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SYNTHESIS OF *O*-α-L-FUCOPYRANOSYL-(1→3)-*O*-β-D-GALACTOPYRANOSYL-(1→4)-2-ACETAMIDO-2-DEOXY-D-GLUCOPYRANOSE (*N*-ACETYL-3′-*O*-α-L-FUCOPYRANOSYLLACTOSAMINE)*

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ABSTRACT

Methyl 2-*O*-benzyl-β-D-galactopyranoside (**6**) was obtained in five, good yielding steps from methyl β-D-galactopyranoside (**1**). Treatment of **1** with *tert*-butylchlorodiphenylsilane in *N,N*-dimethylformamide in the presence of imidazole afforded a 6-(*tert*-butyldiphenylsilyl) ether, which was converted into its 3,4-*O*-isopropylidene derivative (**3**). Benzylation of **3** with benzyl bromide–silver oxide in *N,N*-dimethylformamide, and subsequent cleavage of its acetal and ether groups then afforded **6**. On similar benzylation, followed by the same sequence of deprotection, benzyl 2-acetamido-3,6-di-*O*-benzyl-4-*O*-[6-*O*-(*tert*-butyldiphenylsilyl)-3,4-*O*-isopropylidene-β-D-galactopyranosyl]-2-deoxy-α-D-glucopyranoside gave the 2-*O*-benzyl derivative (**10**). Compound **10** was converted into its 4,6-*O*-benzylidene acetal (**11**). Glycosylation (catalyzed by halide-ion) of **11** with 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide afforded the fully protected trisaccharide derivative (**13**). Cleavage of the benzylidene and then the benzyl groups of **13** furnished the title trisaccharide (**16**). The structure of **16** was established by ^{13}C-n.m.r. spectroscopy.

INTRODUCTION

The trisaccharide α-L-Fuc*p*-(1→3)-β-D-Gal*p*-(1→4)-D-GlcNAc has been reported to occur as part of the complex oligosaccharides isolated from human milk[2]. Kobata *et al.*[2] suggested the possibility of the existence of a unique L-fucosyltransferase that is responsible for the biosynthesis of the α-L-Fuc*p*-(1→3)-D-Gal linkages in such glycoconjugates. Our continued interest in the study of human L-fucosyltransferases with the aid of α-L-fucosylated derivatives as reference

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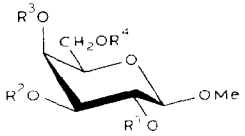
compounds and low-molecular-weight oligosaccharides as acceptor substrates warranted the synthesis of *O*-α-L-fucopyranosyl-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranose (**16**). Our interest in the synthesis of this trisaccharide was also enhanced because of our recent observation[3] that antisera raised against our synthetic disaccharide conjugate, α-L-Fuc*p*-(1→3)-β-D-Gal*p*OC$_6$H$_4$N=N-BSA, preferentially reacts with human colon adenocarcinoma cells. A similar observation was previously made by Miyauchi *et al.*[4] for the antiserum raised against 3′-*O*-α-L-fucopyranosyllactose [α-L-Fuc*p*-(1→3)-β-D-Gal*p*-(1→4)-D-Glc]. We describe herein the synthesis of trisaccharide **16**.

RESULTS AND DISCUSSION

For the synthesis of trisaccharide **16**, the readily accessible[5] benzyl 2-acetamido-3,6-di-*O*-benzyl-4-*O*-[6-*O*-(*tert*-butyldiphenylsilyl)-3,4-*O*-isopropylidene-β-D-galactopyranosyl]-2-deoxy-α-D-glucopyranoside (**7**) was the intermediate of choice for obtaining an *N*-acetyllactosamine derivative having free OH-3′. However, in an attempt to standardize the reaction conditions, we carried out first the subsequent transformations with methyl β-D-galactopyranoside (**1**). Thus, treatment of **1** with *tert*-butylchlorodiphenylsilane in *N*,*N*-dimethylformamide, in the presence of imidazole, afforded a good yield of the 6-(*tert*-butyldiphenylsilyl) ether **2**. This was readily converted, in high yield, into its 3,4-*O*-isopropylidene derivative **3**. Benzylation with benzyl bromide in *N*,*N*-dimethylformamide, in the

