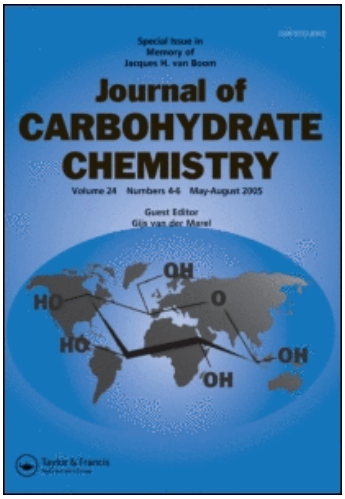

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Synthesis of *O*-α-L-Fucopyranosyl-(1→2)-O-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranose. The H-Blood Group Specific Trisaccharide

Surjit S. Rana[a]; Rashmi Vig[a]; Khushi L. Matta[a]
[a] Department of Gynecologic Oncology, New York State Department of Health, Roswell Park Memorial Institute, Buffalo, New York, U.S.A.

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SYNTHESIS OF O-α-L-FUCOPYRANOSYL-(1→2)-O-β-D-GALACTOPYRANOSYL-(1→4)-2-ACETAMIDO-2-DEOXY-D-GLUCOPYRANOSE. THE H BLOOD-GROUP SPECIFIC TRISACCHARIDE†

Surjit S. Rana, Rashmi Vig, and Khushi L. Matta*

Department of Gynecologic Oncology
Roswell Park Memorial Institute
New York State Department of Health
Buffalo, New York 14263 (U.S.A.)

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ABSTRACT

Different reaction conditions were investigated for the preparation of benzyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (5). Compound 5 on reaction with 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide afforded the 4-O-substituted 2-acetamido-2-deoxy-β-D-glucopyranosyl derivative which, on O-deacetylation, gave benzyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-4-O-β-D-galactopyranosyl-β-D-glucopyranoside (8). The trimethylsilyl (Me_3Si) derivative of 8, on treatment with pyridine-acetic anhydride-acetic acid for 2 days, gave the disaccharide derivative having an O-acetyl group selectively introduced at the primary position and Me_3Si groups at the secondary positions. The latter groups were readily cleaved by treatment with aqueous acetic acid in methanol to afford benzyl 2-acetamido-4-O-(6-O-acetyl-β-D-galactopyranosyl)-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside, which on isopropylidenation gave the desired, key intermediate benzyl 2-acetamido-4-O-(6-O-acetyl-3,4-O-isopropylidene-β-D-galactopyranosyl)-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (12). Reaction of 12 with 2,3,4-tri-O-benzyl-α-L-fucopyranosyl bromide under catalysis by bromide ion afforded the trisaccharide derivative from which the title trisaccharide was

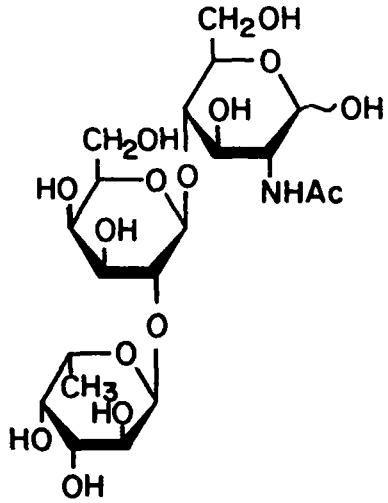
† Synthetic Studies in Carbohydrates, Part XXX. For Part XXIX, see ref. 1.

obtained by systematic removal of the protective groups. The structures of the final trisaccharide and of various intermediates were established by ^{1}H and ^{13}C NMR spectroscopy.

INTRODUCTION

The trisaccharide unit α-L-Fuc-(1→2)-β-D-Gal-(1→4)-β-D-GlcNAc has been found to be a part of the carbohydrate moiety of various glycoconjugates.[2] Rege and coworkers[3] isolated the title trisaccharide by degradation of human blood-group H specific substance. The structure of the trisaccharide 16 was established by its chemical synthesis.[4] Rosevear et al.[5] described an enzymatic preparation of ^{13}C-enriched trisaccharide 16, by action of GDP-fucose on the acceptor disaccharide, β-D-Gal-(1→4)-D-GlcNAc, in the presence of purified β-D-galactoside α (1→2)-fucosyltransferase.

In our laboratory, the title trisaccharide was required for substrate specificity of human α-L-fucosidase.[6] In this report, we describe another facile approach for its synthesis.



