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Chemical Modification of Lactose. XV.[1] Syntheses of O-α- and O-β-L-Fucopyranosyl-(1→3)-O-β-D-galactopyranosyl-(1→4)-D-glucopyranoses (3′-O-α- and 3′-O-β-L-Fucopyranosyllactoses)

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1,6-Anhydro-4′,6′-O-benzylidene-3′-O-tosyl-β-lactose was converted into 1,6-anhydro-2,2′,3-tri-O-benzyl-4′,6′-O-benzylidene-β-lactose (**3**) in 74.1% yield by benzylation followed by detosylation. Condensation of **3** with 2,3,4-tri-O-acetyl-α-L-fucopyranosyl bromide in benzene–nitromethane in the presence of mercuric cyanide and molecular sieves gave the corresponding trisaccharide derivatives (**6** and **11**) bearing α- and β-L-fucopyranosyl linkages in 37.1 and 43.1% yields, respectively. Compounds **6** and **11** were isolated by column chromatography on silica gel. The title sugars (**10** and **15**) were prepared in 4 steps from **6** and **11**, respectively; hydrogenolytic removal of the benzylidene and benzyl groups, acetylation, cleavage of the 1,6-anhydro-β-ring to β-acetate, and deacetylation.

^{1}H-NMR and ^{13}C-NMR spectral data of **10**, **15**, and the intermediates are also described.

Keywords——synthesis; 3′-O-α-L-fucopyranosyllactose; 3′-O-β-L-fucopyranosyllactose; human milk oligosaccharide; glycosylation; lactosan derivatives; 1,6-anhydro-β-trisaccharide derivatives; ^{1}H-NMR; ^{13}C-NMR

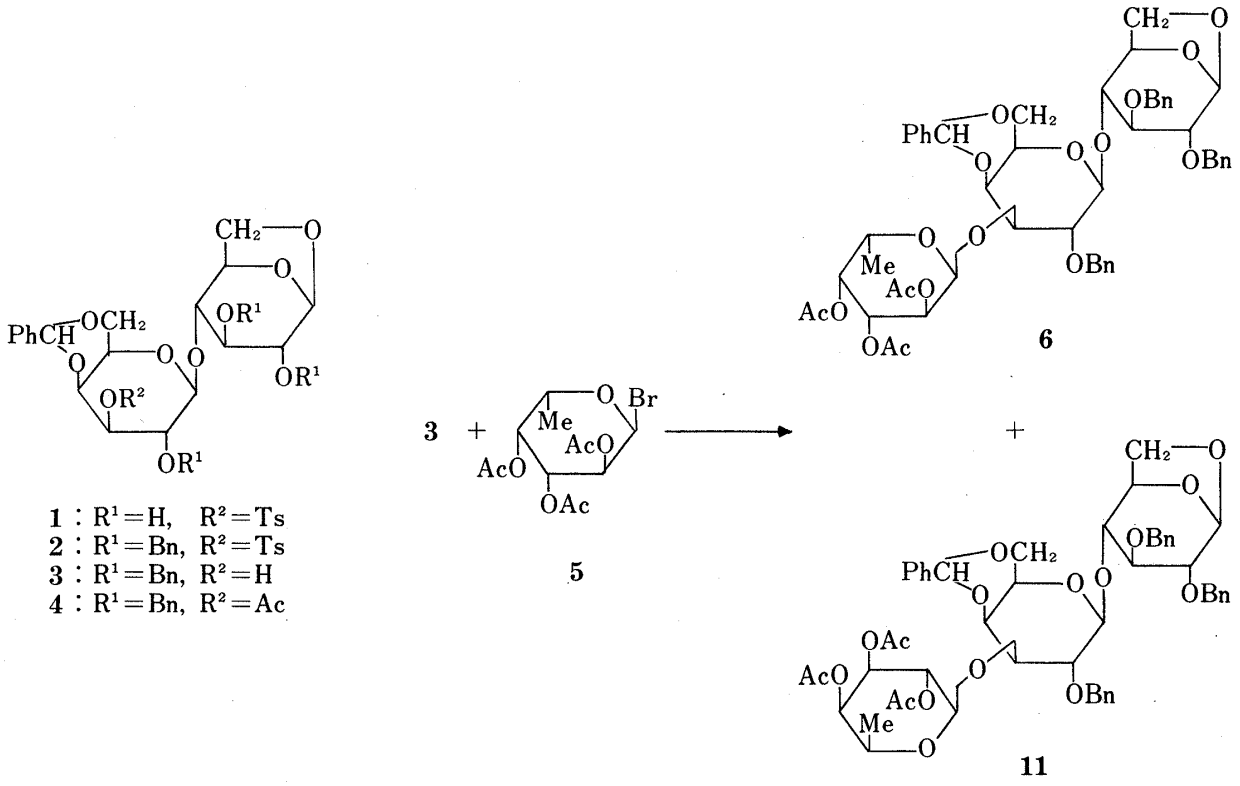
The fucosyl derivatives of lactose and lacto-N-tetraose are the major components of oligosaccharides in human milk.[2] Among these shown to be present in human milk, 2′-O-α-L-fucopyranosyllactose and 3-O-α-L-fucopyranosyllactose have the simplest structures. Therefore, the title sugars are positional isomers of fucosyllactoses in human milk with α- and β-L-fucopyranosyl linkages. Recently, the occurrence, in nature, of higher oligosaccharides that are structurally derived from lactose and one or more L-fucopyranosyl groups has also been reported.[3]

As an extension of our project of synthesizing oligosaccharides in human milk and their analogs, and those bearing α-L-fucopyranose such as 3-O-[4] and 6-O-α-L-fucopyranosyl-di-N-acetylchitobioses,[5] we now describe syntheses and physical properties of the title sugars (**10** and **15**).

