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SYNTHESIS OF 3- AND 2′-FUCOSYL-LACTOSE AND 3,2′-DIFUCOSYL-LACTOSE FROM PARTIALLY BENZYLATED LACTOSE DERIVATIVES

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ABSTRACT

O-β-D-Galactopyranosyl-(1→4)-*O*-[α-L-fucopyranosyl-(1→3)]-D-glucose has been synthesised by reaction of benzyl 2,6-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside with 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide in the presence of mercuric bromide, followed by hydrogenolysis. Benzylation of benzyl 3′,4′-*O*-isopropylidene-β-lactoside, *via* tributylstannylation, in the presence of tetrabutylammonium bromide or *N*-methylimidazole, gave benzyl 2,6-di-*O*-benzyl-4-*O*-(6-*O*-benzyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (**6**). α-Fucosylation of **6** in the presence of tetraethylammonium bromide provided either benzyl 2,6-di-*O*-benzyl-4-*O*-[6-*O*-benzyl-3,4-*O*-isopropylidene-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (**13**, 73%) or a mixture of **13** (42%) and benzyl 2,6-di-*O*-benzyl-4-*O*-[6-*O*-benzyl-3,4-*O*-isopropylidene-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-3-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-β-D-glucopyranoside (**16**, 34%). α-Fucosylation of **13** in the presence of mercuric bromide and 2,6-di-*tert*-butyl-4-methylpyridine gave **16** (73%). Hydrogenolysis and acid hydrolysis of **13** and **16** afforded *O*-α-L-fucopyranosyl-(1→2)-*O*-β-D-galactopyranosyl-(1→4)-D-glucose and *O*-α-L-fucopyranosyl-(1→2)-*O*-β-D-galactopyranosyl-(1→4)-*O*-[α-L-fucopyranosyl-(1→3)]-D-glucose, respectively.

INTRODUCTION

We have reported[1] that partial benzylation of benzyl 3′,4′-*O*-isopropylidene-β-lactoside (**1**), under phase-transfer conditions, gave benzyl 2,6-di-*O*-benzyl-4-*O*-(2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (**4**, 38%). Partial benzylation of either benzyl β-lactoside (**2**) or benzyl hepta-*O*-acetyl-β-lactoside (**3**), under similar conditions, gave benzyl 2,6-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**5**, 25%). Compounds **4** and **5** were starting materials for the synthesis of 3-*O*-methyl-lactose, which is presently being used for the evaluation *in vivo* of intestinal lactase[1,2]. We now report the synthesis of 3-fucosyl-lactose (**9**), 2′-fucosyl-lactose (**17**), and 3,2′-

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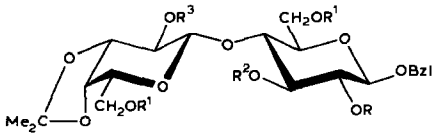

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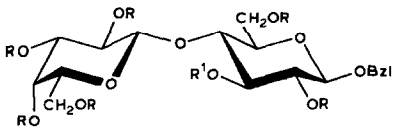
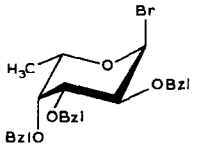
4 R = $R^1 = R^3 = Bzl, R^2 = H$
6 R = $R^1 = Bzl, R^2 = R^3 = H$
10 R = $Bzl, R^1 = R^2 = R^3 = H$
11 R = $Bzl, R^1 = R^2 = R^3 = Ac$
12 R = $R^1 = Bzl, R^2 = R^3 = Ac$



2 R = $R^1 = H$
3 R = $R^1 = Ac$
5 R = $Bzl, R^1 = H$



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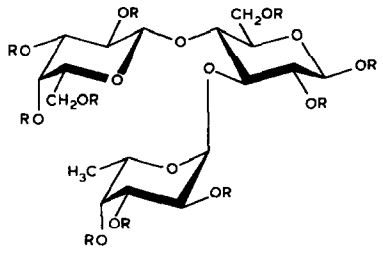


