylidene-β-lactoside[8] (**1**) as the starting material for the preparation of **4**. Regioselective benzylation of the dibutylstannylene derivative, obtained by azeotropic removal[9] of water from a mixture of **1** and 2.5 mol. equiv. of dibutyltin oxide in benzene, with benzyl bromide in the presence of tetrabutylammonium bromide[9], gave, as the major product, the 2,3′-di-*O*-benzyl-4′,6′-*O*-benzylidene derivative **2**, isolated crystalline in 52% yield after column chromatographic separation. Methylation[10] of **2** with methyl iodide and sodium hydride in *N,N*-dimethylformamide gave the crystalline 2,3′-di-*O*-benzyl-4′,6′-*O*-benzylidene-3,6,2′-tri-*O*-methyl derivative **3**. Hydrogenolysis of **3**, followed by hydrolysis, reduction with sodium borohydride, and acetylation, produced an equimolar mixture of the peracetates of 3,6-di-*O*-methyl-D-glucitol and 2-*O*-methyl-D-galactitol (g.l.c.), proving the structure of **2**.

Initial attempts to synthesize **4** by preferential benzoylation of HO-6 of **2** with 1.1 mol. equiv. of benzoyl chloride in pyridine or in chloroform in the presence of triethylamine[11] were unsuccessful. Subsequently, we found that the introduction of a benzoyl group at position 6 in **2** is achieved satisfactorily by treating the com-

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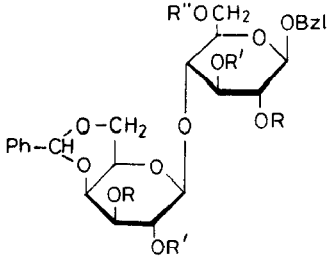
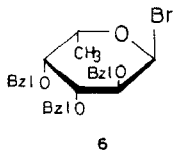
pound with 5 mol. equiv. of *N*-benzoylimidazole[12] in chloroform for 5 h at reflux. This procedure gave crystalline **4** in 91% yield after column chromatography. In the ^{13}C-n.m.r. spectrum of **4**, the downfield shift of 2.0 p.p.m. exhibited by C-6 suggested the position of substitution. The location of the benzoyl group was confirmed by the methylation of **4** with diazomethane–boron trifluoride etherate[13] to give the crystalline 6-*O*-benzoyl-2,3′-di-*O*-benzyl-4′,6′-*O*-benzylidene-3,2′-di-*O*-methyl derivative **5**. Compound **5**, on successive *O*-debenzoylation, hydrogenolysis, hydrolysis, reduction with sodium borohydride, and acetylation, furnished a 1:1 mixture of the peracetates of 3-*O*-methyl-D-glucitol and 2-*O*-methyl-D-galactitol (g.l.c.).

Glycosylation of **4** with **6** in 1,2-dichloroethane and *N*,*N*-dimethylformamide, in the presence of tetraethylammonium bromide and molecular sieve[6,7,14], for 3 days at room temperature, gave a mixture shown by t.l.c. to contain a major product, accompanied by traces of a marginally faster migrating, unidentified substance that could not be removed by chromatography. Therefore, the mixture was *O*-debenzoylated to facilitate the separation of the major product, and the debenzoylated material was chromatographed on a column of silica gel to give the amorphous tetrasaccharide derivative **7** in 68% yield. The ^{1}H-n.m.r. spectrum of **7**





6

1 R = R′ = R″ = H

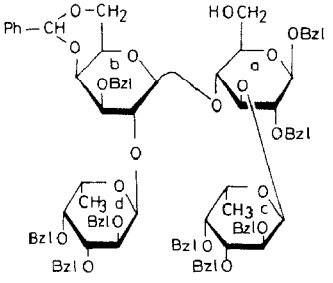
2 R = Bzl, R′ = R″ = H

3 R = Bzl, R′ = R″ = Me

4 R = Bzl, R′ = H, R″ = Bz

5 R = Bzl, R′ = Me, R″ = Bz

Bzl = $PhCH_2$



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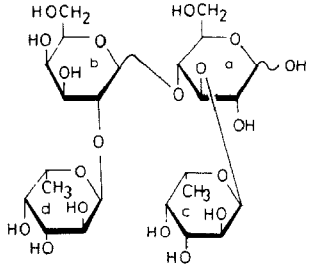

8

showed two doublets at δ 5.69 ($J_{1,2}$ 3.78 Hz) and 5.52 ($J_{1,2}$ 3.60 Hz), indicating the presence of two α-linked L-fucopyranosyl groups. That no migration of the benzoyl group in **4** had taken place during the glycosylation was indicated by the ^{13}C-n.m.r. spectrum of **7**, which showed the C-6a resonance at δ 60.6.