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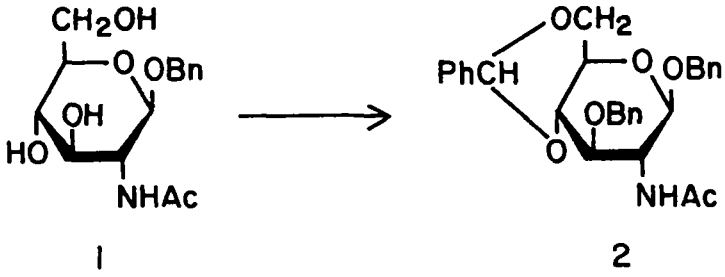
RESULTS AND DISCUSSION

Benzyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (5)[7] was chosen as a suitable sugar for the synthesis of the title

trisaccharide. Easily accessible benzyl 2-acetamido-3-O-benzyl-2-deoxy-β-D-glucopyranoside (3),[7,8] on selective benzylation with benzyl bromide under phase transfer catalysis procedure,[9] gave a mixture of compounds which were separated by silica gel column chromatography and fractionally crystallized to give the desired alcohol 5 in only 43% yield. However, selective benzylation of 3, via a bis (tributylstannyl) derivative,[10] was effective in giving the product 5



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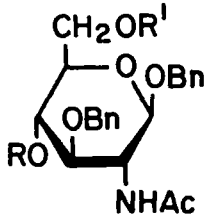
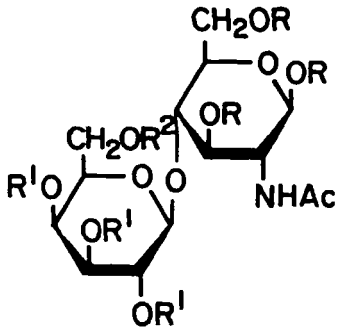
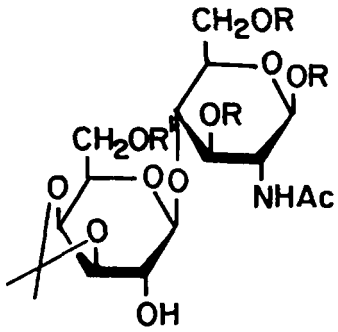


3 $R = R' = H$
4 $R = Bn, R' = H$
5 $R = H, R' = Bn$
6 $R = R' = Bn$

in 65% yield. In another approach, compound 3 was converted into benzyl 2-acetamido-3-O-benzyl-2-deoxy-6-O-tosyl-β-D-glucopyranoside, which on treatment with sodium benzylate in benzyl alcohol,[11] provided 5 in 76% yield.

During our investigation, Garegg and Hultberg[12] reported a novel method of reductive ring-opening of carbohydrate benzylidene acetals with sodium cyanoborohydride in HCl/ether. Under these conditions,





7 R = Bn, $R^1 = R^2 = Ac$

8 R = Bn, $R^1 = R^2 = H$

9 R = Bn, $R^1 = R^2 = SiMe_3$

10 R = Bn, $R^1 = SiMe_3$, $R^2 = Ac$

11 R = Bn, $R^1 = H$, $R^2 = Ac$

12 R = Bn, $R^1 = Ac$

benzyl 2-acetamido-3-O-benzyl-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (2) afforded 5 in 77% yield. The structure of the benzylated derivatives 3, 4 and 5, were established by ^{13}C NMR spectroscopy (Table I).

Recently, various catalysts have been introduced to facilitate the condensation of acetylated sugar halides at the 4-position of suitably protected 2-acetamido-2-deoxy-D-glucopyranosyl derivatives.[13] In the present studies, mercuric cyanide in benzene[14] was found to be effective when 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide was reacted with the alcohol 5 to give the protected disaccharide derivative 7. Compound 7 was saponified without further purification to afford benzyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-4-O-β-D-galactopyranosyl-β-D-glucopyranoside (8) in 79% yield (after chromatographic purification). The ^{13}C NMR spectrum of 8 (Table II), supported the β-D-configuration at the inter-sugar linkage and ruled out the possibility of any contamination with the α-D-galactosyl anomer. As expected, the introduction of the β-D-galactopyranosyl group at the 4-position of 5 caused a deshielding of the inter-sugar aglycon carbon (C-4) by 5.92 ppm.