1,6-Anhydro-4′,6′-O-benzylidene-3′-O-tosyl-β-lactose (**1**), isolated in 15% yield by partial tosylation of 1,6-anhydro-4′,6′-O-benzylidene-β-lactose,[6] is the starting material of our syntheses. Benzylation of **1** afforded 1,6-anhydro-2,2′,3-tri-O-benzyl-4′,6′-O-benzylidene-3′-O-tosyl-β-lactose (**2**) as a syrup. Subsequent detosylation of **2** with sodium amalgam gave 1,6-anhydro-2,2′,3-tri-O-benzyl-4′,6′-O-benzylidene-β-lactose (**3**) as white needles in 74.1% yield based on **1**.

Condensation of **3** (1 mol eq.) with 2,3,4-tri-O-acetyl-α-L-fucopyranosyl bromide[7] (**5**, 2.1 mol eq.) was carried out by stirring in benzene-nitromethane (1 : 1) in the presence of mercuric cyanide and molecular sieves at 60—65° for 24 hr under nitrogen. After 24 hr, more **5** (2.1 mol eq.) was added and the reaction was continued for a further 24 hr. Thin-layer chromatography (TLC) showed the formation of two products, and each product was isolated by column chromatography on silica gel. From the earlier fractions, the trisaccharide bearing the α-L-fucopyranosyl linkage (**6**), O-(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-(1→3)-O-(2-O-benzyl-4,6-O-benzylidene-β-D-galactopyranosyl)-(1→4)-1,6-anhydro-2,3-di-O-benzyl-β-D-glucopyranose, was isolated as an amorphous powder in 37.1% yield. From the later fractions, the trisaccharide bearing the β-L-fucopyranosyl linkage (**11**), O-(2,3,4-tri-O-acetyl-β-L-fucopyranosyl)-

(1→3)-O-(2-O-benzyl-4,6-O-benzylidene-β-D-galactopyranosyl)-(1→4)-1,6-anhydro-2,3-di-O-benzyl-β-D-glucopyranose, was isolated as needles in 43.1% yield. The proton nuclear magnetic resonance (^{1}H-NMR) spectra of **6** and **11** were in full agreement with their proposed structures. Trisaccharide derivatives bearing α-L-fucopyranosidic linkages (**7**—**10**) and those with the β-linkages (**12**—**15**) were then prepared from **6** and **11**, respectively, as described below.



Ac=acetyl, Bn=benzyl, Me=methyl, Ph=phenyl, Ts=tosyl

Chart 1

Compound **6** was converted into the 1,6-anhydro-β-trisaccharide octaacetate (**7**), O-(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-(1→3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-2,3-di-O-acetyl-1,6-anhydro-β-D-glucopyranose, by catalytic hydrogenation (which resulted in debenzylidenation with simultaneous debenzylation), followed by acetylation. Deacetylation of **7** afforded the 1,6-anhydro-β-trisaccharide (**8**), O-α-L-fucopyranosyl-(1→3)-O-β-D-galactopyranosyl-(1→4)-1,6-anhydro-β-D-glucopyranose, as an amorphous powder. The corresponding crystalline 1,6-anhydro-β-trisaccharide octaacetate (**12**) and 1,6-anhydro-β-trisaccharide (**13**) bearing β-L-fucosidic linkages were prepared from **11** by methods analogous to those described for the preparation of the α-isomers (**7** and **8**).

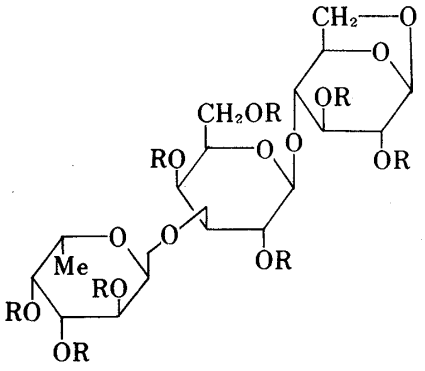
The ^{1}H-NMR spectrum of **8** was measured in deuterium oxide (D_2O) containing deuteromethanol (CD_3OD), and that of **13** was measured in D_2O. The signals due to anomeric protons were assigned by comparison with those of methyl α- and β-L-fucopyranosides and 1,6-anhydro-β-lactose. In **8** or **13**, the anomeric proton due to the fucopyranosidic linkage (H-1″) appeared as a doublet at δ 4.99 or 4.57 ppm with a coupling constant (J=3.5 or 7 Hz, respectively) consistent with the assigned α- or β-L-fucopyranosyl configuration for **8** or **13**. The configurations of the newly established glycosidic linkages were further confirmed by carbon-13 magnetic resonance (^{13}C-NMR) spectroscopy as mentioned later.