Catalytic hydrogenolysis of **7** in acetic acid in the presence of palladium-on-carbon furnished, in 86% yield after column chromatography, the amorphous title tetrasaccharide **8**, homogeneous by t.l.c. and paper chromatography. The optical rotation ($[\alpha]_D$ −103.2° in water) of **8** agreed well with that reported[1], and the resonances observed in the ^{13}C-n.m.r. spectrum were consistent with those reported[15] for this compound.

EXPERIMENTAL

General methods. — Unless stated otherwise, these were as previously described[16]. ^{1}H- And ^{13}C-n.m.r. spectra were recorded with a Hitachi R-90H spectrometer; tetramethylsilane (in chloroform-*d*) and sodium 4,4-dimethyl-4-silapentanoate-d_4 (in deuterium oxide) were the internal standards. The ^{1}H-n.m.r. spectrum of **7** was recorded with a Brucker AM-400 spectrometer, with tetramethylsilane as the internal standard. Retention times in g.l.c. are given[17] relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol. Descending p.c. was performed on Whatman No. 1 paper in 6:4:3 (v/v/v) 1-butanol–pyridine–water, detection being accomplished with the alkaline silver nitrate reagent[18]. The following solvent systems (v/v) were used: (*1*) 1:2 and (*2*) 1:1 benzene–ethyl acetate, (*3*) 1:1 and (*4*) 2:1 hexane–ethyl acetate, (*5*) 3:2:2 and (*6*) 5:7:3 ethyl acetate–2-propanol–water, and (*7*) 5:1:1 chloroform–methanol–water.

Benzyl O-(3-O-benzyl-4,6-O-benzylidene-β-D-galactopyranosyl)-(1→4)-2-O-benzyl-β-D-glucopyranoside (**2**). — A mixture of **1** (ref. 8, 6.50 g, 12.5 mmol) and dibutyltin oxide (7.70 g, 31.2 mmol) in benzene (300 mL) was stirred for 5 h under reflux, and the water formed was collected in a Dean-Stark trap. After concentration of the mixture to ~220 mL, tetrabutylammonium bromide (10.06 g, 31.2 mmol) and benzyl bromide (7.4 mL, 62.4 mmol) were added, and the mixture was stirred for 20 h at 100°, when t.l.c. (solvent *1*) indicated the presence of a major (**2**, R_F 0.42) and several minor products. No starting material was detected. The mixture was evaporated to a syrup, and this was applied to a column of silica gel. Elution with benzene removed the excess of benzyl bromide. Subsequent elution with solvent *2* gave **2** (4.54 g, 52%); m.p. 183–184° (from ethanol), $[\alpha]_D^{26}$ +1.4° (*c* 1.4, chloroform); n.m.r. data (chloroform-*d*): δ_C 103.5 (C-1), 102.2 (C-1′), and 61.8 (C-6). Minor components were discarded.

Anal. Calc. for $C_{40}H_{44}O_{11}$: C, 68.56; H, 6.33. Found: C, 68.52; H, 6.53.

Benzyl O-(3-O-benzyl-4,6-O-benzylidene-2-O-methyl-β-D-galactopyranosyl)-(1→4)-2-O-benzyl-3,6-di-O-methyl-β-D-glucopyranoside (**3**). — A solution of **2** (0.36 g) in anhydrous *N,N*-dimethylformamide (5 mL) was stirred for 1 h with sodium hydride (0.22 g; 50% in mineral oil) at room temperature, and then cooled

to 0°. Methyl iodide (1 mL) was added, and the mixture was stirred for 3 h at room temperature, then diluted with dichloromethane. The solution was washed with water, dried, and evaporated to dryness, and the residue was chromatographed on a column of silica gel with solvent *3*, to give **3** (0.34 g, 89%); m.p. 150–151° (from ethanol), $[\alpha]_D^{27}$ −10.1° (*c* 1.1, chloroform); n.m.r. data (chloroform-*d*): δ_H 7.49–7.20 (m, 20 H, 4 Ph), 5.42 (s, 1 H, PhC*H*), and 3.59, 3.41, and 3.40 (3 s, each 3 H, 3 OMe).