TABLE 1

^{13}C NMR Chemical Shifts[a] (25.2 MHz, Me_2SO-d_6)

Atoms	Compound			
	1[b]	3	4	5
C-1	100.5	100.28	100.25	100.29
C-2	55.3	54.00	54.40	54.00
C-3	74.0	82.57	82.18	82.48
C-4	70.6	69.29	77.68	70.07
C-5	76.9	76.88	75.54	75.54
C-6	61.0	60.76	60.14	72.21
$OCO\underline{C}H_3$	23.0	22.85	22.76	22.84
C = O	168.8	168.61	168.71	168.64
$\underline{C}H_2Ph$	69.3	69.98	69.48	69.42
		73.22	73.61	73.35

[a] In ppm downfield from Me_4Si (internal)

[b] From reference 17

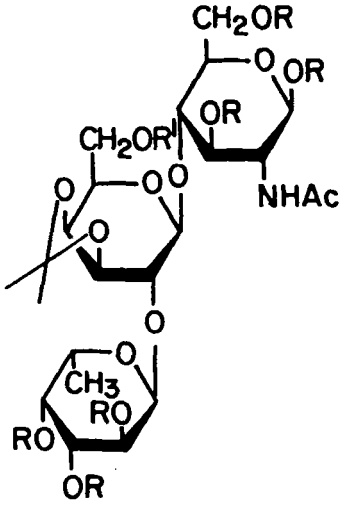
It has been observed that per(trimethylsilyl)ated derivatives can be selectively acetylated at the primary hydroxyl group by using a mixture of pyridine, acetic anhydride, and a small proportion of acetic acid.[15,16] This strategy was successfully applied in our laboratory for a rapid synthesis of the Le^b blood-group antigenic determinant.[17] We have now extended the use of this procedure to obtain benzyl 2-acetamido-4-O-(6-O-acetyl-β-D-galactopyranosyl)-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (11) from compound 8. Thus, the disaccharide derivative 8 was per(trimethylsilyl)ated,[18] to afford the fully protected derivative 9, the IR spectrum of which showed complete absence of hydroxyl groups. Compound 9 on exposure to acetic anhydride, glacial acetic acid, and pyridine for 2 days at room temperature[15] gave 10, having an acetyl group on the primary hydroxyl group; the course of the reaction was monitored by TLC, to circumvent acetylation at the secondary positions.[16] The removal of the remaining trimethylsilyl

TABLE 2

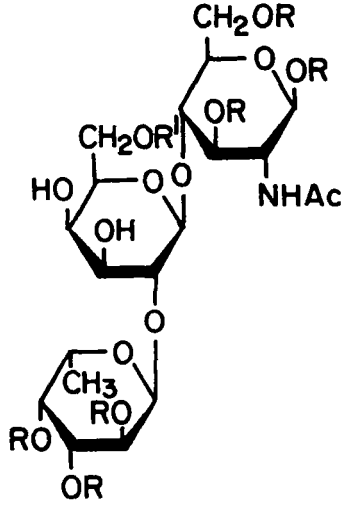
^{13}C NMR Chemical Shifts[a] (25.2 MHz)

Atoms	Compound			
	8	11	16 α	16 β
C-1	100.35	100.32	91.30	95.65
C-2	53.84	53.80	54.67	57.23
C-3	80.26	80.00	71.30	74.35
C-4	75.99	75.95	77.14	76.80
C-5	74.48	74.40	70.42	76.01
C-6	71.98	71.95	60.87	60.94
C=O	168.61	168.62,169.81	175.06	175.35
CH_3	22.78	20.40,22.78	22.76	23.05
C-1'	102.98	102.79	101.00	
C-2'	71.98	71.95	77.14	
C-3'	72.72	71.95	72.46	
C-4'	68.16	68.03	69.94	
C-5'	74.95	72.23	76.01	
C-6'	59.74	63.36	61.90	
C-1"			100.10	
C-2"			69.94	
C-3"			70.42	
C-4"			73.16	
C-5"			67.70	
C-6"			16.12	

a. In ppm downfield from Me_4Si. The solvent was Me_2SO-d_6, except for D_2O for 16. The reference (Me_4Si) was internal for solutions in Me_2SO-d_6, and external for that in D_2O.



13 R=Bn, R'=Ac



14 R=Bn, R'=Ac
15 R=Bn, R'=H

Bn = CH_2Ph

groups in 10, by means of aqueous acetic acid in methanol,[15] gave 11, which was isolated in crystalline form. The downfield shift of 3.62 ppm exhibited by C-6' on acetylation, and the upfield shift of C-5' (2.72 ppm) confirmed the position of substitution in 11. Isopropylidenation[19] of compound 11 with 2,2-dimethoxypropane in N,N-dimethylformamide, in the presence of p-toluenesulfonic acid, gave a product whose ^1H NMR spectrum clearly showed the presence of an isopropylidene group. The ^{13}C NMR spectrum of the compound exhibited a resonance for the acetal carbon atom at 110.09 ppm, and the chemical shifts for the methyl groups were separated by 1.86 ppm. These data support the presence of the five-membered ring[20] of the acetal group. Based on these observations, we assigned structure 12 to the compound.