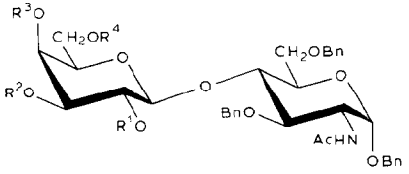


1 $R^1 = R^2 = R^3 = R^4 = H$
2 $R^1 = R^2 = R^3 = H; R^4 = Bu^tPh_2Si$
3 $R^1 = H; R^2, R^3 = CMe_2; R^4 = Bu^tPh_2Si$
4 $R^1 = Bn; R^2, R^3 = CMe_2; R^4 = Bu^tPh_2Si$
5 $R^1 = Bn; R^2, R^3 = CMe_2; R^4 = H$
6 $R^1 = Bn; R^2 = R^3 = R^4 = H$

presence of freshly prepared[6] silver oxide, gave in 73% yield the 2-*O*-benzyl derivative **4**. It has been previously observed[7] that base-catalyzed hydrolysis of the *tert*-butyldiphenylsilyl group was an unavoidable side-reaction during benzylation in the presence of sodium hydride or barium oxide–barium hydroxide. Although such a side-reaction cannot be excluded (some by-products were observed in t.l.c.), it would appear that it was minimized under the present reaction conditions as reflected by the relatively good yield of **4**, and also of **10** (see later). Sequential removal of the *tert*-butyldiphenylsilyl and 3,4-*O*-isopropylidene groups of **4** with

fluoride ion, and hot, 60% aqueous acetic acid, furnished known[8] methyl 2-*O*-benzyl-β-D-galactopyranoside (**6**) in a fair overall yield.

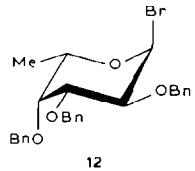
A similar sequence of reactions was adopted for the preparation of **10**. Thus, benzylation of **7** (ref. 5) with silver oxide–benzyl bromide, as described for **3** (to give **4**), afforded in 73% yield syrupy **8**, the ^{1}H-n.m.r. spectrum of which contained signals in support of its overall structure. During this benzylation, some unidentified by-products were also revealed by t.l.c. They may have resulted, at least in part, from the hydrolysis of the Bu^{t}Ph$_2$Si group.



7 R^1 = H; R^2, R^3 = CMe_2; R^4 = Bu^{t}Ph$_2$Si
8 R^1 = Bn; R^2, R^3 = CMe_2; R^4 = Bu^{t}Ph$_2$Si
9 R^1 = Bn; R^2, R^3 = CMe_2; R^4 = H
10 R^1 = Bn; R^2 = R^3 = R^4 = H
11 R^1 = Bn; R^2 = H; R^3, R^4 = PhCH

Removal of the Bu^{t}Ph$_2$Si group of **8** was readily accomplished by treatment with a molar solution of tetrabutylammonium fluoride in oxolane to give **9**. The acetal group was directly cleaved off, without prior purification, to give, in moderate yield, benzyl 2-acetamido-3,6-di-*O*-benzyl-4-*O*-(2-*O*-benzyl-β-D-galactopyranosyl)-2-deoxy-α-D-glucopyranoside (**10**). This was then converted, under the usual reaction conditions[9], into the 4,6-*O*-benzylidene derivative **11**.

Halide-ion-catalyzed glycosylation[10] of **11** with 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide[10,11] (**12**) afforded, in 89% yield, the fully protected trisaccharide derivative **13**, the ^{1}H-n.m.r. spectrum of which exhibited the appropriate signals, diagnostic of its identity. Cleavage of the benzylidene acetal group of **13** with hot, 60% aqueous acetic acid gave diol **14**, the identity of which was further



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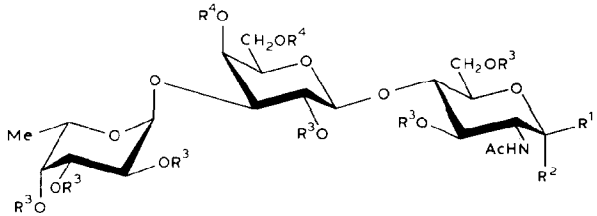

13 R^1 = H; R^2 = OBn; R^3 = Bn; R^4 = PhCH
14 R^1 = R^4 = H; R^2 = OBn; R^3 = Bn
15 R^1 = H; R^2 = OBn; R^3 = Bn; R^4 = Ac
16 R^1, R^2 = H,OH; R^3 = R^4 = H