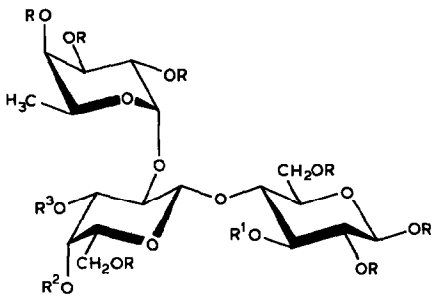
8 R = Bzl
9 (α,β) R = H

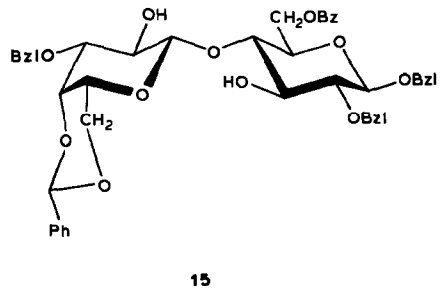
difucosyl-lactose (**18**). Compounds **9**, **17**, and **18** were first isolated from human milk[3–5]. Syntheses of **17** and **18** have been reported[6,7].

RESULTS AND DISCUSSION

Reaction of **5**[1] with 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide[8] (**7**) in the presence of mercuric bromide[9] gave 53% of the protected trisaccharide derivative **8**, the ^{1}H-n.m.r. spectrum of which contained a signal for H-1′ at δ 5.59 ($J_{1',2'}$ 3.3 Hz) and the ^{13}C-n.m.r. spectrum a signal for C-1′ at 97.7 p.p.m. Hydrogenolysis of **8** gave 3-fucosyl-lactose (**9**) in quantitative yield.

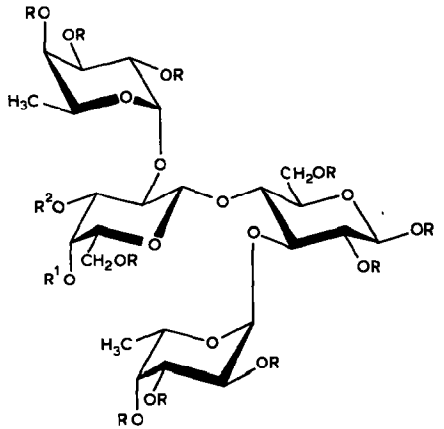
The synthesis of **17** and **18** from the diol **6** was envisaged. Treatment of **1** with tributyltin oxide (3 equiv.) and subsequent reaction with benzyl bromide gave, after 6 days, a complex mixture of products. When the benzylation was carried out in the presence of tetrabutylammonium bromide (0.35 equiv.)[10], 50% of benzyl 2-*O*-benzyl-4-*O*-(3,4-*O*-isopropylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (**10**) was obtained after 22 h. After 7 days, the main product (38%) was **6**. Compound **10** was characterised as the acetate **11**, and was the main product when **1** was treated with dibutyltin oxide (1.2 equiv.) and the product was benzylated in the presence of tetrabutylammonium bromide (0.3 equiv.). Acetylation of **6** gave **12**, the ^{1}H-n.m.r. spectrum of which contained signals at δ 1.92 and 2.00 (2 s), and 5.10