In another approach to the preparation of acetal 12, isopropylidenation of 8 with acetone and copper sulfate in the presence of catalytic amount of concentrated sulfuric acid at room temperature gave a mixture of 3',4'- and 4',6'-O-isopropylidene derivatives. A similar observation has been made by other investigators.[14,21] The

mixture on treatment with anhydrous ethyl acetate in the presence of neutral alumina,[22] followed by silica gel column chromatography, gave the acetal 12 in 27% yield.

Condensation of 12 with 2,3,4-tri-O-benzyl-α-L-fucopyranosyl bromide under bromide ion-catalyzed reaction-conditions[23] for 4 days, followed by the usual processing, afforded 13, which on mild treatment with acid provided benzyl 2-acetamido-4-O-[6-O-acetyl-2-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (14) in 72% yield; it was purified by chromatography on a column of silica gel. The ^1H and ^{13}C NMR spectra confirmed the structure assigned. O-Deacetylation[24] of 14 afforded 15, which, on catalytic hydrogenolysis in glacial acetic acid in the presence of 10% Pd-C, gave the title trisaccharide 16 as an amorphous material in 81% yield. Its structure was confirmed by ^1H and ^{13}C NMR spectroscopy.

EXPERIMENTAL

General Methods. Melting points were determined with a Fisher-Johns apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 polarimeter at room temperature. Ascending TLC was conducted on plates coated with a 0.25-mm layer of silica gel 60 PF-254 (E. Merck, Darmstadt, Germany); the components were located by exposure to UV light, or spraying the plate with 5% sulfuric acid in ethanol and heating. Elemental analyses were performed by Robertson Laboratory, Florham Park, New Jersey, U.S.A. NMR spectra were recorded with a Varian XL-100 instrument; ^1H NMR spectra at 100 MHz and ^{13}C NMR spectra at 25.2 MHz were determined in the Fourier-transform (F.t.) mode; the positions of the peaks are expressed in δ from the signal of tetramethylsilane.

Benzyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (5). Method (a). A mixture of benzyl 2-acetamido-3-O-benzyl-2-deoxy-β-D-glucopyranoside (3, 1.4 g, 3.5 mmol) in dichloromethane (60 mL), 5% sodium hydroxide (5 mL), benzyl bromide (0.72 mL, 6 mmol), and tetrabutylammonium hydrogen sulfate (240 mg, 7 mmol) was boiled under reflux for 2 days, cooled, washed with water (4 x 20 mL), dried (anhydrous Na_2SO_4), and evaporated. The residue was purified by chromatography on a column (2.5 cm x 30 cm) of silica gel by elution with 9:1 (v/v) chloroform-acetone, to give benzyl 2-acetamido-3,4,6-tri-O-benzyl-2-deoxy-β-D-glucopyranoside (6) in 14.8% yield (300 mg); TLC in 9:1 chloroform-acetone, R_F 0.70; $[\alpha]_D$ -14°, (c 1, chloroform); IR showed no absorption in the hydroxyl region; ^1H NMR (Me_2SO-d_6): δ 1.84 (s, 3 H, NAc),

4.85 (d, 1 H, $J_{1,2}$ = 7.5 Hz, H-1), 7.2 - 7.45 (m, 20 H, aromatic), and 8.06 (d, 1 H, $J_{NH,2}$ = 8 Hz, NH).

Anal. Calcd for $C_{36}H_{39}NO_6$: C, 74.33; H, 6.76; N, 2.41. Found: C, 74.24; H, 6.81; N, 2.39.

On elution with 5:1 (v/v) chloroform-acetone, a mixture of compounds 4 and 5 was obtained as a solid mass which was dissolved in minimum amount of hot ethyl acetate. It was kept overnight at 0 - 5°C to give a crystalline compound 4 in 31.5% yield (540 mg), TLC (9:1 chloroform-acetone): R_F 0.33; mp 200 - 201°, $[\alpha]_D$ -9.9° (c 1, chloroform); ^1H NMR (Me_2SO-d_6): δ 1.82 (s, 3 H, NAc) and 7.25 - 7.45 (m, 15 H, aromatic).

Anal. Calcd for $C_{29}H_{33}NO_6$: C, 70.85; H, 6.78; N, 2.85. Found: C, 70.63; H, 6.61; N, 2.76.