The 1,6-anhydro-β-ring of **7** was cleaved by heating to reflux in chloroform with titanium tetrabromide. Subsequent treatment of the resulting crude bromide with mercuric acetate (in order to replace the bromine atom by an acetoxyl group), followed by column chromatographic

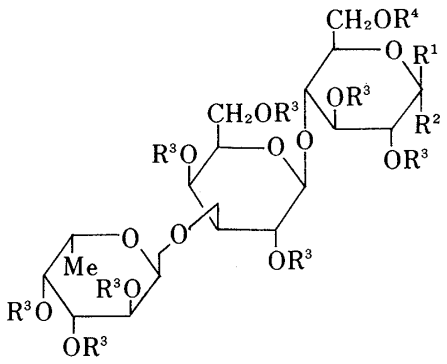
purification, afforded the β-anomer of the trisaccharide nonaacetate (**9**), O-(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-(1→3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-1,2,3-tri-O-acetyl-β-D-glucopyranose, as an amorphous powder in 59.7% yield. The anomeric-proton doublet due to terminus glucose appeared at δ 5.68 ppm with a coupling constant (J=8 Hz) characteristic of the β linkage.

Deacetylation of **9** gave O-α-L-fucopyranosyl-(1→3)-O-β-D-galactopyranosyl-(1→4)-α-D-glucopyranose (3′-O-α-L-fucopyranosyl-α-lactose) (**10**). The product crystallized from aqueous ethanol as fine needles and showed mutarotation, −25.3° (3 min)→−30° (3 hr, constant). Baer and Abbas[3] obtained 3′-O-α-L-fucopyranosyllactose as a white powder, $[\alpha]_D$ −39° (3 min)→−42.6° (2 hr, final).

The trisaccharide nonaacetate (**14**) and trisaccharide (**15**) bearing the β-L-fucopyranosidic linkages were obtainable from **12** by methods similar to those described in the preparations of **9** and **10**. O-β-L-Fucopyranosyl-(1→3)-O-β-D-galactopyranosyl-(1→4)-D-glucopyranose (3′-O-β-L-fucopyranosyllactose) (**15**) crystallized as an anomeric mixture, $[\alpha]_D^{21}$ +67.1° (no mutarotation). Baer and Abbas[8] obtained the α-anomer of 3′-O-β-L-fucopyranosyllactose as a white solid, $[\alpha]_D^{25}$ +54.2° (initial)→+51° (4 hr, constant).

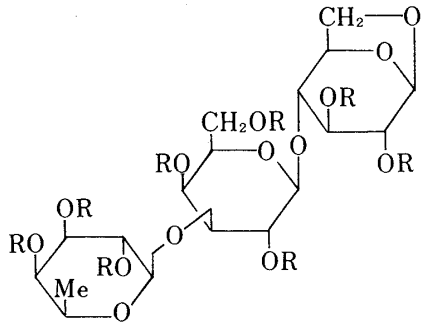


7 : R=Ac
8 : R=H

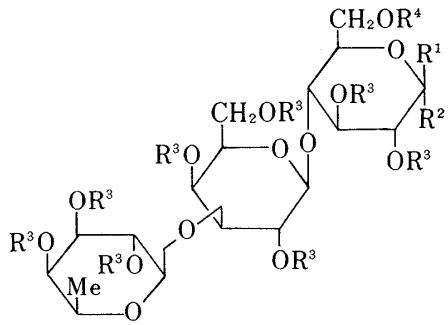


9 : R^1=OAc, R^2=R^4=H, R^3=Ac
10 : R^1=R^3=R^4=H, R^2=OH

Chart 2



12 : R=Ac
13 : R=H



14 : R^1=OAc, R^2=R^4=H, R^3=Ac
15 : R^1,R^2=H, OH, R^3=R^4=H

Chart 3

Partial hydrolysis of **10** or **15** with hydrochloric acid resulted in the liberation of fucose and lactose, while complete hydrolysis liberated fucose, galactose, and glucose: they were identified by TLC.

The ^{13}C-NMR spectral data of the title sugars (**10** and **15**) and the corresponding 1,6-anhydro-β-derivatives (**8** and **13**) are summarized in Table I. The signals were assigned by

comparison with observed values for methyl α- and β-L-fucopyranosides (indicated in Table II) and with literature values for 1,6-anhydro-β-lactose,[9] and α- and β-lactoses.[10] The signals for the corresponding carbon atoms in methyl α-L-fucopyranoside and fucosyl residues of **8** and **10** showed similar chemical shifts, which established that **8** and **10** had an α-L-fucopyranosidic linkage. Similarly, **13** and **15** had a β-L-fucopyranosidic linkage. The resonances for C-3′ of **8**, **13**, **10**, and **15** appeared at 81.3, 80.6, 81.6, and 80.9 ppm, respectively. They were deshielded by 8.2—7.5 or 8.1—7.4 ppm as compared to the chemical shifts for C-3′ of 1,6-anhydro-β-lactose (73.1 ppm) or lactose (73.5 ppm), respectively. The results provide an unequivocal proof of the positions of the newly introduced L-fucosyl linkages in **8**, **10**, **13**, and **15**.

