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Synthesis of benzyl *O*-(2-*O*-methyl-β-D-galactopyranosyl)-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside [benzyl 2′-*O*-methyllacto-*N*-bioside I], and its higher saccharide containing an *O*-(2-*O*-methyl-β-D-galactopyranosyl)-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl group as a potential substrate for (1→4)-α-L-fucosyltransferase*

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ABSTRACT

Treatment of benzyl *O*-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside with *tert*-butylchlorodiphenylsilane afforded the 6′-*O*-*tert*-butyldiphenylsilyl ether, which was converted into the 3′,4′-*O*-isopropylidene derivative. Methylation and subsequent removal of protecting groups afforded benzyl *O*-(2-*O*-methyl-β-D-galactopyranosyl)-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (**7**). The trisaccharide methyl *O*-(2-*O*-methyl-β-D-galactopyranosyl)-(1→3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-β-D-galactopyranoside (**17**) and the tetrasaccharide *O*-(2-*O*-methyl-β-D-galactopyranosyl)-(1→3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-D-glucopyranose (**32**), both containing the 2′-*O*-methyllacto-*N*-biose I unit at the nonreducing end, were synthesized, and the structures of **7**, **17**, and **32** were confirmed by ^{13}C-n.m.r. spectroscopy.

INTRODUCTION

Greater attention is being focused presently upon the enzymic basis for the abberrant accumulation of tumor-associated antigens in tumor cells, so that a crucial enzyme responsible for the synthesis of that antigen may be identified and used as a diagnostic marker for cancer. One such enzyme involved in the synthesis of the chemically characterized epitope of CA19-9 antigen[2–8] is (1→4)-α-L-fucosyltransferase[9]. According to Beyer *et al.*[10], compounds containing the type I (β-D-Gal*p*-(1→3)-D-GlcNAc) sequence, such as lacto-*N*-fucotetraose [β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-Glc] are good acceptors for the specific assays of (1→4)-α-L-fucosyltransferase; however, they also possess an acceptor site at O-2 of the nonreducing terminal D-galactosyl group for (1→2)-α-L-fucosyltransferase.

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In order to achieve a specific, quantitative determination for individual α-L-fucosyltransferases, we initiated a program to obtain by synthesis well defined, low-molecular-weight oligosaccharides capable of acting as acceptors for a single enzyme, even in the presence of other related enzymes. Recently, we reported[11] this approach for the assay of (1→3)-α-L-fucosyltransferase by use of the synthetic acceptor substrate, *O*-(2-*O*-methyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-2-deoxy-D-glucopyranose (2′-*O*-methyllactosamine)[12]. Based upon a similar approach, herein we describe the synthesis of benzyl *O*-(2-*O*-methyl-β-D-galactopyranosyl)-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranose (benzyl 2′-*O*-methyllacto-*N*-bioside I) as a specific acceptor for (1→4)-α-L-fucosyltransferase as well as a related trisaccharide and tetrasaccharide.

RESULTS AND DISCUSSION

Among the various saccharides examined for the determination of the specificity of (1→4)-α-L-fucosyltransferase, the disaccharide β-D-Gal*p*-(1→3)-D-GlcNAc (lacto-*N*-biose I) was found to be an effective acceptor for this enzyme[10]. As a result, 2-*O*-methyllacto-*N*-biose I was chosen to be our first target acceptor compound for the study of this enzyme.

Benzyl 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**1**), previously synthesized in our laboratory[13], was *O*-deacetylated in methanolic sodium methoxide to give, in 94% yield, amorphous benzyl 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-4,6-*O*-isopropylidene-β-D-glucopyranoside (**2**). Treatment of **2** with *tert*-butylchlorodiphenylsilane in *N*,*N*-dimethylformamide, in the presence of imidazole, gave in 85% yield the 6-*O*-*tert*-butyldiphenylsilyl derivative **3** as an amorphous solid, the ^{1}H-n.m.r. spectrum of which contained signals in support of the structure expected.