15

13 R = Bzl, R^1 = H, R^2, R^3 = CMe_2
14 R = Bzl, R^1 = Ac, R^2, R^3 = CMe_2
17 (α,β) R = R^1 = R^2 = R^3 = H



16 R = Bzl, R^1, R^2 = CMe_2
18 (α,β) R = R^1 = R^2 = H

(t, $J_{2,3} \cong J_{3,4}$ = 9.5 Hz) and 4.77 (t, $J_{1',2'} \cong J_{2',3'}$ = 7.5 Hz) for H-3 and H-2', respectively. In the partial benzylation of **1** with benzyl chloride in the presence of potassium hydroxide[11], or with benzyl bromide under phase-transfer conditions[1], HO-2' is the secondary hydroxyl group preferentially benzylated, probably due to the existence of an intramolecular hydrogen-bond[11] HO-2' · · · O-3'. The products of partial benzylation of **1** *via* tributylstannylation[12] in the presence of tetrabutylammonium bromide indicate a marked change of regioselectivity with an enhancement of the reactivity of HO-2. Thus, the reaction allows the preparation of **6**, which is difficult to obtain by other procedures. When *N*-methylimidazole[13] (1 equiv.) was used instead of tetrabutylammonium bromide in the benzylation step, 52% of **10** was obtained after 6.5 h, and 44% of **6** after 2.5 days with a further addition of *N*-methylimidazole (2 equiv.). The presence of *N*-methylimidazole alters the regioselectivity of benzoylation *via* stannylene derivatives[13], and it enhances the reactivity of HO-2 of **1** as efficiently as tetrabutylammonium bromide.

Reaction of **6** with the bromide **7** (2 equiv.) in the presence of tetraethylammonium bromide (2 equiv.) afforded the trisaccharide derivative **13** (73%) after

50 h; no trace of **16** could be detected. The ^{1}H-n.m.r. spectrum of **13** contained signals at δ 5.54 ($J_{1'',2''}$ 3.1 Hz, H-1'') and 1.10, and the ^{13}C-n.m.r. spectrum contained a signal at 96.0 (C-1''). Acetylation of **13** gave the monoacetate **14**, the ^{1}H-n.m.r. spectrum of which contained a downfield signal at δ 5.01 (t, $J_{2,3} \cong J_{3,4} = 9.5$ Hz) for H-3. When the glycosylation reaction was carried out using 4.7 equiv. of tetraethylammonium bromide, as recently reported[7] for the fucosylation of **15**, only 19% of the tetrasaccharide derivative **16** was isolated; the trisaccharide derivative **13** was also isolated (55%). The ^{1}H-n.m.r. spectrum of **16** contained signals at δ 5.64 ($J_{1',2'}$ 3.6 Hz, H-1'), 5.51 ($J_{1''',2'''}$ 3.6 Hz, H-1'''), 1.11, and 1.07. The ^{13}C-n.m.r. spectrum showed signals at 97.7 (C-1') and 95.6 p.p.m. (C-1'''). Attempts to improve the yield of **16** using more-active catalysts [$Hg(CN)_2$ and $HgBr_2$], according to the *in situ* anomerisation procedure[14], gave complex mixtures of glycosylation and decomposition products. The rather low reactivity of HO-3 of **6**, in comparison with that of **15**[7], could be due to the 3',4'-*O*-isopropylidene group. In order to evaluate the influence of the 3',4'-*O*-isopropylidene group, **4** and **5** were each treated with the bromide **7** (3 equiv.) in the presence of tetraethylammonium bromide (4 equiv.); HO-3 in **5** was more reactive (t.l.c.) than that in **4**, the ratio of unreacted **4** and **5** after 6 days being 3:1. Thus, the difficulty in obtaining the tetrasaccharide derivative **16** from **6** may be due partly to the presence of the 3',4'-*O*-isopropylidene group, although the steric hindrance as a consequence of the fast glycosylation of HO-2' should not be ignored. Condensation of **6** with **7** in the presence of a large excess (14 equiv.) of tetraethylammonium bromide gave, after 7 days, **16** (34%) and **13** (42%). Hydrogenolysis of **16** and **13** followed by acid hydrolysis afforded 3,2'-difucosyl-lactose (**18**) and 2'-fucosyl-lactose (**17**), respectively, in almost quantitative yields. The ^{13}C-n.m.r. spectra and $[\alpha]_D$ values of **17** and **18** accorded with reported data[4,5,15].

The tetrasaccharide derivative **16** was also synthesised (37%) from the trisaccharide derivative **13** by reaction with **7** (4 equiv.) in the presence of mercuric bromide for 1.5 h. Longer reaction times resulted in decomposition of **7** without any improvement in the yield of **16**. When the sterically hindered base 2,6-di-*tert*-butyl-4-methylpyridine was used, together with a larger proportion of mercuric bromide, 73% of **16** was obtained from **13** after 24 h.