TABLE I

PROPOSED ^{13}C-N.M.R. CHEMICAL SHIFTS[a]

Residue	*Compound*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	CH_3CO	OCH_3 *or* $C=O$
	α-D-GlcNAc[b]	90.33	54.12	70.96	70.27	71.80	60.96	22.50	169.08
	β-D-GlcNAc	95.22	57.08	74.12	70.63	76.48	60.96	22.86	169.08
	α-L-Fuc*p*OMe[c]	100.05	67.96	69.54	71.39	65.53	16.37		54.37
α-D-Glc*p*NAc	**16**	90.32	53.71	69.73	81.74	68.50	60.49	22.54	168.98
β-D-Glc*p*NAc		95.30[d]	56.48	72.14	81.74	74.71	60.49	22.95	168.98
β-D-Gal*p*		103.51	69.73	82.21	67.62	75.38	60.08		
α-L-Fuc*p*		101.46	68.08	69.83	71.74	66.29	16.48		

[a]For solutions in ($^2H_6$)Me_2SO with Me_4Si as the internal standard. [b]Values from ref. 5. [c]Values from ref. 12. [d]This signal had a substantially reduced (<one-fifth) intensity by comparison to that at δ 90.32.

confirmed by its conversion into analytically pure diacetate **15**. Hydrogenolysis of the benzyl groups of **14** in glacial acetic acid and in the presence of 10% Pd–C then furnished amorphous **16**, the ^{13}C-n.m.r. spectrum of which was in accord with the structure assigned (see Table I). In the ^{13}C-n.m.r. spectrum of **16**, the presence of an α-L-fucopyranosyl group was accounted for by the carbon atom signal at δ 101.46 attributable to C-1″, whereas the presence of a β-D-galactopyranosyl residue was evident by the signal at δ 103.51 assigned to C-1′. On the other hand, the 2-acetamido-2-deoxy-D-glucopyranose residue appeared to exist preponderantly in the α configuration, as evidenced by the intensity of the signal for C-1α (δ 90.32), which was more than five-times greater than that of the signal at δ 95.30, attributable to C-1β. That C-3′ and C-4 were the sites of glycosylation could readily be seen by the occurrence of their respective signals at low (δ 82.21 and 81.74, respectively) fields.

EXPERIMENTAL

General methods. — Melting points were determined with a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured at ~26° with a Perkin–Elmer 241 polarimeter. T.l.c. was conducted on aluminum sheets precoated with 0.2-mm layers of Silica Gel 60F-254 (E. Merck, Darmstadt, Germany); the components were located either by exposure to u.v. light, or by spraying the plates with 5% H_2SO_4 in ethanol and heating. Silica gel used for column chromatography was Baker Analyzed (60–200 mesh). The following solvent systems (v/v) were used for chromatography: (*A*) 3:2 ethyl acetate–hexane, (*B*) 19:1 chloroform–methanol, and (*C*) 13:6:1 chloroform–methanol–water. N.m.r. spectra were recorded at ~25°; ^{1}H-n.m.r. spectra at 90 MHz with a Varian EM-390, and ^{13}C-n.m.r. spectra at 67.8 MHz, with a JEOL FX-270 instrument. The positions of the peaks (δ) are expressed from the Me_4Si signal. Organic solutions were generally dried with anhydrous Na_2SO_4. Ag_2O was prepared by the method of Helferich and Klein[6].

Elemental analyses were performed by Robertson Laboratory, Florham Park, New Jersey, U.S.A.

*Methyl 6-O-(*tert*-butyldiphenylsilyl)-β-D-galactopyranoside* (**2**). — To a stirred solution of methyl β-D-galactopyranoside (**1**; 0.58 g, 3 mmol) and imidazole (0.45 g, 6.6 mmol) in anhydrous *N,N*-dimethylformamide (10 mL) was added *tert*-butylchlorodiphenylsilane (0.86 mL, 3.3 mmol), and stirring was continued for 1 h at room temperature. The mixture was then poured into ice–water, and the solid that separated was filtered off, and thoroughly washed with water, followed by hexane to give **2** (1.1 g, 85%), amorphous, $[\alpha]_D^{26}$ −22° (*c* 0.43, 2:3 chloroform–methanol); ^{1}H-n.m.r. ($CDCl_3$): δ 7.80–7.25 (m, 10 H, arom.), 3.45 (s, 3 H, OMe), and 1.06 (s, 9 H, CMe_3).