Acetalation of triol **3** with 2,2-dimethoxypropane in *N*,*N*-dimethylformamide, in the presence of 4-toluenesulfonic acid, afforded the isopropylidene derivative **4**. Methylation of **4** with methyl iodide in dichloromethane–*N*,*N*-dimethylformamide, in the presence of freshly prepared silver oxide, gave in 65% yield the 2-*O*-methyl derivative **5**. The use of silver oxide as catalyst in the methylation reaction appeared to give a better yield than the use of sodium hydride or barium oxide–barium hydroxide; the hydrolysis[14] of the *tert*-butylsilyl group as well as *N*-alkylation was substantially reduced. Removal of the *tert*-butyldiphenylsilyl group of **5** with a M solution of tetrabutylammonium fluoride in oxolane furnished, in high yield, the amorphous derivative **6**, which on treatment with 60% aqueous acetic acid at 60° afforded in 91% yield the desired 2-*O*-methyl derivative **7**, the ^{13}C-n.m.r. spectrum of which was consistent with the structure assigned (see Table I).

Similarly to the earlier described procedure, we also successfully accomplished the synthesis of the trisaccharide **17** and of the tetrasaccharide **32**, the later compound being a modified analog of lacto-*N*-tetraose. Isopropylidenation of methyl 3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside[15] (**8**) with 2,2-dimethoxypropane in *N*,*N*-dimethylformamide in the presence of 4-tolu-

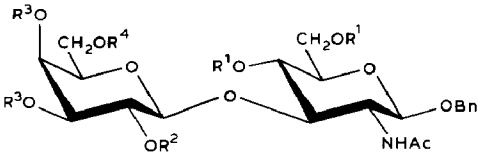
TABLE I

Proposed ^{13}C-n.m.r. chemical shifts (δ)[a]

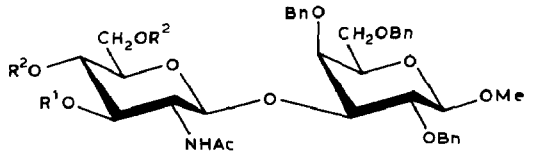
Residue or group	*Compoud*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*2-O-Me*	*1-O-Me*	*NAc*
β-D-Glc*p*NAcOBn	**7**[b]	100.26	53.67	84.39	68.79	76.49	60.80			22.80
2-*O*-*Me*-β-D-Gal*p*-(1→3)		103.77	80.71	72.34	68.11	75.30	60.31	60.03		
β-D-Gal*p*OMe		106.75	72.58	85.08	71.17	77.51	63.42		60.01	
β-D-GlcpNac-(1→3)	**17**[c]	105.43	57.48	85.23	71.36	78.02	63.73			25.04
2-*O*-Me-β-D-Gal*p*-(1→3)		106.26	83.57	75.10	71.36	78.02	63.73	63.42		
α-D-Glc*p*		94.62	74.21	73.96	81.22	72.94	62.94			
β-D-Glc*p*		98.55	76.62	77.18	81.22	78.01	63.38			
β-D-Gal*p*-(1→4)	**32**[c]	106.25	71.33	84.81	71.15	77.61	63.75–			
β-D-Glc*p*NAc-(1→3)		105.53	57.44	85.21	71.40	77.70	63.44			25.05
2-*O*-Me-β-D-Galp-(1→3)		105.76	83.56	75.10	71.33	78.02	63.75	63.66		

[a] Carbonyl and aromatic resonances are not shown. [b] For a solution in Me_2SO-d_6 with Me_4Si as the internal standard. [c] For solutions in D_2O with Me_4Si as the external standard.