Thus, the preparation of the diol **6**, in reasonable yield, *via* tributylstannylation using *N*-methylimidazole as catalyst in the benzylation step, permits the synthesis of 2'-fucosyl-lactose (**17**) and 3,2'-difucosyl-lactose (**18**) in one step using well established glycosylation methods.

EXPERIMENTAL

Material and methods. — Melting points were measured in capillary tubes and are uncorrected. T.l.c. was performed on silica gel GF_{254} (Merck) with detection by charring with sulfuric acid. Column chromatography was performed on Merck Type I (70–230 mesh) or Macherey Nagel Type II (230–400 mesh) silica gel.

^{1}H-N.m.r. spectra (300 MHz) were recorded with a Varian XL-300 spectrometer, and ^{13}C-n.m.r. spectra with a Varian XL-300 (75 MHz) or Bruker WP-80 (20 MHz) spectrometer. Optical rotations were determined with a Perkin–Elmer 141 polarimeter.

*Benzyl 2,6-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-*D*-galactopyranosyl)-3-*O*-(2,3,4-tri-*O*-benzyl-α-*L*-fucopyranosyl)-β-*D*-glucopyranoside* (**8**). — A mixture of **5** (0.3 g, 0.3 mmol), mercuric bromide (0.06 g, 0.17 mmol), molecular sieves Type 4A (0.85 g), and dichloromethane (6 mL) was stirred for 1 h at room temperature under argon. A solution of the fucopyranosyl bromide **7** (0.35 g, 0.7 mmol) in dichloromethane (6 mL) was then slowly added during 3 h. After 18 h, methanol (2 mL) was added, and the mixture was stirred for 2 h and then filtered through Celite. The solids were thoroughly washed with dichloromethane, and the combined filtrate and washings were washed successively with aqueous sodium hydrogencarbonate, aqueous 10% sodium iodide, and water, dried, and concentrated. Column chromatography (Type II, 7:1 hexane–ethyl acetate) of the residue gave **8** (0.23 g, 53%), isolated as a syrup, $[\alpha]_D^{20}$ −43° (*c* 0.9, chloroform). N.m.r. data ($CDCl_3$): ^{1}H, δ 5.59 (d, 1 H, $J_{1',2'}$ 3.3 Hz, H-1′), 1.09 (d, 3 H, $J_{5',6'}$ 7 Hz, H-6′); ^{13}C (75 MHz), δ 102.7, 102.2 (C-1,1″), 97.7 (C-1′), 82.7, 82.4, 80.1, 79.6, 78.7, 75.8, 75.5, 75.4, 75.3, 75.1, 75.0, 73.9, 73.3, 73.0, 72.9, 72.8, 72.4, 72.2, 70.9, 67.9, 67.8, 65.8, and 16.6.

Anal. Calc. for $C_{88}H_{92}O_{15}$: C, 76.06; H, 6.67. Found: C, 76.21; H, 6.76.

Further elution with 7:2 hexane–ethyl acetate gave **5** (0.11 g).

O-β-D-Galactopyranosyl-(1→4)-O-[α-L-fucopyranosyl-(1→3)]-D-glucose (**9**). — A solution of **8** (0.21 g) in ethanol (25 mL) and ethyl acetate (2 mL) was shaken under hydrogen at 2 atm. pressure for 7 h at room temperature in the presence of 10% Pd/C (0.15 g). The suspension was filtered through Celite and then concentrated under diminished pressure, to give **9** as a highly hygroscopic, white solid (64 mg, 86%), which was homogeneous in t.l.c. (1:1 chloroform–methanol). Column chromatography (Type I, 3:3:1 ethyl acetate–2-propanol–water) of this material gave **9**, $[\alpha]_D^{20}$ −43° (constant) (*c* 0.4, methanol). ^{13}C-N.m.r. data (D_2O, 50°, 75 MHz): δ 102.65 (C-1″β), 102.6 (C-1″α), 99.35 (C-1′α), 99.2 (C-1′β), 96.7 (C-1β), 92.9 (C-1α), 78.1 (C-3β), 76.4 (C-4β), 76.3 (C-4α), 75.8 (C-5″α), 75.75 (C-3α and C-5′β), 73.7 (C-5β and C-3″β), 73.6 (C-3″α), 73.4 (C-2β), 72.85 (C-4′α,β), 72.1 (C-2″α,β), 71.9 (C-2α), 70.2, 70.1 (C-3′α,β), 69.2 (C-4″α,β), 69.0 (C-2′α,β), 67.3 (C-5′α,β), 62.3 (C-6″α,β), 60.8 (C-6β), 60.7 (C-6α), and 16.1 (C-6′α,β).

