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SYNTHESIS OF THE ISOMERIC TRISACCHARIDES, METHYL *O*-α-L-FUCOPYRANOSYL-(1→3, 4, AND 6)-*O*-(2-ACETAMIDO-2-DEOXY-β-D-GLUCOPYRANOSYL)-(1→3)-β-D-GALACTOPYRANOSIDE*

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ABSTRACT

Benzylation of methyl 3-*O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside with benzyl bromide in *N,N*-dimethylformamide in the presence of sodium hydride afforded methyl 3-*O*-(2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**3**). Reductive ring-opening of the benzylidene group of **3** gave methyl 3-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**4**). Cleavage of the 4,6-acetal group of **3** with hot, 80% aqueous acetic acid afforded the diol (**5**). Compounds **3**, **4**, and **5** were each subjected to halide ion-catalyzed glycosylation with 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide to produce the corresponding trisaccharide derivatives, which, on catalytic hydrogenation, furnished the title trisaccharides, respectively.

INTRODUCTION

During the past few years, a variety of glycoconjugates having an L-fucopyranosyl group α-(1→3)-linked to a 2-acetamido-2-deoxy-D-glucopyranosyl residue have been reported as tumor-associated antigens. It was largely due to the work of Hakomori and assoc.[2–6] that the structure of a number of such glycoconjugates has been elucidated. Their studies on glycolipids of human cancer tissues have established that accumulation of highly fucosylated, extended polylactosamine glycolipids is characteristic for certain types of human cancers, suggesting that malignant transformation may involve an increase in the amount or activity

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of the fucosyltransferase invoked in the biosynthesis of such glycoconjugates[6].

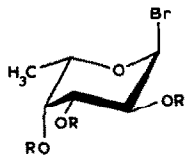
Recently, we reported the synthesis[1,7] and use[7] of 2-acetamido-2-deoxy-4-*O*-(2-*O*-methyl-β-D-galactopyranosyl)-D-glucopyranose (*N*-acetyl-2′-*O*-methyllactosamine) as a specific acceptor for (1→3)-α-L-fucosyltransferase from human serum. We also utilized the same compound in a clinical investigation for the assay of the enzyme activity in the sera and saliva of some ovarian cancer patients[8]. However, more recent findings suggested the presence of a fucosyltransferase that is capable of incorporating L-fucose into our synthetic disaccharide, methyl 3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-galactopyranoside[9] [β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*OMe]. A similar enzyme activity that incorporates L-fucose into *p*-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside (β-D-Glc*p*NAcOC$_6$H$_4$NO$_2$-4) has also been reported[10]. Thus, in an effort to confirm the linkage in the enzymic product obtained by the action of the fucosyltransferase on the aforementioned disaccharide unit, we synthesized the three isomeric trisaccharides expected on incorporation of L-fucose into the GlcNAc residue. Such compounds are also expected to be useful in specificity studies of an antibody raised against a related, synthetic antigen that we are currently investigating.

RESULTS AND DISCUSSION

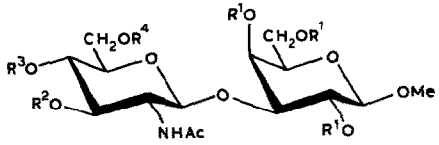
Methyl 3-*O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside[11] (**2**) was benzylated by the general procedure of Brimacombe[12] to afford, in 71.5% yield, the 3-*O*-benzyl derivative **3**. Reductive ring-opening[13] of the 4,6-benzylidene acetal group of **3**, in acidic medium, in the presence of sodium cyanoborohydride then gave, in ~82% yield, methyl 3-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**4**). The ^{1}H-n.m.r. spectra of both **3** and **4** contained signals in support of the overall structures expected. Thus, whereas the spectrum of **3** contained a one-proton resonance at δ 5.50, attributable to the benzylidene methine proton, that of **4** was devoid of such a resonance. However, in both of the spectra, the ratio of the aromatic protons remained the same, a clear indication that the acetal group of **3** was not completely removed, but rather rearranged to give the dibenzyl derivative **4**.