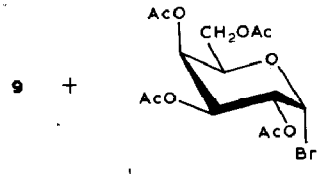
enesulfonic acid afforded the 4,6-*O*-acetal derivative **9** in 85% yield. The ^{1}H-n.m.r. spectra of **9** contained two signals each of three protons (at δ 1.40 and 1.48) attributable to the presence of an isopropylidene ring. The reaction of **9** with 2,3,4,6-tetra-*O*-acetyl-*α*-D-galactopyranosyl bromide in freshly distilled, anhydrous acetonitrile in the presence of mercuric cyanide for 8 h at room temperature afforded the trisaccharide derivative **10**, which was not isolated but directly *O*-deacetylated to give, in 62% yield, the trisaccharide derivative **11**. A similar sequence of reactions was adopted for the preparation of **16** from **11** as described earlier for the synthesis of **6** from **2**. Thus, treatment of **11** with *tert*-butylchlorodiphenylsilane in *N*,*N*-dimethylformamide, as described for **2** (to give **3**) gave, in 70% yield, the amorphous derivative **12**. The overall structure of **12** was clearly evident from its ^{1}H-n.m.r. spectrum. Acetalation of **12** with 2,2-dimethoxypropane in *N*,*N*-dimethylformamide, in the presence of 4-toluenesulfonic acid, gave the isopropylidene derivative **13**. Methylation of **13** with silver oxide–methyl iodide, as described for **4** (to give **5**), afforded in 55% yield amorphous **14**. Sequential removal of the protecting groups of **14** furnished methyl *O*-(2-*O*-methyl-*β*-D-galactopyranosyl)-(1→3)-*O*-(2-acetamido-2-deoxy-*β*-D-glucopyranosyl)-(1→3)-*β*-D-galactopyranoside (**17**), by way of intermediates **15** and **16**, respectively. The ^{13}C-n.m.r. spectrum of **17** was also in agreement with the structure assigned (see Table I). For the synthesis of *O*-(2-*O*-methyl-*β*-D-galactopyranosyl)-(1→3)-*O*-(2-acetamido-2-deoxy-*β*-D-glucopyranosyl)-(1→3)-*O*-*β*-D-galactopyranosyl-(1→4)-D-glucopyranose (**32**), benzyl 2,3,6,2′,4′,6′-hexa-*O*-benzyl-*β*-lactopyranoside (**21**) was required as a starting material. The stanylation of benzyl *β*-D-lactoside[16] (**18**) with one molar equivalent of dibutyltin oxide, followed by reaction with allyl bromide afforded the 3′-*O*-allyl compound **19**. This was benzylated with benzyl bromide in oxolane in the presence of potassium hydroxide and Crown ether[17] to afford, in 80% yield, compound **20**. Removal of the 3′-*O*-allyl group of **20** with 10% palladium-on charcoal afforded the intermediate compound **21**. Glycosylation of this compound with 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-*α*-D-glucopyrano)-[2,1-*d*]-2-oxazoline afforded, in 65% yield, the trisaccharide derivative **22**. *O*-Deacetylation of **22** followed by acetalation with 2,2-dimethoxypropane gave the 4,6-*O*-isopropylidene derivative **24**, the ^{1}H-n.m.r. spectrum of which

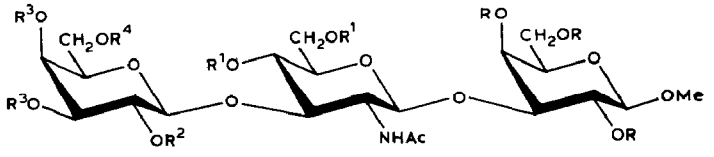


1 $R^1 = CMe_2, R^2 = R^3 = R^4 = Ac$
2 $R^1 = CMe_2, R^2 = R^3 = R^4 = H$
3 $R^1 = CMe_2, R^2 = R^3 = H, R^4 = Bu^tPh_2Si$
4 $R^1 = R^3 = CMe_2, R^2 = H, R^4 = Bu^tPh\ Si$
5 $R^1 = R^3 = CMe_2, R^2 = Me, R^4 = Bu^tPh_2Si$
6 $R^1 = R^3 = CMe_2, R^2 = Me, R^4 = H$
7 $R^1 = R^3 = R^4 = H, R^2 = Me$