Anal. Calc. for $C_{23}H_{32}O_6 \cdot 0.5\ H_2O$: C, 62.54; H, 7.54. Found: C, 62.33; H, 7.90.

*Methyl 6-O-(*tert*-butyldiphenylsilyl)-3,4-O-isopropylidene-β-D-galactopyranoside* (**3**). — To a solution of **2** (3.6 g) in dry acetone (20 mL) were added 2,2-dimethoxypropane (20 mL) and 4-toluenesulfonic acid monohydrate (0.6 g). The mixture was stirred for 1.5 h at room temperature, made neutral by the addition of triethylamine, and evaporated. The residue was dissolved in chloroform, the solution washed with water, dried, and concentrated. The concentrate was applied to a column of silica gel and eluted with chloroform to give amorphous **3** (3.6 g, 92%), $[\alpha]_D^{26}$ −1.5° (*c* 0.7, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.80–7.25 (m, 10 H, arom.), 3.50 (s, 3 H, OMe), 1.53 and 1.36 (s, 3 H each, CMe_2), and 1.03 (s, 9 H, CMe_3).

Anal. Calc. for $C_{26}H_{36}O_6Si$: C, 66.06; H, 7.69. Found: C, 66.16; H, 7.81.

*Methyl 2-O-benzyl-6-O-(*tert*-butyldiphenylsilyl)-3,4-O-isopropylidene-β-D-galactopyranoside* (**4**). — A mixture of **3** (4.6 g), benzyl bromide (8 mL), and freshly prepared[6] Ag_2O (8 g) in *N,N*-dimethylformamide (70 mL) was stirred for 16 h at ~45°. The solids were removed by filtration (Celite bed) and thoroughly washed with *N,N*-dimethylformamide, and the filtrate and washings were combined and evaporated to dryness. The residue was stirred in chloroform, the solid material that separated was filtered off, and the solution was successively washed with water, aqueous $Na_2S_2O_3$, and water, dried, and evaporated to give a syrup, which showed in t.l.c. (toluene) a major product, faster-migrating than **3**; some faster- and some slower-migrating contaminants were also revealed in t.l.c. The crude product mixture was applied to a column of silica gel. Elution with 3:7 (v/v) hexane–toluene gave syrupy **4** (4 g, 73%), $[\alpha]_D^{26}$ +16.5° (*c* 1.4, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.80–7.25 (m, 15 H, arom.), 3.50 (s, 3 H, OMe), 1.25 (s, 6 H, CMe_2), and 1.10 (s, 9 H, CMe_3).

Anal. Calc. for $C_{33}H_{42}O_6Si$: C, 70.42; H, 7.54. Found: C, 70.20; H, 7.67.

Methyl 2-O-benzyl-3,4-O-isopropylidene-β-D-galactopyranoside (**5**). — A solution of **4** (4 g) in anhydrous oxolane (40 mL) was treated with M tetrabutylammonium fluoride in oxolane (3 mL), and the stirring was continued for 4 h at room temperature. The mixture was evaporated to dryness, and the residue was purified in a column of silica gel with chloroform as the eluent to give **5** (2 g, 87%),

a white amorphous solid, $[\alpha]_D^{26}$ +45° (*c* 0.97, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.35–7.20 (m, 5 H, arom.), 3.50 (s, 3 H, OMe), and 1.33 and 1.30 (s, 3 H each, CMe_2).

Anal. Calc. for $C_{17}H_{24}O_6$: C, 62.94; H, 7.47. Found: C, 62.65; H, 7.43.

Methyl 2-O-benzyl-β-D-galactopyranoside (**6**). — Compound **5** (2 g) in 60% aqueous acetic acid (40 mL) was heated for 1 h at ~70°. The acetic acid was evaporated under reduced pressure, the last traces being removed by coevaporation with several added portions of toluene. The residue was purified in a column of silica gel with solvent *B* as the eluent to afford **6** (1.6 g, 91%), m.p. 146–148° (acetone–hexane), $[\alpha]_D^{26}$ +7.0° (*c* 0.92, methanol); lit.[8] m.p. 148–149°, $[\alpha]_D^{24}$ +9.24° (*c* 0.73, methanol).