TABLE I. ^{13}C-NMR Chemical Shifts δ (ppm) from TMS

Compound	Carbon								
	C-1	C-2	C-3	C-4	C-5	C-6	C-1′	C-2′	C-3′
8[a]	102.7	71.2	72.7	79.1	75.1	66.0	102.9	70.7	81.3
13[b]	102.7	71.2	72.7	78.9	75.3	66.4	101.7	70.4	80.6
1,6-Anhydro-β-lactose[9]	102.0	70.6	72.0	78.3	74.6	65.7	102.6	71.2	73.1
10(α)[c]	93.0	72.4	72.7	79.8	71.3	61.3	103.9	71.7	81.6
10(β)[c]	97.0	75.1	75.6	79.7	76.0	61.4			
15(α)[d]	93.1	72.5	72.3	79.4	71.3	61.3	101.7	71.7	80.9
15(β)[d]	97.0	75.1	75.6	79.4	76.0	61.3			
Lactose(α)[10]	92.7	72.2	72.4	79.3	71.0	61.0	103.6	72.0	73.5
Lactose(β)[10]	96.2	74.8	75.3	79.2	75.6	61.1	103.7	72.0	73.5

Compound	Carbon								
	C-4′	C-5′	C-6′	C-1″	C-2″	C-3″	C-4″	C-5″	C-6″
8[a]	67.9	76.2	61.9	101.7	72.9	69.6	71.5	67.9	16.3
13[b]	67.4	76.3	62.3	103.0	72.6	74.1	72.3	71.4	16.7
1,6-Anhydro-β-lactose[9]	69.2	75.8	61.6						
10(α)[c]	69.7[e]	76.5	62.2	102.1	73.0	69.9[e]	70.7	68.4	16.6
10(β)[c]									
15(α)[d]	67.3	76.3	62.1	103.8	72.5	74.1	72.3	70.7	16.6
15(β)[d]									
Lactose(α)[10]	69.5	76.2	62.0						
Lactose(β)[10]	69.5	76.2	62.0						

a) O-α-L-Fucopyranosyl-(1→3)-O-β-D-galactopyranosyl-(1→4)-1,6-anhydro-β-D-glucopyranose.
b) O-β-L-Fucopyranosyl-(1→3)-O-β-D-galactopyranosyl-(1→4)-1,6-anhydro-β-D-glucopyranose.
c) O-α-L-Fucopyranosyl-(1→3)-O-β-D-galactopyranosyl-(1→4)-D-glucopyranose.
d) O-β-L-Fucopyranosyl-(1→3)-O-β-D-galactopyranosyl-(1→4)-D-glucopyranose.
e) Assignments for C-4′ and C-3″ may be reversed.

TABLE II. ^{13}C-NMR Chemical Shifts δ(ppm) from TMS

	C-1	C-2	C-3	C-4	C-5	C-6	OMe
Methyl α-L-fucoside[a]	100.7	73.0	69.1	70.8	67.6	16.5	56.3
Methyl β-L-fucoside[b]	104.9	72.6	74.2	72.1	71.7	16.6	58.4

a) Assignments of each carbon were made by reference to Lemieux and Driguez.[11]
b) Assignments of each carbon were made by reference to Tsai and Behrman.[7]

After this work had been completed, Baer and Abbas[3] reported the syntheses of the title sugars. They prepared the fucosyllactose derivatives bearing an α-L-fucopyranosyl linkage by condensation of 1,2,2′,3,6,6′-hexa-O-acetyl-α,β-lactose (**16**) with 2,3,4-tri-O-benzyl-α-L-

fucopyranosyl bromide by a bromide ion-catalyzed reaction, while those bearing the β-linkage were prepared by condensation of **5** and **16** under Koenigs–Knorr conditions. The final products were separated as amorphous solids. They reported no ^{13}C-NMR spectral data throughout the paper.

Experimental

Instruments used and conditions for chromatography were the same as in Part XIII[12] unless otherwise indicated. TLC was performed with the following solvent combinations (v/v): (A), $CHCl_3$-acetone (3:1); (B), benzene–ether (1:1); (C), ether–AcOEt (3:1); (D), $CHCl_3$–ether–MeOH (10:10:1). Solvent combinations for elution on column chromatography with Kiesel-gel 60 (Merck, 70—230 mesh) are shown as v/v.