Anal. Calc. for $C_{43}H_{50}O_{11}$: C, 69.52; H, 6.78. Found: C, 69.70; H, 6.90.

Hydrogenolysis of a portion of **8** in acetic acid in the presence of palladium-on-charcoal, followed by hydrolysis with 0.5M sulfuric acid for 10 h at 100°, neutralization with barium carbonate, reduction with sodium borohydride, acetylation, and g.l.c. of the resulting products, gave peaks corresponding the peracetates of 3,6-di-*O*-methyl-D-glucitol (*T* 4.37, 50%) and 2-*O*-methyl-D-galactitol (*T* 8.08, 50%).

Benzyl O-(3-O-benzyl-4,6-O-benzylidene-β-D-galactopyranosyl)-(1→4)-6-O-benzoyl-2-O-benzyl-β-D-glucopyranoside (**4**). — A solution of benzoyl chloride (5.82 mL, 50 mmol) in purified chloroform (20 mL) was added dropwise at 0° to a stirred solution of imidazole (6.81 g, 100 mmol) in chloroform (70 mL). The precipitate that separated was filtered off and washed with chloroform (20 mL). The combined filtrate and washings were added to a solution of **2** (7.01 g, 10 mmol) in chloroform (50 mL), and the mixture was boiled for 5 h under reflux. The solution was cooled, washed successively with aqueous sodium hydrogencarbonate and water, dried, and evaporated. The residual syrup was chromatographed on a column of silica gel with solvent *3*, to give **4** (7.32 g, 91%); m.p. 70–72° (from acetone–petroleum ether–ether), $[\alpha]_D^{26}$ −1.0° (*c* 1.9, chloroform); n.m.r. data (chloroform-*d*): δ_C 103.8 (C-1), 101.7 (C-1'), and 63.8 (C-6).

Anal. Calc. for $C_{47}H_{48}O_{12}$: C, 70.14; H, 6.01. Found: C, 70.29; H, 6.10.

Benzyl O-(3-O-benzyl-4,6-O-benzylidene-2-O-methyl-β-D-galactopyranosyl)-(1→4)-6-O-benzoyl-2-O-benzyl-3-O-methyl-β-D-glucopuranoside (**5**). — Diazomethane in dichloromethane was gradually added to a stirred solution (cooled to −10°) of **4** (0.70 g) in dichloromethane containing a few drops of boron trifluoride etherate, until a faint yellow color persisted, and the mixture was kept for 1 h at room temperature. Polymethylene was filtered off, and the filtrate was washed successively with aqueous sodium hydrogencarbonate and water, dried, and evaporated. The resulting syrup was eluted from a column of silica gel with solvent *4*, to give **5** (0.63 g, 88%); m.p. 144–145° (from ethanol–hexane), $[\alpha]_D^{27}$ +2.9° (*c* 0.7, chloroform); n.m.r. data (chloroform-*d*): δ_H 8.05–7.20 (m, 25 H, 5 Ph), 5.33 (s, 1 H, PhC*H*), and 3.62 and 3.53 (2 s, each 3 H, 2 OMe).

Anal. Calc. for $C_{49}H_{52}O_{12}$: C, 70.66; H, 6.29. Found: C, 70.78; H, 6.10.

Successive *O*-debenzoylation of a portion of **5** with sodium methoxide in methanol, hydrogenolysis, hydrolysis, reduction with sodium borohydride, and acetylation, gave compounds that had the retention times of the peracetates of 2-*O*-methyl-D-galactitol (*T* 8.07, 50%) and 3-*O*-methyl-D-glucitol (*T* 9.59, 50%).