Cleavage of the 4,6-benzylidene group of **3** in hot, 80% aqueous acetic acid gave, in high yield, methyl 3-*O*-(2-acetamido-3-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**5**). By contrast to that of **4**, the ^{1}H-n.m.r. spectrum of **5** contained, as expected, a twenty-proton multiplet at δ 7.10–7.50, confirming the complete hydrolysis of the acetal group of **3**.

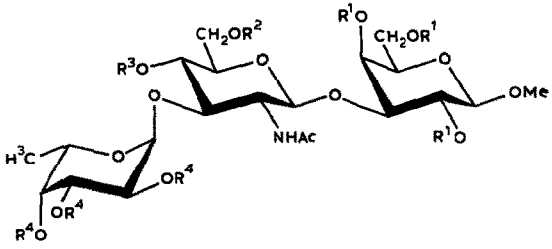
Glycosylation (catalyzed by bromide ion) of compound **2** with 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide[14,15] (**1**) gave, in 60.5% yield after column chromatographic purification, the fully protected trisaccharide derivative **6**, the ^{1}H-n.m.r. spectrum of which was in conformity with the overall structure expected. Hydrogenolytic cleavage of the benzyl and benzylidene groups of **6** in glacial acetic



1 R = Bn



2 R^1 = Bn, R^2 = H, R^3, R^4 = PhCH
3 R^1 = R^2 = Bn, R^3, R^4 = PhCH
4 R^1 = R^2 = R^4 = Bn, R^3 = H
5 R^1 = R^2 = Bn, R^3 = R^4 = H



6 R^1 = R^3 = Bn, R^2, R^4 = PhCH
7 R^1 = R^2 = R^3 = R^4 = H

acid, and in the presence of 10% palladium-on-carbon then furnished, in ~82% yield, methyl *O*-α-L-fucopyranosyl-(1→3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-β-D-galactopyranoside (**7**). The ^{13}C-n.m.r. spectrum of amorphous **7** was in accord with the structure assigned; see Table I.

A similar glycosylation with bromide **1** of **4** afforded, in 57.5% yield, the trisaccharide derivative **8**. Amorphous **8**, which was not fully characterized, was hydrogenolyzed in glacial acetic acid in a manner analogous to that described for **6** (to give **7**), to furnish methyl *O*-α-L-fucopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-β-D-galactopyranoside (**9**). The ^{13}C-n.m.r. spectrum of **9** was also consistent with the structure assigned; see Table I.

A similar reaction of diol **5** with bromide **1** was virtually complete in one day and afforded, in ~79% yield, the trisaccharide derivative **10** as an amorphous solid, the ^{1}H-n.m.r. spectrum of which was, likewise, in accord with the overall structure expected. Hydrogenolysis of the benzyl groups of **10**, followed by column-chromatographic purification on silica gel gave, in ~77% yield, methyl *O*-α-L-fucopyranosyl-(1→6)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-β-D-galactopyranoside (**11**), the ^{13}C-n.m.r. spectrum of which was also in agreement with the structure assigned; see Table I.

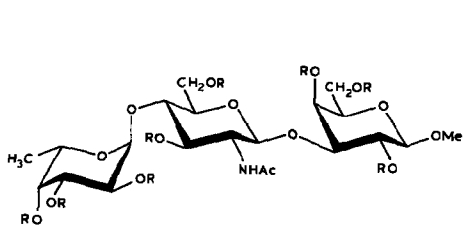
^{13}C-N.m.r. Assignments. — The assignments of the ^{13}C-n.m.r. resonances for trisaccharides **7**, **9**, and **11** were made by comparing their spectra with those of methyl α-L-fucopyranoside and methyl 3-*O*-(2-acetamido-2-deoxy-β-D-gluco-