Anal. Calc. for $C_{18}H_{32}O_{15}$: C, 44.26; H, 6.60. Found: C, 44.05; H, 6.75.

*Benzylation of benzyl 4-*O*-(3,4-*O*-isopropylidene-β-*D*-galactopyranosyl)-β-*D*-glucopyranoside* (**1**). — A mixture of **1** (1 g, 2.1 mmol), tributyltin oxide (3.25 mL, 6.3 mmol), and molecular sieves Type 3A (7.5 g) in toluene (70 mL) was stirred for 20 h at 120°. After cooling to 95–100°, benzyl bromide (10 mL) and *N*-methylimidazole (0.17 mL, 2.1 mmol) were added and the mixture was stirred under argon for 6.5 h. T.l.c. (ethyl acetate) then revealed **10** as a major product. The molecular sieve was collected and washed with chloroform–methanol, and the

combined filtrate and washings were concentrated under vacuum. Column chromatography (Type I, ethyl acetate) of the residue gave **10** (0.62 g, 52%), m.p. 77–79°, $[\alpha]_D^{25}$ −3° (*c* 0.7, methanol).

Anal. Calc. for $C_{29}H_{38}O_{11}$: C, 61.91; H, 6.81. Found: C, 62.00; H, 6.95.

Conventional treatment of **10** (0.13 g) with acetic anhydride–pyridine gave **11** (0.14 g), isolated as a syrup, $[\alpha]_D^{20}$ +28° (*c* 0.7, chloroform). N.m.r. data ($CDCl_3$): ^1H, δ 7.20–7.40 (m, 10 H, 2 Ph), 5.15 (t, 1 H, $J_{2,3} \cong J_{3,4} \cong 9.1$ Hz, H-3), 4.82 (m, 1 H, H-2′), 4.54 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1), 4.45 (dd, 1 H, $J_{5,6a}$ 1.7, $J_{6a,6b}$ 12.1 Hz, H-6a), 4.34 (d, 1 H, $J_{1',2'}$ 7.5 Hz, H-1′), 4.28 (d, 2 H, $J_{5',6'}$ 6.2 Hz, H-6′a,6′b), 4.18 (dd, 1 H, $J_{5,6b}$ 5.1, $J_{6a,6b}$ 12.1 Hz, H-6b), 4.12 (m, 2 H, H-3′,4′), 3.89 (dt, 1 H, $J_{4',5'}$ 1.7, $J_{5',6'}$ 6.2 Hz, H-5′), 3.64 (m, 2 H, H-4,5), 3.39 (dd, 1 H, $J_{1,2}$ 7.7, $J_{2,3}$ 9.1 Hz, H-2), 2.13, 2.08, 2.05, 1.96 (4 s, each 3 H, 4 Ac), 1.54 and 1.32 (2 s, each 3 H, Me); ^{13}C (20 MHz), δ 170.6, 170.5, 169.8, 169.1 (CO), 138.2, 137.0 (C-ipso), 128.5, 128.3, 128.0, 127.7 (aromatic), 110.8 (CMe_2), 102.1, 100.0 (C-1,1′), 79.1, 78.6, 77.0, 74.1, 73.1, 72.9, 71.2, 70.9, 63.1, 62.7, 27.3, 26.1, and 20.8.

Anal. Calc. for $C_{37}H_{46}O_{15}$: C, 60.81; H, 6.34. Found: C, 61.10; H, 6.25.