Addition of a few drops of hexane to the mother liquor, gave compound 5 (740 mg, 43.2% yield) which was further recrystallized from ether, mp 170-171°, $[\alpha]_D$ -35.9° (c 1, chloroform) [lit.[7] mp 181°C, $[\alpha]_D$ -37° (c 1.7, chloroform)]; tlc (9:1 chloroform-acetone): R_F 0.44; ^1H NMR data (Me_2SO-d_6): δ 1.83 (s, 3 H, NAc), 2.90 (d, 1 H, J = 3 Hz, D_2O-exchangeable, OH-4), 4.90 (d, 1 H, $J_{1,2}$ = 6 Hz, H-1), 5.60 (d, 1 H, $J_{NH,2}$ = 8 Hz, NH), and 7.28 - 7.42 (m, 15 H, aromatic).

Anal. Calcd for $C_{29}H_{33}NO_6$: C, 70.85; H, 6.78; N, 2.85. Found: C, 70.92; H, 6.89; N, 2.82.

Method (b). A mixture of 3 (4.01 g, 10 mmol) and bis(tributylstannyl) oxide (4.47 g, 7.5 mmol) in toluene (200 mL) was refluxed for 4 h with continuous removal of water, then concentrated to half volume, and cooled to 80°C. Benzyl bromide (5.13 g, 30 mmol) and tetrabutylammonium bromide (1.61 g, 5 mmol) were added and the mixture was stirred under nitrogen at 80°C for 2 days. Further addition of benzyl bromide (5.13 g, 30 mmol) and tetrabutylammonium bromide (1.61 g, 5 mmol) was made after 24 h. The solution was cooled, diluted with chloroform, washed with 10% aqueous $NaHCO_3$ solution, water, dried (Na_2SO_4), and evaporated to dryness. The residue was purified by chromatography on a column (3.0 cm x 60 cm) of silica gel, with elution with 5:1 (v/v) chloroform-acetone, to give 5 in 65% yield (3.2 g).

Method (c). To a solution of 3 (1.605 g, 4 mmol) in anhydrous pyridine (15 mL) at 0°C was added tosyl chloride (1.114 g, 6 mmol). After 6 h, the mixture was stirred for 1 h in the presence of ice (10 g), and concentrated. A solution of the residue in chloroform was washed with water, aqueous sodium hydrogen carbonate, water, dried, and evaporated to dryness. The residue was purified by chromatography on a column (2.5 cm x 30 cm) of silica gel, eluting with 9:1 (v/v) chloroform-acetone

to afford crystalline benzyl 2-acetamido-3-O-benzyl-2-deoxy-6-O-tosyl-β-D-glucopyranoside in 75% yield (1.6 g); mp 145-146°C, $[\alpha]_D$ -4° (c 1, methanol); TLC (9:1 chloroform-acetone): R_F 0.43; ^1H NMR data (Me_2SO-d_6): δ 1.80 (s, 3 H, NAc), 2.39 (s, 3 H, Me), 5.58 (d, 1 H, $J_{NH,2}$ = 7 Hz, NH), and 7.3 - 7.96 (m, 14 H, aromatic protons).

Anal. Calcd for $C_{29}H_{33}NO_8S$: C, 62.69; H, 5.99; N, 2.52; S, 5.76. Found: C, 62.60; H, 5.93; N, 2.43; S, 6.02.

A solution of the tosylate of 3 (1.5 g) in N,N-dimethylformamide (10 mL) containing 6 mL of M sodium benzylate in benzyl alcohol was kept for 2 h at 90°C, and then cooled, concentrated, diluted with chloroform (150 mL), washed with aqueous sodium hydrogen carbonate, saturated aqueous sodium chloride, water, dried (Na_2SO_4), and concentrated. The residue was dissolved in 4:1 (v/v) ethanol-water and treated with charcoal, filtered and evaporated to give 5 in 76% yield (1.0 g).

Method (d). A solution of the benzylidene acetal 2 (2.445 g, 5 mmol) and sodium cyanoborohydride (2.828 g, 45 mmol) in dry tetrahydrofuran (75 mL) containing powdered 3 A° molecular sieves (10 g), was cooled to 0°. Hydrogen chloride in diethyl ether was added until the solution was acidic (pH paper, gas evolution). After 1.5 h at 0°C, when TLC (5:1 (v/v) chloroform-acetone) indicated complete reaction, the mixture was poured into ice-water, and the product was extracted with chloroform (5 x 30 mL). The combined extracts were washed with saturated, aqueous sodium hydrogen carbonate, dried over magnesium sulfate, filtered, dried, and concentrated *in vacuo*. Chromatography of the residue on a column (2.5 cm x 60 cm) of silica gel gave, in 77.4% yield (1.9 g), a pure compound that was identical to compound 5 on the basis of spectral data.

Benzyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (7). A solution of the alcohol 5 (2.95 g, 6 mmol) and mercuric cyanide (1.52 g, 6 mmol) in dry benzene (60 mL) was boiled under nitrogen atmosphere until ~30 mL of the solvent had distilled. A solution of 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide (2.47 g, 6 mmol) in dry benzene (30 mL) was rapidly added, and the mixture was refluxed for 2 days. A further addition of bromide (1.24 g, 3 mmol) in dry benzene (30 mL) was made after 24 h. The reaction mixture was cooled to room temperature, diluted with benzene, washed with 10% aqueous potassium iodide solution and water, dried (Na_2SO_4), and evaporated. The solid residue was used as such for the next reaction.

Benzyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-4-O-(β-D-galacto-pyranosyl)-β-D-glucopyranoside (8). A solution of 7 (5 g) in absolute methanol (50 mL) was treated with M sodium methoxide (5 mL), and the solution was kept overnight at room temperature with occasional shaking, made neutral with acetic acid, and evaporated; a few additions and evaporations of dry toluene produced a solid mixture containing the glycosyl bromide, monosaccharide 5, and disaccharide 8. The residue was dissolved in CH_3OH (5 mL), and $CHCl_3$ (45 mL) was added with stirring. After stirring for 1 h at room temperature, the precipitate was removed by filtration, and the filtrate was evaporated to dryness, giving a solid which was purified by chromatography on a column (3.0 cm x 60 cm) of silica gel, with elution first with 5:1 chloroform-acetone, to remove unreacted compound 5, and then with 9:1 chloroform-methanol, to afford disaccharide 8 (3.1 g) in 79% yield (from 5); m.p. 140 - 141°C (from acetone-ether), $[\alpha]_D$ -8.6° (c 1.4, Me_2SO); TLC (9:1 chloroform-methanol) R_F 0.41; ^1H NMR (Me_2SO-d_6): δ 1.78 (s, 3 H, NAc), 7.2 - 7.4 (m, 15 H, aromatic), and 7.83 (1 H, NH).

Anal. Calc for $C_{35}H_{43}NO_{11}$: C, 64.30; H, 6.63; N, 2.14. Found: C, 64.14; H, 6.53; N, 2.00.

Benzyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-4-O-[2,3,4,6-tetra-O-(trimethylsilyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (9). A solution of compound 8 (2 g) in absolute pyridine (60 mL) was treated with hexamethyldisilazane (24 mL), warmed to dissolve the suspension, and cooled to 5°C; then, chlorotrimethylsilane (10 mL) was added from a syringe. The mixture was stirred for 24 h at 60°C, cooled and filtered to remove a white precipitate. The filtrate was evaporated, and the residue was subjected to a few additions and evaporations of dry toluene, taken up in hexane, and the suspension filtered to remove a trace of precipitate. The filtrate was evaporated to dryness, to afford amorphous 9 (2.78 g) in 96% yield; $[\alpha]_D$ -1.8° (c 2.4, chloroform); the IR spectrum showed the complete absence of hydroxyl groups; ^1H NMR ($CDCl_3$): δ 0.1 - 0.2 (cluster of singlets, 36 H, 4 $SiMe_3$), 1.91 (s, 3 H, NAc), and 7.33 (m, 15 H, aromatic).

Benzyl 2-acetamido-4-O-[6-O-acetyl-2,3,4-tri-O-(trimethylsilyl)-β-D-galactopyranosyl]-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (10). A solution of compound 9 (4.712 g, 5 mmol) in absolute pyridine (10 mL) and acetic anhydride (7.5 mL) was stirred at room temperature in the presence of glacial acetic acid (0.6 g, 10 mmol), and the reaction was monitored by TLC in 4:1 (v/v) ether-hexane. After 2 days, the solution was evaporated under diminished pressure. A solution of the solid

residue in chloroform (200 mL) was washed with water, dried (anhydrous magnesium sulfate), and evaporated. The solid mass was purified by chromatography on a column (3.0 cm x 60 cm) of silica gel, with elution with 4:1 (v/v) ether-hexane, to afford amorphous 10 (3.7 g, 81%), $[\alpha]_D$ +0.2° (c 1.6, chloroform); ^{1}H NMR ($CDCl_3$): δ 0.1 - 0.2 (cluster of singlets, 27 H, 3 $SiMe_3$), 1.90 (s, 3 H, NAc), 2.05 (s, 3 H, Ac), 7.2 - 7.4 (m, 15 H, aromatic).