TABLE I

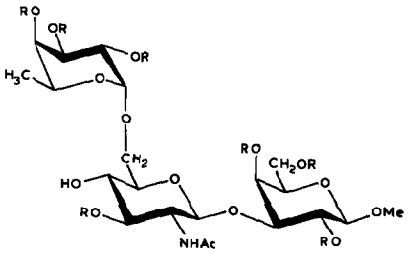
PROPOSED ^{13}C-N.M.R. CHEMICAL SHIFTS[a]

Residue	*Compound*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*OCH_3*	*CH_3CO*	*C=O*
α-L-Fuc*p*	[b]	100.05	67.96	69.54	71.39	65.53	16.37	54.37		
β-D-Gal*p*OMe	[c]	103.79	69.29	82.22	67.02	74.69	60.22	55.56		
β-D-Glc*p*NAc		101.88	56.38	74.22	70.28	76.57	60.77		22.98	169.94
β-D-Gal*p*OMe	**7**	103.83	69.19	82.19	67.00	74.65	60.21	55.64		
β-D-Glc*p*NAc-(1→3)		101.38	55.01	81.47	68.59	76.22	60.58		22.96	170.29
α-L-Fuc*p*-(1→3)		99.40	67.98	69.63	71.46	66.30	16.26			
β-D-Gal*p*OMe	**9**	104.01	69.35	82.51	67.11	74.71	60.10	55.76		
β-D-Glc*p*NAc-(1→3)		102.01	56.70	72.54	77.71	75.27	60.24		22.99	170.40
α-L-Fuc*p*-(1→4)		99.35	68.08	69.48	71.69	66.25	16.32			
β-D-Gal*p*OMe	**11**	104.11	70.86	82.25	67.53	74.86	60.42	55.78		
β-D-Glc*p*NAc-(1→3)		102.05	56.29	74.20	69.30	75.27	68.23		23.01	170.27
α-L-Fuc*p*-(1→6)		99.92	67.70	69.72	71.58	65.89	16.44			

[a]For solutions in di($^2H_3$)methylsulfoxide with Me_4Si as the internal standard. [b]Methyl α-L-fucopyranoside. [c]Methyl 3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-galactopyranoside[11]. The chemical shifts for the latter two compounds were included for comparison purposes.



8 R = Bn
9 R = H



10 R = Bn
11 R = H

pyranosyl)-β-D-galactopyranoside, reported in Table I. In the ^{13}C-n.m.r. spectra of the three trisaccharides, the resonances for C-1 and C-1′ were all in the region normally expected for β-D-glycosidic linkages; compare the corresponding values for the anomeric carbon atoms of the parent disaccharide. On the other hand, the resonances for C-1″ for all three compounds were observed at δ 90.40–90.70, a clear indication of an α-L configuration for the newly introduced L-fucopyranosyl groups in compounds **7**, **9**, and **11**. In the ^{13}C spectrum of **7**, the resonance for C-3 of the 2-acetamido-2-deoxy-D-glucose (GlcNAc) residue suffered a downfield shift of 7.25 p.p.m., by comparison to that of its counterpart in the spectrum of the parent disaccharide β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*OMe, evidencing that O-3′ was the site of glycosylation. However, in the spectra of both **9** and **11**, analogous downfield shifts of 7.43 and 7.46 p.p.m., respectively, were observed for the C-4 and C-6 resonances of their corresponding GlcNAc residues, confirming that fucosylation had occurred at O-4′ and O-6′, respectively.