When benzylation was continued, more *N*-methylimidazole (0.34 mL, 4.2 mmol) was added after 24 h, and the stirring was continued for 2.5 days at 95–100°; t.l.c. (2:1 chloroform–ethyl acetate) then revealed **6** as the main product. The molecular sieve was collected and washed with warm chloroform, and the combined filtrate and washings were concentrated. The residue was stirred with hexane (100 mL) and kept overnight at −10°. The hexane was decanted and column chromatography (Type I, 3:1 chloroform–ethyl acetate) of the syrupy residue gave **6** (0.69 g, 44%), isolated as a syrup, $[\alpha]_D^{20}$ −8° (*c* 1, chloroform).

Anal. Calc. for $C_{43}H_{50}O_{11}$: C, 69.52; H, 6.78. Found: C, 69.24; H, 6.97.

Conventional treatment of **6** with acetic anhydride–pyridine gave benzyl 3-*O*-acetyl-4-*O*-(2-*O*-acetyl-6-*O*-benzyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-2,6-di-*O*-benzyl-β-D-glucopyranoside (**11**), isolated as a syrup, $[\alpha]_D^{20}$ +17.5° (*c* 0.85, chloroform). N.m.r. data ($CDCl_3$): ^1H, δ 7.20–7.40 (m, 20 H, 4 Ph), 5.10 (t, 1 H, $J_{2,3} \cong J_{3,4}$ 9.5 Hz, H-3), 4.77 (t, 1 H, $J_{1',2'} \cong J_{2',3'} = 7.65$ Hz, H-2′), 4.51 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.28 (d, 1 H, $J_{1',2'}$ 8 Hz, H-1′), 4.08 (dd, 1 H, $J_{3',4'}$ 5.4, $J_{4',5'}$ 1.7 Hz, H-4′), 3.90 (dd, 1 H, $J_{2',3'}$ 7.4, $J_{3',4'}$ 5.4 Hz, H-3′), 3.83 (t, 1 H, $J_{3,4} \cong J_{4,5} = 9.5$ Hz, H-4), 3.73 (m, 5 H, H-6a,6b,5′,6′a,6′b), 3.44 (m, 1 H, H-5), 3.41 (dd, 1 H, $J_{2,3}$ 9.5, $J_{1,2}$ 7.5 Hz, H-2), 2.00, 1.92 (2 s, each 3 H, 2 Ac), 1.52 and 1.32 (2 s, each 3 H, Me); ^{13}C (75 MHz), δ 169.9, 168.9 (CO), 138.2, 138.0, 137.2 (C-ipso), 128.7, 128.6, 128.4, 128.2, 128.1, 127.9, 127.8, 127.6 (aromatic), 110.4 (CMe_2), 102.4, 99.8 (C-1,1′), 27.5, 26.3 (2 Me), and 20.9 p.p.m. (2 Ac).

Anal. Calc. for $C_{47}H_{54}O_{13}$: C, 68.26; H, 6.58. Found: C, 68.05; H, 6.75.

*Benzyl 2,6-di-*O*-benzyl-4-*O*-[6-*O*-benzyl-3,4-*O*-isopropylidene-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside* (**13**). — To a mixture of **6** (0.4 g, 0.54 mmol), tetraethylammonium bromide (0.23 g, 1.08 mmol), molecular sieves Type 4A (2.4 g), and dichloromethane (10 mL) was added a solution of the bromide **7** (0.6 g, 1.2 mmol) in dichloromethane (5 mL) and *N,N*-

dimethylformamide (14 drops). The mixture was stirred for 50 h at room temperature in the dark and under argon, methanol (1 mL) was then added, and the mixture was stirred for 1 h. The solids were collected and washed with dichloromethane, and the combined filtrate and washings were washed successively with aqueous sodium hydrogencarbonate and water, dried, and concentrated. Column chromatography (Type I, 7:2 hexane–ethyl acetate) of the residue gave **13** (0.34 g, 55%), $[\alpha]_D^{20}$ −47° (*c* 0.45, chloroform). N.m.r. data ($CDCl_3$): ^1H, δ 5.54 (d, 1 H, $J_{1'',2''}$ 3.1 Hz, H-1″), 1.47, 1.33 (2 s, 6 H, Me), 1.10 (d, 3 H, $J_{5'',6''}$ 6.6 Hz, H-6″); ^{13}C (20 MHz), δ 139.1, 138.8, 137.6 (C-ipso), 128.4, 128.2, 128.0, 127.8, 127.4, 127.3 (aromatic), 110.4 (CMe_2), 102.1, 101.3 (C-1,1′), 96.0 (C-1″), 81.7, 81.0, 79.9, 79.3, 77.9, 76.3, 75.5, 74.9, 73.8, 73.7, 73.0, 72.5, 71.2, 69.4, 66.5, 27.9 and 26.5 (Me), and 16.6 (C-6″).