Benzyl 2-acetamido-4-O-(6-O-acetyl-β-D-galactopyranosyl)-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (11). A mixture of 10 (4.56 g, 5 mmol), methanol (25 mL), and 30% acetic acid (30 mL) was stirred for 5 h at room temperature, and then evaporated to dryness. The solid residue was purified by chromatography on a column (3.0 cm x 60 cm) of silica gel with elution with 9:1 (v/v) chloroform-ethanol, to get compound 11 (2.78 g) in 80% yield; mp 150-151°C (from methanol-ether), $[\alpha]_D$ +3.58° (c 1.1, chloroform); ^{1}H NMR (Me_2SO-d_6): δ 1.80 (s, 3 H, NAc), 1.90 (s, 3 H, Ac), 7.2 - 7.4 (m, 15 H, aromatic), and 7.90 (1 H, NH).

Anal. Calcd for $C_{37}H_{45}NO_{12}$: C, 63.87; H, 6.52; N, 2.01. Found: C, 63.71; H, 6.44; N, 2.09.

Benzyl 2-acetamido-4-O-(6-O-acetyl-3,4-O-isopropylidene-β-D-galactopyranosyl)-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (12). A solution of crystalline 11 (1 g) and 2,2-dimethoxypropane (2 mL) in dry N,N-dimethylformamide (15 mL) containing p-toluenesulfonic acid (20 mg) was stirred for 1 h at 60-65°C, made neutral with triethylamine, and evaporated. A solution of the solid residue in chloroform (100 mL) was washed with water (2 x 25 mL), dried (anhydrous Na_2SO_4), and evaporated to dryness. The solid mass was purified by chromatography on a column (2.5 cm x 30 cm) of silica gel, with elution with 4:1 (v/v) chloroform-acetone, to give 12 in 65% yield (0.69 g), amorphous, $[\alpha]_D$ +18.45° (c 1, chloroform); TLC (3:1 chloroform-acetone): R_F 0.60; ^{1}H NMR ($CDCl_3$): δ 1.33 and 1.48 (2 s, 6 H, isopropylidene methyls), 1.78 (s, 3 H, NAc), 1.93 (s, 3 H, Ac), 5.67 (d, 1 H, $J_{NH,2}$ = 7.5 Hz, NH), 7.2 - 7.4 (m, 15 H, aromatic); ^{13}C NMR (Me_2SO-d_6): δ 20.68 ($OCO\underline{C}H_3$), 23.37 ($NHCO\underline{C}H_3$), 26.20, 28.06 $>C(\underline{C}H_3)_2$, 55.96 (C-2), 63.32 (C-6'), 99.32 (C-1), 102.00 (C-1'), 110.09 ($>\underline{C}Me_2$), and 170.35 (C=O).

Anal. Calcd for $C_{40}H_{49}NO_{12}$: C, 65.29; H, 6.71; N, 1.90. Found: C, 65.04; H, 6.82; N, 1.99.

In another experiment, a solution of compound 8 (1.306 g, 2 mmol) in dry acetone (150 mL) containing concentrated sulfuric acid (0.22 g); anhydrous copper sulfate (7.2 g) was then added, and the mixture was stirred at room temperature for 24 h. TLC (19:1 chloroform-ethanol)

then showed traces of the starting material and a major spot (R_F 0.47) along with two minor slow moving products. The mixture was filtered, neutralized by stirring with sodium carbonate, filtered again, and evaporated to give a thick syrup. This material was dissolved in ethyl acetate (200 mL), and was stirred for 2 days at 70°C in the presence of neutral alumina (20 g). The reaction mixture was filtered, washed with ethyl acetate, and evaporated. The residue was purified by chromatography on a column (2.5 cm x 30 cm) of silica gel, with elution with 4:1 (v/v) chloroform-acetone to give an amorphous material (400 mg, 27%) that was identical to compound 12 on the basis of spectral data.

Benzyl 2-acetamido-4-O-[6-O-acetyl-3,4-O-isopropylidene-2-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (13). A suspension of acetal 12 (0.977 g, 1.33 mmol) in dry dichloromethane (25 mL) was stirred for 3 h at room temperature in the presence of tetraethylammonium bromide (0.555 g, 2.65 mmol and molecular sieves 4 A (5 g). A solution of freshly prepared 2,3,4-tri-O-benzyl-α-L-fucopyranosyl bromide (1.32 g, 2.65 mmol) in dichloromethane (25 mL) and dry $HCONMe_2$ (30 mL) was added, and the mixture was stirred under dry nitrogen for 4 days at room temperature. Methanol (20 mL) was added, the mixture was stirred for 4 h; the solids were removed by filtration, and the filtrate was evaporated. A solution of the solid residue in dichloromethane (150 mL) was successively washed with $NaHCO_3$ solution and water, dried (anhydrous Na_2SO_4), and evaporated. The residue was used, as such, for the next operation, as we were unable to separate compound 13 from 2,3,4-tri-O-benzyl-L-fucose.