EXPERIMENTAL

General methods. — Melting points were determined with a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured at 25–27° with a Perkin–Elmer 241 polarimeter. N.m.r. spectra were recorded at ~25°; ^{1}H-n.m.r. spectra with a Varian EM-390, and ^{13}C-n.m.r. spectra either with a Varian XL-100 or with a Bruker WP-200 instrument, at 25.2 or 50.3 MHz, respectively. The positions of the signals are expressed from the Me_4Si signal. T.l.c. was conducted on aluminum sheets, precoated with a 0.2-mm layer 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*) 2:1 chloroform–methanol, (*B*) 13:6:1 chloroform–methanol–water, and (*C*) 5:4:1 chloroform–methanol–water. Organic solutions were generally dried with anhydrous sodium sulfate. Sodium hydride used in alkylation reactions was a 60% dispersion in mineral oil. Elemental analyses were performed by Robertson Laboratory,

Florham Park, New Jersey, U.S.A.; or by Galbraith Laboratories, Inc., Knoxville, Tennessee, U.S.A.

Methyl 3-O-(2-acetamido-3-O-benzyl-4,6-O-benzylidene-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-O-benzyl-β-D-galactopyranoside (**3**). — To a stirred solution of methyl 3-*O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside[11] (**2**; 7.5 g, 10 mmol) in *N,N*-dimethylformamide (100 mL) was added NaH (1.0 g, 25 mmol) portionwise, and stirring was continued for 0.5 h at room temperature. The mixture was then cooled (~0°; bath), and benzyl bromide (1.5 mL, 12.5 mmol) was cautiously added, and the stirring continued for 16 h at room temperature. After careful addition of methanol to decompose the excess of NaH, the mixture was evaporated, and the residue dissolved in chloroform. The solution was washed with water, dried, and evaporated, and the residue applied to a column of silica gel. On elution with chloroform, evaporation of the fractions corresponding to the product gave a solid which was redissolved in a small volume of dichloromethane. Addition of hexane caused the precipitation of **3** (6 g, 71.5%), amorphous, $[\alpha]_D^{25}$ −10.9° (*c* 2.7, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.50–7.10 (m, 25 H, arom.), 5.50 (s, 1 H, PhCH), 3.50 (s, 3 H, OMe), and 1.50 (s, 3 H, NAc).

Anal. Calc. for $C_{50}H_{55}NO_{11}$: C, 71.00; H, 6.51; N, 1.66. Found: C, 71.11; H, 6.61; N, 1.54.

Methyl 3-O-(2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-O-benzyl-β-D-galactopyranoside (**4**). — To a cold (0°, bath) stirred mixture of **3** (5.5 g), sodium cyanoborohydride (4 g), and powdered 3A molecular sieves (15 g) in dry oxolane (100 mL) was added, dropwise, a saturated solution of HCl in ether (50 mL), and stirring was continued for 15 min. T.l.c. (9:1 chloroform–acetone) revealed the disappearance of **3**, and the presence of a major product, slower-migrating than **3**; a trace of a slower-migrating contaminant (presumably due to complete cleavage of the acetal group of **3**) was also revealed in t.l.c. The mixture was diluted with chloroform, and the solids were filtered off (a bed of glass wool) and washed with chloroform. The filtrate and washings were combined and washed with cold water, cold saturated $NaHCO_3$, and water, dried, and concentrated to a small volume. The concentrate was applied to a column of silica gel and eluted with 19:1 chloroform–acetone. The fractions corresponding to the product were evaporated to give a residue which was dissolved in ethyl acetate. Addition of ether caused the precipitation of **4** (4.5 g, 81.6%), white powder, $[\alpha]_D^{25}$ −12.7° (*c* 2.5, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.50–7.00 (m, 25 H and arom.), 3.50 (s, 3 H, OMe), and 1.50 (s, 3 H, NAc).

Anal. Calc. for $C_{50}H_{57}NO_{11}$: C, 70.81; H, 6.78; N, 1.65. Found: C, 70.94; H, 6.57; N, 1.59.

Methyl 3-O-(2-acetamido-3-O-benzyl-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-O-benzyl-β-D-galactopyranoside (**5**). — Compound **4** (4.5 g) in 80% aqueous acetic acid (150 mL) was stirred for 1 h at ~80°. Acetic acid was evaporated under diminished pressure, the last traces being removed by co-evaporation with several

added portions of toluene to leave a residue that was dissolved in chloroform. Addition of hexane precipitated **5** (3.7 g, 91.6%), amorphous, $[\alpha]_D^{25}$ −12.5° (*c* 1.3, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.10–7.50 (m, 20 H, arom.), 3.50 (s, 3 H, OMe), and 1.50 (s, 3 H, NAc).