Anal. Calc. for $C_{70}H_{78}O_{15}$: C, 72.52; H, 6.78. Found: C, 72.69; H, 7.07.

Continued elution with 7:3 hexane–ethyl acetate gave **6** (0.11 g).

Benzyl 3-O-acetyl-2,6-di-O-benzyl-4-O-[6-O-benzyl-3,4-O-isopropylidene-2-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (**14**). — Acetylation of **13** conventionally gave **14**, isolated as a syrup, $[\alpha]_D^{20}$ −30° (*c* 0.6, chloroform). N.m.r. data ($CDCl_3$): ^1H, δ 5.49 (d, 1 H, $J_{1'',2''}$ 3.8 Hz, H-1″), 5.01 (t, 1 H, $J_{2,3} \cong J_{3,4}$ = 9.5 Hz, H-3), 4.50 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.14 (d, 1 H, $J_{1',2'}$ 8.2 Hz, H-1′), 4.06 (dd, 1 H, $J_{1'',2''}$ 3.8, $J_{2'',3''}$ 9.8 Hz, H-2″), 3.79 (t, 1 H, $J_{3,4} \cong J_{4,5}$ = 9.5 Hz, H-4), 3.35 (dd, 1 H, $J_{1',2'}$ 8.2, $J_{2',3'}$ 6.3 Hz, H-2′), 3.41 (dd, 1 H, $J_{1,2}$ 7.5, $J_{2,3}$ 9.5 Hz, H-2), 1.90 (s, 3 H, Ac), 1.44, 1.31 (2 s, each 3 H, Me), and 1.11 (d, 3 H, $J_{5'',6''}$ 6.6 Hz, H-6″); ^{13}C (20 MHz), δ 170.0 (CO), 110.1 (CMe_2), 102.7, 100.2 (C-1,1′), 95.2 (C-1″), 27.9, 26.6 (2 Me), 20.9 (Ac), and 16.5 (C-6″).

Anal. Calc. for $C_{72}H_{80}O_{16}$: C, 71.98; H, 6.71. Found: C, 72.08; H, 7.01.

O-α-L-Fucopyranosyl-(1→2)-O-β-D-galactopyranosyl-(1→4)-D-glucose (**17**). — A solution of **13** (0.25 g) in ethanol (40 mL) was hydrogenolysed (2 atm.) over 10% Pd/C (0.25 g) at room temperature. The catalyst was collected and washed with methanol, and the combined filtrate and washings were concentrated. The residue was treated with aqueous 20% acetic acid (4 mL) at 90° for 1 h, and then concentrated to give **17** (75 mg, 75%) as a white solid that was homogeneous in t.l.c. (1:1 chloroform–methanol). Column chromatography (Type I, 1:1 chloroform–methanol) of this product gave **17**, $[\alpha]_D^{20}$ −43° (initial)→ −48° (72 h; *c* 0.47, water), lit.[4] $[\alpha]_D^{20}$ −53.5° (initial)→ −57.5° (72 h; *c* 2, water). ^{13}C-N.m.r. data (D_2O, 75 MHz): δ 101.1 (C-1′), 100.2 (C-1″), 96.8 (C-1β), 92.7 (C-1α), and 16.2 (C-6″).