1,6-Anhydro-2,2′,3-tri-O-benzyl-4′,6′-O-benzylidene-β-lactose (3)——1) 1,6-Anhydro-2,2′,3-tri-O-benzyl-4′,6′-O-benzylidene-3′-O-tosyl-β-lactose (**2**): Benzyl bromide (11.4 ml, 96.1 mmol) was added dropwise with stirring at 0° to a mixture of 1,6-anhydro-4′,6′-O-benzylidene-3′-O-tosyl-β-lactose (**1**)[6] (3 g, 5.3 mmol), BaO (6.6 g, 43 mmol), and $Ba(OH)_2 \cdot 8H_2O$ (2.82 g, 8.95 mmol) in dry N,N-dimethylformamide (60 ml). Stirring was continued at room temperature for 48 hr, then the mixture was poured into ice-H_2O (300 ml), and after being stirred for 24 hr, it was filtered, and the filtrate was extracted with CH_2Cl_2 (3×100 ml). The extracts were washed successively with H_2O, 10% H_2SO_4, H_2O, aq. $NaHCO_3$, and H_2O, dried ($MgSO_4$), and concentrated to give a syrup, that was chromatographed on a column with benzene–ether (5:1). After benzyl alcohol had emerged, **2** was eluted and isolated as a syrup (5.2 g).

2) Detosylation of **2**: 2% sodium amalgam (20 g) was added with stirring to a solution of **2** (5.2 g) in dry MeOH (150 ml), and stirring was continued at room temperature overnight. The mixture was filtered, and the filtrate was neutralized with glacial AcOH. After removal of the solvent, the residue was treated with CH_2Cl_2 (100 ml) and H_2O (100 ml) under stirring to effect dissolution. The organic layer was separated, washed with H_2O (3×100 ml), dried ($MgSO_4$), and concentrated to a syrup (3.87 g), which was crystallized from EtOH. Recrystallization from EtOH gave **3** (2.68 g, 74.1% based on **1**) as white needles, mp 78—80°, $[\alpha]_D^{21}$ −41.5° (c=0.88, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 3280 (OH). ^1H-NMR ($CDCl_3$): 7.20—7.68 (20H, m, aromatic protons). TLC: *Rf* 0.52 (solvent A), 0.07 (B), 0.22 (C), 0.32 (D). *Anal.* Calcd for $C_{40}H_{42}O_{10}$: C, 70.37; H, 6.20. Found: C, 70.42; H, 6.02.

3′-O-Acetyl-1,6-anhydro-2,2′,3-tri-O-benzyl-4′,6′-O-benzylidene-β-lactose (4)——Acetylation of **3** (150 mg, 0.22 mmol) was carried out with Ac_2O (2 ml) and pyridine (2 ml) at room temperature overnight. The mixture was then concentrated by repeated co-distillation with toluene. The residue was dissolved in CH_2Cl_2, successively washed with H_2O, 10% H_2SO_4, H_2O, aq. $NaHCO_3$, and H_2O, dried ($MgSO_4$), and concentrated to give **4** (135 mg, 84.8%) as an amorphous powder, $[\alpha]_D^{23}$ +7.5° (c=1.7, $CHCl_3$). ^1H-NMR ($CDCl_3$): 2.02 (3H, s, OAc), 7.08—7.52 (20H, m, aromatic protons). TLC: *Rf* 0.78 (solvent A), 0.24 (B), 0.58 (C), 0.57 (D). *Anal.* Calcd for $C_{42}H_{44}O_{11}$: C, 69.60; H, 6.12. Found: C, 69.66; H, 5.82.

O-(2,3,4-Tri-O-acetyl-α-L-fucopyranosyl)-(1→3)-O-(2-O-benzyl-4, 6-O-benzylidene-β-D-galactopyranosyl)-(1→4)-1,6-anhydro-2,3-di-O-benzyl-β-D-glucopyranose (6) **and O-(2,3,4-Tri-O-acetyl-β-L-fucopyranosyl)-(1→3)-O-(2-O-benzyl-4,6-O-benzylidene-β-D-galactopyranosyl)-(1→4)-1,6-anhydro-2,3-di-O-benzyl-β-D-glucopyranose** (11)——Crystalline 2,3,4-tri-O-acetyl-α-L-fucopyranosyl bromide (**5**)[7] (3 g, 8.5 mmol), mercuric cyanide (2.4 g, 9.5 mmol), and powdered 4 Å molecular sieves (3 g) were successively added to a solution of **3** (2.75 g, 4 mmol) in dry benzene–nitromethane (1:1, v/v, 60 ml). The suspension was stirred under nitrogen at 60—65° for 24 hr. After 24 hr, more **5** (3 g) and mercuric cyanide (2.4 g) were added and stirring was continued for a further 24 hr. The mixture was diluted with benzene (100 ml), filtered, and the filtrate was successively washed with H_2O, aq. $NaHCO_3$, and H_2O, then dried ($MgSO_4$), and concentrated to give an amorphous powder (7.18 g) that was chromatographed on a column with ether–AcOEt (15:1). From the earlier fractions, **6** (1.43 g, 37.1%) was isolated as an amorphous powder, $[\alpha]_D^{22}$ −66.8° (c=0.9, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 1735 (OAc). ^1H-NMR ($CDCl_3$): 0.95 (3H, d, $J_{5'',6''}$=6.5 Hz, C$\underline{H}_3$), 1.64, 1.99, 2.13 (9H, all s, OAc×3), 5.45 (1H, s, H-1, β-Glc), 7.19—7.54 (20H, m, aromatic protons). TLC: *Rf* 0.69 (solvent A), 0.10 (B), 0.41 (C), 0.49 (D). *Anal.* Calcd for $C_{52}H_{58}O_{17}$: C, 65.40; H, 6.12. Found: C, 65.25; H, 6.11.