Anal. Calc. for $C_{43}H_{51}NO_{11}$: C, 68.14; H, 6.80; N, 1.85. Found: C, 67.88; H, 6.93; N, 1.67.

Methyl O-*(2,3,4-tri-*O*-benzyl-α-*L*-fucopyranosyl)-(1→3)-*O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-*D*-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-*D*-galacto-pyranoside* (**6**). — A solution of 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide[14,15] [(**1**; 11 g, 22.1 mmol); freshly prepared from the 1-(*p*-nitrobenzoate[15])], and tetraethylammonium bromide (4.6 g, 21.9 mmol) in dichloromethane (110 mL) was stirred for 0.5 h with 4A molecular sieves (20 g) under protection from light and moisture. Then, a solution of **2** (8 g, 10.6 mmol) in dichloromethane (56 mL) was added, followed by ethyldiisopropylamine (3.9 mL, 22.3 mmol), and the mixture stirred for 2 days at room temperature. Further amounts of **1** (2 g), tetraethylammonium bromide (1 g), and ethyldiisopropylamine (0.9 mL) 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 combined, and washed with water, aqueous $NaHCO_3$, and water, dried, and concentrated to a small volume. The concentrate was applied to a column of silica gel and eluted with a solvent gradient consisting of 15–30% ethyl acetate in hexane. On evaporation, the fractions corresponding to the product gave a syrup which was taken up in ether. Addition of hexane precipitated **6** (7.5 g, 60.5%), amorphous, $[\alpha]_D^{25}$ −62.9° (*c* 0.61, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.50–7.10 (m, 35 H, arom.), 5.46 (s, 1 H, PhCH), 3.46 (s, 3 H, OMe), 1.43 (s, 3 H, NAc), and 0.86 (d, 3 H, *J* 6 Hz, CMe).

Anal. Calc. for $C_{70}H_{77}NO_{15}$: C, 71.70; H, 6.63; N, 1.19. Found: C, 71.53; H, 6.69; N, 1.36.

Continued elution of the column with ethyl acetate gave unchanged **2** (1 g).

Methyl O-α-L-*fucopyranosyl-(1→4)-*O*-(2-acetamido)-2-deoxy-β-*D*-glucopyra-nosyl)-(1→3)-β-*D*-galactopyranoside* (**7**). — A solution of **6** (4 g) in glacial acetic acid (100 mL) was shaken under H_2 at ~345 kPa for 2 days at room temperature in the presence of 10% Pd–C (4 g). The suspension was filtered through a bed of Celite, the solid thoroughly washed with glacial acetic acid, and the filtrate and washings were combined, and evaporated under diminished pressure. The residue was applied to a column of silica gel. Elution with 3:2:2 ethyl acetate–2-propanol–water, and evaporation of the fractions corresponding to the product gave a solid which was dissolved in aqueous ethanol. Addition of ether precipitated **7** (1.53 g, 82.3%), amorphous, $[\alpha]_D^{25}$ −65.8° (*c* 0.4, water); for ^{13}C-n.m.r. spectra, see Table I.

Anal. Calc. for $C_{21}H_{37}NO_{15} \cdot H_2O$: C, 44.9; H, 7.01; N, 2.48. Found: C, 45.12; H, 6.84; N, 2.41.