Benzyl 2,6-di-O-benzyl-4-O-[6-O-benzyl-3,4-O-isopropylidene-2-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-glucopyranoside (**16**). — (*a*) *From* **6**. To a mixture of **6** (0.5 g, 0.67 mmol), tetraethylammonium bromide (1.2 g, 5.7 mmol), molecular sieves Type 4A (0.9 g), and *N,N*-dimethylformamide (1.1 mL) was added a solution of the bromide **7** (1.3 g, 2.6 mmol) in dichloromethane (4.5 mL) and ethyldi-isopropylamine (0.5 mL, 3.8 mmol), and the mixture was stirred under argon at room

temperature in the dark. After 3 days, a solution of more **7** (0.43 g, 0.86 mmol) in dichloromethane (3 mL) and tetraethylammonium bromide (0.8 g, 3.8 mmol) were added and, after 7 days, the mixture was treated with methanol (1 mL). The mixture was worked-up as described above for the synthesis of **13**. Column chromatography (Type II, 7:1 hexane–ethyl acetate) of the product gave **16** contaminated with a slower-moving component. After further column chromatography (Type II, 7:2 hexane–ethyl acetate), pure **16** (0.36 g, 34%) was obtained.

Further elution of the column gave **13** (0.33, 42%).

(*b*) *From* **13**. A mixture of **13** (0.3 g, 0.26 mmol), dichloromethane (5 mL), mercuric bromide (0.053 g, 0.13 mmol), 2,6-di-*tert*-butyl-4-methylpyridine (0.21 g, 1 mmol), and molecular sieves Type 4A (0.7 g) was stirred for 1 h. A solution of the bromide **7** (0.5 g, 1 mmol) in dichloromethane (5 mL) was then added during 3 h under argon, in the dark, and at room temperature. After 3.5, 4.5, and 7.5 h, more mercuric bromide (0.05, 0.05, and 0.08 g) was added, 2,6-di-*tert*-butyl-4-methylpyridine (0.1 g) was also added after 3.5 h, and the stirring was continued for 24 h. Methanol (1 mL) was then added and the mixture was worked-up as described for the synthesis of **8**. Column chromatography (Type II, 7:2 hexane–ethyl acetate) of the product gave **16** (0.3 g, 73%), $[\alpha]_D^{20}$ −59.5° (*c* 0.6, chloroform). N.m.r. data ($CDCl_3$): ^1H, δ 5.64 (d, 1 H, $J_{1',2'}$ 3.6 Hz, H-1'), 5.51 (d, 1 H, $J_{1''',2'''}$ 3.6 Hz, H-1'''), 1.35 (s, 6 H, CMe_2), 1.11 and 1.07 (2 d, 6 H, $J_{5,6}$ 6.4 Hz, H-6' and H-6'''); ^{13}C (20 MHz), δ 109.7 (CMe_2), 102.9, 100.3 (C-1,1''), 97.7, 95.6 (C-1',1'''), 28.1, 26.4 (2 Me), and 16.8 (C-6' and C-6''').

Anal. Calc. for $C_{97}H_{106}O_{19}$: C, 73.93; H, 6.78. Found: C, 74.23; H, 6.67.

Further elution gave **13** (0.04 g).

O-α-L-*Fucopyranosyl-(1→2)*-O-β-D-*galactopyranosyl-(1→4)*-O-[α-L-*fucopyranosyl-(1→3)*]-D-*glucose* (**18**). — A solution of **16** (0.29 g) in ethanol (35 mL) and ethyl acetate (6 mL) was hydrogenolysed (2 atm.) in the presence of 10% Pd/C (0.3 g) for 4 h at room temperature. The catalyst was collected on Celite and washed with methanol, and the combined filtrate and washings were concentrated. A solution of the residue in aqueous 20% acetic acid (4 mL) was kept for 0.5 h at 85–90° and then concentrated. Toluene was evaporated from the residue and column chromatography (Type I, 5:4 methanol–chloroform) then gave **18** as a white solid, $[\alpha]_D^{20}$ −100° (*c* 0.5, water); lit.[5] $[\alpha]_D^{20}$ −106° (*c* 1, water). N.m.r. data (D_2O): ^{13}C (20 MHz), δ 101.0 (C-1''), 100.3 (C-1'''), 99.1 (C-1'), 96.8 (C-1β), 92.9 (C-1α), and 16.3 (C-6' and C-6''').

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