From the later fractions with the same solvent, **11** was isolated as an amorphous powder (1.66 g, 43.1%) that crystallized from AcOEt–ether–petr. ether as white needles, mp 106—108°, $[\alpha]_D^{21}$ +1.3° (c=1.4, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 1741 (OAc). ^1H-NMR ($CDCl_3$): 1.10 (3H, d, $J_{5'',6''}$=6.5 Hz, C$\underline{H}_3$), 1.55, 1.95, 2.16 (9H, all s, OAc×3), 5.43 (1H, s, H-1, β-Glc), 7.14—7.44 (20H, m, aromatic protons). TLC: *Rf* 0.59 (solvent A), 0.04 (B), 0.25 (C), 0.36 (D). *Anal.* Calcd for $C_{52}H_{58}O_{17}$: C, 65.40; H, 6.12. Found: C, 65.10; H, 6.05.

O-(2,3,4-Tri-O-acetyl-α-L-fucopyranosyl)-(1→3)-O-(2,4,6-tri-O-acetyl-β-galactopyranosyl)-(1→4)-2,3-di-O-acetyl-1,6-anhydro-β-D-glucopyranose (7)——A solution of **6** (860 mg, 0.9 mmol) in dry MeOH (20 ml) was hydrogenated overnight in the presence of a Pd catalyst, freshly prepared[13] from $PdCl_2$ (500 mg), at room temperature under atmospheric pressure. After filtration, the filtrate was concentrated to an amorphous powder (600 mg) that was acetylated with Ac_2O (10 ml) and pyridine (10 ml) at room temperature overnight. The mixture was poured into ice-H_2O (100 ml), stirred for 3 hr, and the whole was extracted with CH_2Cl_2 (3×30 ml). The extracts were successively washed with H_2O, 10% H_2SO_4, H_2O, aq. $NaHCO_3$, and H_2O,

dried ($MgSO_4$), and concentrated to an amorphous powder (821 mg) that was chromatographed on a column with $CHCl_3$–acetone (12: 1). The eluate provided **7** (660 mg, 90.9%) as an amorphous powder, $[\alpha]_D^{23}$ −82.2° (c=0.5, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 1728 (OAc). ^{1}H-NMR ($CDCl_3$): 1.15 (3H, d, $J_{5'',6''}$=6.5 Hz, C$\underline{H}_3$), 1.97, 2.04, 2.11, 2.16, 2.20 (24H, all s, OAc×8), 5.44 (1H, s, H-1, β-Glc). TLC: *Rf* 0.48 (solvent A), 0.04 (B), 0.25 (C), 0.33 (D). *Anal.* Calcd for $C_{34}H_{46}O_{22}$: C, 50.62; H, 5.75. Found: C, 50.38; H, 5.67.

O-α-L-Fucopyranosyl-(1→3)-O-β-D-galactopyranosyl-(1→4)-1,6-anhydro-β-D-glucopyranose (8)——0.5 N Methanolic MeONa (0.6 ml) was added dropwise under stirring to a chilled solution of **7** (120 mg, 0.15 mmol) in dry MeOH (6 ml). After being stirred overnight at room temperature, the mixture was neutralized with Amberlite IR-120 (H^+) resin, filtered, and concentrated to dryness to give **8** as a hydroscopic amorphous powder (62 mg, 87.9%), $[\alpha]_D^{19}$ −89.4° (c=0.9, H_2O). IR ν_{max}^{KBr} cm^{-1}: 3330 (OH). ^{1}H-NMR (D_2O–CD_3OD): 1.04 (3H, d, $J_{5'',6''}$=6.5 Hz, C$\underline{H}_3$), 4.33 (1H, d, $J_{1',2'}$=7 Hz, H-1′, β-Gal), 4.99 (1H, d, $J_{1'',2''}$=3.5 Hz, H-1″, α-Fuc), 5.18 (1H, s, H-1, β-Glc). ^{13}C-NMR: see Tables I and II. TLC: *Rf* 0.42 (70% aq. 2-PrOH–AcOEt, 2: 1, v/v). *Anal.* Calcd for $C_{18}H_{30}O_{14}\cdot 2H_2O$: C, 42.68; H, 6.76. Found: C, 42.38; H, 6.78.