Benzyl 2-acetamido-3,6-di-O-benzyl-4-O-(2-O-benzyl-β-D-galactopyranosyl)-2-deoxy-α-D-glucopyranoside (**10**). — A mixture of benzyl 2-acetamido-3,6-di-*O*-benzyl-4-*O*-[6-*O*-(*tert*-butyldiphenylsilyl)-3,4-*O*-isopropylidene-β-D-galactopyranosyl]-2-deoxy-α-D-glucopyranoside[5] (**7**; 8.7 g), Ag_2O (15 g), and benzyl bromide (12 mL) in *N,N*-dimethylformamide (100 mL) was stirred for 16 h at ~45°. After processing as described for **3** (to give **4**), examination of the product by t.l.c. (2:3, v/v, ethyl acetate–hexane) revealed the presence of a major product faster-migrating than **7**; a trace of **7** and some faster-migrating contaminants were also revealed in t.l.c. The mixture was purified in a column of silica gel with 1:3 (v/v) ethyl acetate–hexane as the eluent to give syrupy **8** (7 g, 73%), $[\alpha]_D^{26}$ +70° (*c* 0.76, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.43–7.13 (m, 30 H, arom.), 1.75 (s, 3 H, NAc), 1.33 and 1.36 (s, 3 H each, CMe_2), and 1.03 (s, 9 H, CMe_3).

A stirred solution of **8** in dry oxolane (100 mL) was treated with a molar solution of tetrabutylammonium fluoride in oxolane (12 mL), and the stirring was continued for 5 h at room temperature. The mixture was evaporated to dryness to give intermediate **9** as a syrup which was dissolved, without purification, in 60% aqueous acetic acid (200 mL) and stirred for 4 h at ~70°. The acetic acid was evaporated under diminished pressure, the last traces being removed by coevaporation with several portions of toluene. The residue was applied to a column of silica gel and eluted with solvent *B* to give **10** (3 g, 59%), a white amorphous solid, $[\alpha]_D^{26}$ +94° (*c* 0.67, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.35–7.25 (m, 20 H, arom.) and 1.85 (s, 3 H, NAc).

Anal. Calc. for $C_{42}H_{49}NO_{11} \cdot 0.5\ H_2O$: C, 66.99; H, 6.57; N, 1.86. Found: C, 66.90; H, 6.31; N, 1.91.

Benzyl 2-acetamido-3,6-di-O-benzyl-4-O-(2-O-benzyl-4,6-O-benzylidene-β-D-galactopyranosyl)-2-deoxy-α-D-glucopyranoside (**11**). — To a stirred solution of **10** (0.83 g) in *N,N*-dimethylformamide (10 mL) were added 4-toluenesulfonic acid monohydrate (0.12 g) and α,α-dimethoxytoluene (0.5 mL). The stirring was continued for 8 h at room temperature, more portion of 4-toluenesulfonic acid (0.21 g) and α,α-dimethoxytoluene (0.5 mL) were added, and the stirring was continued for 16 h at room temperature. The acid was neutralized with a little triethylamine, and the solution evaporated to dryness. The residue was then dissolved in chloroform,

the chloroform solution was washed with water, dried, and evaporated. The residue was purified in a column of silica gel with 1:1 (v/v) ethyl acetate–hexane as the eluent to give a solid, which was dissolved in ethyl acetate. Addition of ether–hexane caused the precipitation of **11** (0.5 g, 54%), amorphous, $[\alpha]_D^{26}$ +53° (*c* 0.88, chloroform).

Anal. Calc. for $C_{49}H_{53}NO_{11}$: C, 70.73; H, 6.43; N, 1.68. Found: C, 70.47; H, 6.46; N, 1.77.