Benzyl 2-acetamido-4-O-[6-O-acetyl-2-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (14). A mixture of 13 (2.5 g) and 80% acetic acid (100 mL) was stirred for 45 minutes at 100°C, cooled, and the solvent evaporated. Several additions and evaporations of water, and then of toluene, gave a solid mass which was dissolved in chloroform (200 mL). The solution was washed with water, dried, and evaporated. The residue was purified by chromatography on a column (2.5 cm x 60 cm) of silica gel, eluting first with chloroform, then with 19:1 (v/v) chloroform-acetone (to remove the 2,3,4-tri-O-benzyl-L-fucose), and finally with 9:1 (v/v) chloroform-acetone, giving 14 in an overall yield of 72%; amorphous, $[\alpha]_D$ -39.8° (c 0.8, chloroform); tlc (6:1 chloroform-acetone) R_F 0.43; ^1H NMR (Me_2SO-d_6): δ 1.1 (d, 3 H, J = 6 Hz, CMe), 1.82 (s, 3 H, NAc), 1.95 (s, 3 H, Ac), 5.53 (1 H, H-1"), 7.2 - 7.45 (m, 30 H, aromatic), and 7.96 (d, 1 H, $J_{NH,2}$ = 8 Hz, NH); ^{13}C NMR (Me_2SO-d_6):

δ 16.29 (C-6"), 20.48 (OCOCH$_3$), 22.76 (NHCOCH$_3$), 53.66 (C-2), 63.07 (C-6'), 77.47 (C-2'), 77.73 (C-4), 79.66 (C-3), 95.92 (C-1"), 100.05 (C-1), 100.54 (C-1'), 168.60 (OCOCH$_3$), and 169.79 (NHCOCH$_3$).

Anal. Calcd for $C_{64}H_{73}NO_{16}$: C, 69.11; H, 6.62; N, 1.26. Found: C, 69.36; H, 6.46; N, 1.16.

Benzyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-4-O-[2-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (15). A solution of compound 14 (450 mg) in absolute methanol (25 mL) containing Amberlyst A-26 (OH^-) resin (100 mg) was stirred at room temperature until the reaction was complete, as judged by TLC. After 6 h, the solution was filtered and the filtrate was evaporated to dryness, to afford amorphous 15 in 92% yield (400 mg); $[\alpha]_D$ -65.4° (c 0.9, acetone); ^{1}H NMR (Me_2SO-d_6): δ 1.09 (d, 3 H, J = 6 Hz, CMe), 1.82 (s, 3 H, NAc), 5.55 (1 H, H-1"), 7.33 (m, 30 H, aromatic), and 7.94 (d, 1 H, $J_{NH,2}$ = 8 Hz, NH); ^{13}C NMR (Me_2SO-d_6): δ 16.29 (C-6"), 22.77 (NHCOCH$_3$), 53.63 (C-2), 59.67 (C-6'), 77.51 (C-2'), 77.81 (C-4), 80.01 (C-3), 95.86 (C-1"), 100.15 (C-1), 100.59 (C-1'), and 168.56 (C=O).

Anal. Calcd for $C_{62}H_{71}NO_{15}$: C, 69.58; H, 6.69; N, 1.31. Found: C, 69.71; H, 6.65; N, 1.18.

O-α-L-Fucopyranosyl-(1→2)-O-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranose (16). A solution of 15 (300 mg) in glacial acetic acid (30 mL) was hydrogenolyzed in the presence of 10% Pd-C for 2 days. The suspension was filtered, and the filtrate evaporated to dryness. The residue was purified by chromatography on a column (1.5 cm x 30 cm) of silica gel, eluting with 60:40:9 (v/v) chloroform-methanol-water, to give the title trisaccharide 16 in 81% yield (120 mg), amorphous, $[\alpha]_D$ -55.9° (c 1.2, water), [lit.[4] $[\alpha]_D$ -46.5° (c 0.5, water)]; tlc (55:45:10 chloroform-methanol-water) R_F 0.65; ^{1}H NMR (D_2O): δ 1.67 (d, 3 H, J = 6 Hz, CMe), 2.50 (s, 3 H, NAc), 5.02 (d, 1 H, $J_{1',2'}$ = 7 Hz, H-1'), 5.68 (0.6 H, H-1α), and 5.78 (1 H, H-1").

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