O-(2,3,4-Tri-O-acetyl-α-L-fucopyranosyl)-(1→3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-1,2,3-tri-O-acetyl-β-D-glucopyranose (9)——Crystalline titanium tetrabromide (*ca.* 1 g) was added to a solution of **7** (220 mg, 0.27 mmol) in dry $CHCl_3$ (6 ml). The mixture was boiled to reflux for 5 hr under stirring with exclusion of moisture, cooled, and diluted with $CHCl_3$ (20 ml). The solution was washed with chilled H_2O (3×30 ml), dried ($MgSO_4$), and concentrated to an amorphous powder (267 mg). The residue was treated with AcOH (8 ml) and mercuric acetate (270 mg) overnight at room temperature, poured into ice-H_2O (50 ml), and the whole was extracted with CH_2Cl_2 (3×20 ml). The extracts were washed with aq. $NaHCO_3$ and H_2O, dried ($MgSO_4$), and evaporated to dryness; TLC with solvent A showed the presence of **9**, a small amount of unreacted **7**, and unidentified by-products. The residue was chromatographed on a column with $CHCl_3$–acetone (5: 1). After **7** (29 mg, 13.2%) had been recovered from the earlier fractions, **9** was isolated from the later fractions as an amorphous powder (141 mg, 59.7%), $[\alpha]_D^{21}$ −58.3° (c=0.5, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3430 (OH), 1736 (OAc). ^{1}H-NMR ($CDCl_3$): 1.14 (3H, d, $J_{5'',6''}$=6.5 Hz, C$\underline{H}_3$), 1.98, 2.03, 2.09, 2.16, 2.22 (27H, all s, OAc×9), 5.68 (1H, d, $J_{1,2}$=8 Hz, H-1, β-Glc). TLC: *Rf* 0.31 (solvent A), 0.33 (C), 0.31 (D). *Anal.* Calcd for $C_{36}H_{50}O_{24}\cdot 1/2H_2O$: C, 49.37; H, 5.87. Found: C, 49.25; H, 5.75.

O-α-L-Fucopyranosyl-(1→3)-O-β-D-galactopyranosyl-(1→4)-α-D-glucopyranose (3′-O-α-L-Fucopyranosyl-α-lactose) (10)——Deacetylation of **9** (100 mg, 0.11 mmol) in dry MeOH (5 ml) with 0.5 N methanolic MeONa (0.5 ml) as described for the preparation of **8** gave an amorphous powder which crystallized from aq. EtOH as fine needles (51 mg, 91.8%). The product began to brown at 245° and decomposed at 255—257°, $[\alpha]_D^{20}$ −25.3° (3 min)→−30° (3 hr, constant, c=0.3, H_2O). IR ν_{max}^{KBr} cm^{-1}: 3390 (OH). ^{1}H-NMR (D_2O): 1.67 (3H, d, $J_{5'',6''}$=6.5 Hz, C$\underline{H}_3$), 4.93 (1H, br. d, overlap with ring protons, H-1′, β-Gal), 5.62 (>1H, d, $J_{1,2}$ and $_{1'',2''}$ =3.5 Hz, H-1(α) and H-1″, α-Glc and α-Fuc). The H-1(β) signal overlapped with HOD and could not be assigned. ^{13}C-NMR: see Tables I and II. TLC: *Rf* 0.49 (2-PrOH–acetone–0.1 M lactic acid, 2: 2: 1, v/v),[14] 0.32 (70% aq. 2-PrOH–AcOEt, 2: 1, v/v). *Anal.* Calcd for $C_{18}H_{32}O_{15}\cdot 1/2H_2O$: C, 43.46; H, 6.69. Found: C, 43.24; H, 6.67.

O-(2,3,4-Tri-O-acetyl-β-L-fucopyranosyl)-(1→3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-2,3-di-O-acetyl-1,6-anhydro-β-D-glucopyranose (12)——Compound **11** (200 mg, 0.21 mmol) in dry MeOH (10 ml) was hydrogenated in the presence of a Pd catalyst, freshly prepared[13] from $PdCl_2$ (200 mg), as described for the preparation of **7** to give an amorphous powder (127 mg). The product was then acetylated with Ac_2O (2 ml) and pyridine (2 ml), and the resulting crude acetate (173 mg) was chromatographed on a column with $CHCl_3$-acetone (10: 1) to isolate **12** (163 mg, 96.3%) that crystallized from EtOH as colorless needles, mp 230°, $[\alpha]_D^{21}$ −6.4° (c=0.9, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 1728 (OAc). ^{1}H-NMR ($CDCl_3$): 1.21 (3H, d, $J_{5'',6''}$=6.5 Hz, C$\underline{H}_3$), 1.97, 2.05, 2.08, 2.09, 2.12, 2.13, 2.16 (24H, all s, OAc×8), 5.47 (1H, s, H-1, β-Glc). TLC: *Rf* 0.32 (solvent A), 0.14 (C), 0.22 (D). *Anal.* Calcd for $C_{34}H_{46}O_{22}$: C, 50.62; H, 5.75. Found: C, 50.47; H, 5.66.

O-β-L-Fucopyranosyl-(1→3)-O-β-D-galactopyranosyl-(1→4)-1,6-anhydro-β-D-glucopyranose (13)——Deacetylation of **12** (100 mg, 0.12 mmol) in dry MeOH (5 ml) with 0.5 N methanolic MeONa (0.5 ml) as described for the preparation of **8** gave **13** (53 mg, 91.4%) as an amorphous powder. The product crystallized from EtOH as white needles, mp 250—252°, $[\alpha]_D^{18}$ −14.4° (c=0.5, H_2O). IR ν_{max}^{KBr} cm^{-1}: 3380 (OH). ^{1}H-NMR (D_2O): 1.31 (3H, d, $J_{5'',6''}$=6.5 Hz, C$\underline{H}_3$), 4.57 (1H, d, $J_{1'',2''}$=7 Hz, H-1″, β-Fuc), 4.64 (1H, d, $J_{1',2'}$= 8 Hz, H-1′, β-Gal), 5.50 (1H, s, H-1, β-Glc). ^{13}C-NMR: see Tables I and II. TLC: *Rf* 0.37 (70% aq. 2-PrOH–AcOEt, 2: 1, v/v). *Anal.* Calcd for $C_{18}H_{30}O_{14}$: C, 45.96; H, 6.43. Found: C, 45.72; H, 6.62.