Benzyl O-*(2,3,4-tri*-O-*benzyl-α*-L-*fucopyranosyl)-(1→3)*-O-*(2*-O-*benzyl-4,6*-O-*benzylidene-β*-D-*galactopyranosyl)-(1→4)-2-acetamido-3,6-di*-O-*benzyl-2-deoxy-α*-D-*glucopyranoside* (**13**). — A solution of 2,3,4-tri-*O*-benzyl-L-fucopyranosyl bromide[10,11] [**12**; 0.8 g, 1.6 mmol; freshly prepared from the 1-(4-nitrobenzoate)[10]], and tetraethylammonium bromide (0.33 g, 1.6 mmol) in dichloromethane (20 mL) was stirred for 0.5 h with 4A molecular sieves (2.5 g) under protection from light and moisture. Then, a solution of **11** (0.65 g, 0.78 mmol) in dichloromethane (10 mL) was added, followed by ethyldiisopropylamine (0.2 g, 1.6 mmol), and the mixture was stirred for 18 h at room temperature. Further amounts of **12** (0.8 g, 1.6 mmol), tetraethylammonium bromide (0.33 g, 1.6 mmol), and ethyldiisopropylamine (0.2 g, 1.6 mmol) were added, and the stirring was continued for a total of 4 days. The mixture was filtered through Celite, the solids were thoroughly washed with dichloromethane, and the filtrate and washings were combined, successively washed with water, aqueous $NaHCO_3$, and water, dried, and evaporated. The residue was purified in a column of silica gel with 3:2 ethyl acetate–hexane as the eluent to afford amorphous **13** (0.87 g, 89%), $[\alpha]_D^{26}$ +21° (*c* 0.36, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.35–7.20 (m, 40 H, arom.), 5.45 (s, 1 H, PhC*H*), 1.80 (s, 3 H, NAc), and 1.10 (d, 3 H, *J* 6 Hz, Me).

Anal. Calc. for $C_{76}H_{81}NO_{15}$: C, 73.10; H, 6.55; N, 1.12. Found: C, 72.90; H, 6.28; N, 1.06.

Benzyl O-*(2,3,4-tri*-O-*benzyl-α*-L-*fucopyranosyl)-(1→3)-(2*-O-*benzyl-β*-D-*galactopyranosyl)-(1→4)-2-acetamido-3,6-di*-O-*benzyl-2-deoxy-α*-D-*glucopyranoside* (**14**). — Compound **13** (0.8 g) was stirred in 60% aqueous acetic acid (50 mL) for 4 h at ~70°. The acetic acid was evaporated under diminished pressure, and several portions of toluene were added to, and evaporated from, the residue which was then applied to a column of silica gel. On elution with 9:1 (v/v) ethyl acetate–hexane, evaporation of the fractions corresponding to the product gave **14** (0.51 g, 69%), a white solid, $[\alpha]_D^{26}$ +1.8° (*c* 0.89, chloroform).

Anal. Calc. for $C_{69}H_{77}NO_{15}$: C, 71.41; H, 6.70; N, 1.21. Found: C, 71.25; H, 6.46; N, 1.11.

Benzyl O-*(2,3,4-tri*-O-*benzyl-α*-L-*fucopyranosyl)-(1→3)-(4,6-di*-O-*acetyl-2*-O-*benzyl-β*-D-*galactopyranosyl)-(1→4)-2-acetamido-3,6-di*-O-*benzyl-2-deoxy-α*-D-*glucopyranoside* (**15**). — A solution of **14** (0.1 g) in 1:2 acetic anhydride–pyridine (3 mL) was kept for 16 h at room temperature, and then evaporated under diminished pressure, the last traces of the solvents being removed by coevaporation with several portions of toluene. The residue was purified by preparative t.l.c. with 3:2

(v/v) ethyl acetate–hexane as the irrigant to afford **15** (0.8 g, 75%), amorphous, $[\alpha]_D^{26}$ +15° (*c* 0.94, chloroform).

Anal. Calc. for $C_{73}H_{81}NO_{17}$: C, 70.44; H, 6.57; N, 1.13. Found: C, 70.16; H, 6.36; N, 1.10.

O-α-L-Fucopyranosyl-(1→3)-O-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranose (**16**). — A mixture of **14** (0.4 g) and 10% Pd–C (0.4 g) in glacial acetic acid (20 mL) was shaken under H_2 at ~345 kPa for 3 days at room temperature. The suspension was filtered through a bed of Celite, the solid was thoroughly washed with glacial acetic acid, and the filtrate and washings were combined and evaporated under diminished pressure to give a solid residue which showed in t.l.c. (solvent *C*) some faster-migrating impurities (presumably due to incomplete hydrogenolysis). The crude product was applied to a column of silica gel. On elution with solvent *C*, evaporation of the fractions corresponding to the product gave **16** (0.15 g, 84%), a white amorphous solid, $[\alpha]_D^{26}$ −31° (*c* 0.81, dimethyl sulfoxide); ^{13}C-n.m.r., see Table I.

Anal. Calc. for $C_{20}H_{35}NO_{15}\cdot H_2O$: C, 43.86; H, 6.82; N, 2.56. Found: C, 43.85; H, 6.54; N, 2.58.

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