Methyl O-α-L-*fucopyranosyl-(1→3)-*O*-(2-acetamido)-2-deoxy-β-*D*-glucopyra-nosyl)-(1→3)-β-*D*-galactopyranoside* (**9**). — Compound **4** (3.5 g, 4.1 mmol) was treated with bromide **1** (6.1 g, 8.2 mmol) in dichloromethane (65 mL) in the pre-

sence of tetraethylammonium bromide (2.5 g, 11.9 mmol), ethyldiisopropylamine (2.1 mL, 12.1 mmol), and 4A molecular sieves (9 g) in a manner analogous to that described for **2** (to give **6**). After the aforedescribed processing, the crude reaction product was applied to a column of silica gel and eluted with a solvent gradient consisting of 30–50% ethyl acetate in hexane. Evaporation of the fractions corresponding to the product yielded **8** (3 g, 57.5%), amorphous, $[\alpha]_D^{25}$ −43.5° (*c* 2.0, chloroform), which was utilized without any further characterization in the next step.

A solution of **8** (2.1 g) in glacial acetic acid (50 mL) was hydrogenolyzed in the presence of 10% Pd–C (2 g) as described for **6** (to give **7**). The crude product mixture was purified in a column of silica gel by elution first with solvent *B*, to remove some faster-migrating contaminants, and then with solvent *C*. Chromatographically pure (t.l.c., solvent *C*) **9** was obtained, after lyophilization, as an amorphous solid (0.75 g, 87.2%), $[\alpha]_D^{25}$ −82.2° (*c* 2.1, water); for ^{13}C-n.m.r. data, see Table I.

Anal. Calc. for $C_{21}H_{37}NO_{15} \cdot 1.5\ H_2O$: C, 44.20; H, 7.08; N, 2.46. Found: C, 44.10; H, 7.06; N, 2.44.

Methyl O-*(2,3,4-tri*-O-*benzyl*-α-L-*fucopyranosyl)-(1→6)*-O-*(2-acetamido-3*-O-*benzyl-2-deoxy*-β-D-*glucopyranosyl)-(1→3)-2,4,6-tri*-O-*benzyl*-β-D-*galactopyranoside* (**10**). — Compound **5** (3.2 g, 4.2 mmol) was treated with bromide **1** (4.5 g, 9.0 mmol) in dichloromethane (65 mL) in the presence of tetraethylammonium bromide (1.8 g, 8.6 mmol), ethyldiisopropylamine (1.5 mL, 8.6 mmol), and 4A molecular sieves (9 g), for 24 h at room temperature. After processing as described above for the reaction of **2**, the crude product mixture was subjected to chromatography on silica gel by use of 1:1 ethyl acetate–hexane as the eluent. Evaporation of the fractions-containing product then gave a solid residue, which was dissolved in chloroform. Addition of hexane precipitated **10** (3.9 g, 78.6%), amorphous, $[\alpha]_D^{25}$ −36° (*c* 3.4, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.50–7.00 (m, 35 H, arom.), 3.50 (s, 3 H, OMe), 1.50 (s, 3 H, NAc), and 1.03 (d, 1 H, *J* 6 Hz, CMe).

Anal. Calc. for $C_{70}H_{79}NO_{15}$: C, 71.16; H, 6.73; N, 1.19. Found: C, 71.06; H, 6.75; N, 1.19.

Methyl O-α-L-*fucopyranosyl-(1→6)*-O-*(2-acetamido-2-deoxy*-β-D-*glucopyranosyl)-(1→3)*-β-D-*galactopyranoside* (**11**). — A solution of **10** (3.1 g) in glacial acetic acid (60 mL) was shaken under H_2 at ~345 kPa for 2 days at room temperature in the presence of 10% Pd–C (3 g). After processing as described for **6** (to give **7**), the crude product was purified in a column of silica gel by successive elution with solvents *A*, *B*, and *C*, to furnish **11** (1.1 g, 76.6%), amorphous, $[\alpha]_D^{25}$ −55.8° (*c* 1.5, water); for ^{13}C-n.m.r. data, see Table I.

Anal. Calc. for $C_{21}H_{37}NO_{15}$: C, 46.40; H, 6.87; N, 2.58. Found: C, 46.18; H, 6.91; N, 2.48.

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