Benzyl O-*(2,3,4-tri-*O*-benzyl-α-*L*-fucopyranosyl)-(1→2)-*O*-(3-*O*-benzyl-4,6-*O*-benzylidene-β-*D*-galactopyranosyl)-(1→4)-*O*-[2,3,4-tri-*O*-benzyl-α-*L*-fucopyranosyl-(1→3)]-2-*O*-benzyl-β-*D*-glucopyranoside* (**7**). — A solution of **4** (4.50 g, 5.6 mmol) in dry 1,2-dichloroethane (60 mL) and *N,N*-dimethylformamide (20 mL) was stirred for 2 h at room temperature in the presence of tetraethylammonium bromide (5.50 g, 26.2 mmol) and molecular sieve 4A (30 g). A solution of **6** [freshly prepared from 15.4 g (26.4 mmol) of the 1-*p*-nitrobenzoate[5,6]] in 1,2-dichloroethane (40 mL) was added, and the mixture was stirred for 3 days at room temperature. Methanol (10 mL) was added, and the mixture was stirred for 6 h. The solids were removed by filtration and washed with dichloromethane. The combined filtrate and washings were washed successively with aqueous sodium hydrogencarbonate and water, dried, and evaporated. The residue was dissolved in methanol (70 mL) and dichloromethane (30 mL), and treated with M sodium methoxide (3 mL). The solution was kept overnight at room temperature, made neutral with acetic acid, and evaporated to dryness. The residue was fractionated on a column of silica gel with solvent *3*, to give **7** as an amorphous powder (5.82 g, 68%); $[\alpha]_D^{26}$ −86.9° (*c* 1.6, chloroform); n.m.r. data (chloroform-*d*): δ_H 7.58–6.97 (m, 50 H, 10 Ph), 5.69 (d, 1 H, $J_{1,2}$ 3.78 Hz, H-1 of α-L-Fuc*p*), 5.52 (d, 1 H, $J_{1,2}$ 3.60 Hz, H-1 of α-L-Fuc*p*), 5.47 (s, 1 H, PhC*H*), 1.21 (d, 3 H, $J_{5,6}$ 6.48 Hz, Me), and 1.14 (d, 3 H, $J_{5,6}$ 6.49 Hz, Me); δ_C 103.1 (C-1a), 99.5 (C-1b), 98.5 (C-1d), 97.7 (C-1c), 60.6 (C-6a), 16.6 (Me), and 16.2 (Me).

Anal. Calc. for $C_{94}H_{100}O_{19}$: C, 73.61; H, 6.57. Found: C, 73.86; H, 6.70.

O-α-L-*Fucopyranosyl-(1→2)*-O-β-D-*galactopyranosyl-(1→4)*-O-[α-L-*fucopyranosyl-(1→3)*]-D-*glucose* (**8**). — A solution of **7** (2.01 g) in acetic acid (50 mL) was hydrogenolyzed in the presence of 10% palladium-on-charcoal (2 g) at normal pressure for 3 days at room temperature. The catalyst was filtered off and washed with methanol, and the combined filtrate and washings were evaporated. Purification of the product by elution from a column of silica gel with solvent *5* gave a syrup, which, on precipitation from ethanol, afforded **8** as an amorphous powder (0.71 g, 86%); $[\alpha]_D^{26}$ −103.2° (*c* 1.0, water); lit.[1] $[\alpha]_D^{20}$ −106° (*c* 1, water), $[\alpha]_D^{25}$ −102° (*c* 1, water); t.l.c.: R_F 0.20, $R_{lactose}$ 0.65 in solvent *5*, R_F 0.34, $R_{lactose}$ 0.72 in solvent *6*, and R_F 0.23, $R_{lactose}$ 0.67 in solvent *7*; p.c.: $R_{lactose}$ 0.44; n.m.r. data (deuterium oxide): δ_C 102.7 (C-1b), 102.0 (C-1d), 101.0 and 100.9 (C-1c), 98.6 (C-1aβ), 94.7 (C-1aα), 79.8 (C-3aβ), 79.1 (C-2b), 77.4 (C-3aα), 64.1 (C-6b), 62.6 (C-6aα,β), and 18.2 (C-6c, 6d).

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