O-(2,3,4-Tri-O-acetyl-β-L-fucopyranosyl)-(1→3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-1,2,3-tri-O-acetyl-β-D-glucopyranose (14)——Crystalline titanium tetrabromide (*ca.* 800 mg) was added to a solution of **12** (200 mg, 0.25 mmol) in dry $CHCl_3$ (6 ml), and the mixture was processed as described for the preparation of **9** to give an amorphous powder (217 mg). After treatment with AcOH (6 ml) and mercuric acetate (220 mg) as described for **9**, the resulting amorphous powder (210 mg) was chromatographed on a column with $CHCl_3$–acetone (12: 1). From the earlier fractions, unreacted **12** (22 mg, 11%) was recovered. Further elution with the same solvent eluted **14** and, on removing the solvent, **14** was isolated as an amorphous powder (114 mg, 53%). The product crystallized from MeOH–ether as white needles, $[\alpha]_D^{21}$ +13.1° (c=0.31, $CHCl_3$). It became colorless at 133° and then melted at 138°. IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1742 (OAc). ^{1}H-NMR ($CDCl_3$): 1.21 (3H, d, $J_{5'',6''}$=6.5 Hz, C$\underline{H}_3$), 1.96, 2.04, 2.09, 2.11, 2.16 (27H, all s, OAc×9), 5.71 (1H,

d, $J_{1,2}=8$ Hz, H-1, β-Glc). TLC: *Rf* 0.18 (solvent A), 0.17 (C), 0.19 (D). *Anal.* Calcd for $C_{36}H_{50}O_{24}\cdot H_2O$: C, 48.87; H, 5.92. Found: C, 49.16; H, 6.20.

O-β-L-Fucopyranosyl-(1→3)-O-β-D-galactopyranosyl-(1→4)-D-glucopyranose (3′-O-β-L-Fucopyranosyllactose) (15)——Deacetylation of **14** (120 mg, 0.14 mmol) in dry MeOH (5 ml) with 0.5 N methanolic MeONa (0.5 ml) as described for the preparation of **8** gave **15** as an amorphous powder (60 mg, 91%) that crystallized from H_2O-2-PrOH–AcOEt as fine needles, mp 265—267° (dec.), $[\alpha]_D^{21}$ +67.1° (no mutarotation, $c=0.29$, H_2O). [lit.[8] $[\alpha]_D^{25}$ +54.2° (initial)→+51° (4 hr, constant, $c=0.3$, H_2O)]. IR ν_{max}^{KBr} cm^{-1}: 3360 (OH). ^{1}H-NMR (D_2O): 1.72 (3H, d, $J_{5'',6''}=6.5$ Hz, C$\underline{H}_3$), 5.10 (<1H, d, $J_{1,2}=8$ Hz, H-1(β), β-Glc), 5.66 (<1H, d, $J_{1,2}=3.7$ Hz, H-1(α), α-Glc), H-1′ and H-1″ signals overlapped with HOD and could not be assigned. ^{13}C-NMR: see Tables I and II. TLC: *Rf* 0.48 (2-PrOH–acetone–1 M lactic acid, 2: 2: 1, v/v), 0.30 (70% aq. 2-PrOH–AcOEt, 2: 1, v/v). *Anal.* Calcd for $C_{18}H_{32}O_{15}\cdot H_2O$: C, 42.69; H, 6.77. Found: C, 42.53; H, 6.60.

TLC of the Acid Hydrolysate of 10 or 15——1) Partial Hydrolysis: A mixture of **10** or **15** (2 mg) and 0.1 N HCl (2 ml) was heated at 95° for 30 min, then evaporated to dryness. The residue was dissolved in a small amount of H_2O, and subjected to TLC with 2-PrOH–acetone–1 M lactic acid (2: 2: 1, v/v) lactose (*Rf* 0.47) and fucose (*Rf* 0.65) were identified.

2) Complete Hydrolysis: A mixture of **10** or **15** (2 mg) and 3 N HCl (2 ml) was heated at 95° for 3 hr. Galactose (*Rf* 0.55), glucose (*Rf* 0.59), and fucose (*Rf* 0.65) were identified in the hydrolysate.

Measurement of ^{13}C-NMR Spectra——The ^{13}C-NMR spectra were measured at 25 MHz with a JEOL JNM-FX-100 spectrometer in the pulse Fourier transform mode. The spectrum of **8** was measured in D_2O containing CD_3OD and the spectra of **10**, **13**, **15**, and methyl α- and β-L-fucopyranosides were measured in D_2O at room temperature. Tetramethylsilane (TMS) was used as an external standard; chemical shifts are given in ppm from TMS. The results are shown in Tables I and II.

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