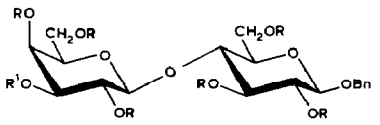


8 $R^1 = R^2 = H$
9 $R^1 = H, R^2 = CMe_2$

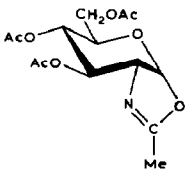




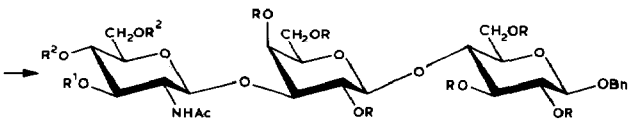
10 R = Bn, $R^1 = CMe_2$, $R^2 = R^3 = R^4 = Ac$
11 R = Bn, $R^1 = CMe_2$, $R^2 = R^3 = R^4 = H$
12 R = Bn, $R^1 = CMe_2$, $R^2 = R^3 = H$, $R^4 = Bu^tPh_2Si$
13 R = Bn, $R^1 = R^3 = CMe_2$, $R^2 = H$, $R^4 = Bu^tPh_2Si$
14 R = Bn, $R^1 = R^3 = CMe_2$, $R^2 = Me$, $R^4 = Bu^tPh_2Si$
15 R = Bn, $R^1 = R^3 = CMe_2$, $R^2 = Me$, $R^4 = H$
16 R = Bn, $R^1 = R^3 = R^4 = H$, $R^2 = Me$
17 R = $R^1 = R^3 = R^4 = H$, $R^2 = Me$



21 +

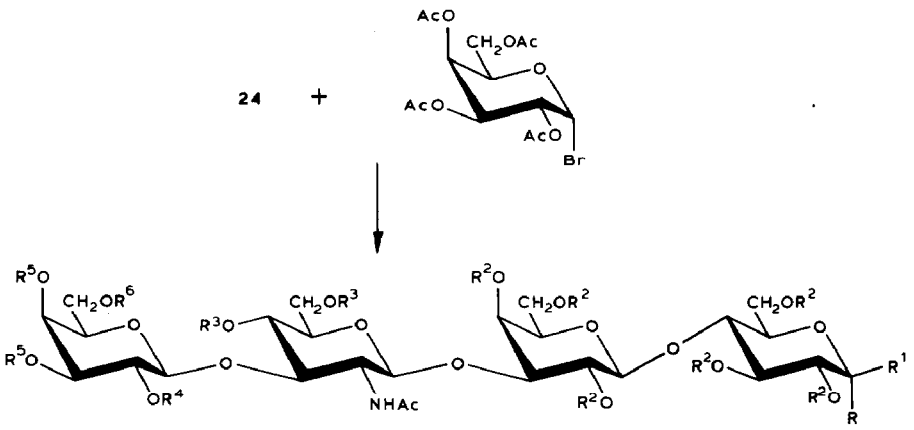


18 R = $R^1 = H$
19 R = H, $R^1 = CH_2-CH=CH_2$
20 R = Bn, $R^1 = CH_2-CH=CH_2$
21 R = Bn, $R^1 = H$



22 R = Bn, $R^1 = R^2 = Ac$
23 R = Bn, $R^1 = R^2 = H$
24 R = Bn, $R^1 = H$, $R^2 = CMe_2$

contained signals in support of the overall structure expected. On glycosylation with 2,3,4,6-tetra-*O*-acetyl-*α*-D-galactopyranosyl bromide and processing in a manner analogous to that described for **9** (to give **10**), compound **24** afforded the tetrasaccharide derivative **25**, which was not isolated but directly *O*-deacetylated to give, in 60% yield, the tetrasaccharide derivative **26**. A similar sequence of reaction was performed for the synthesis of **31** from **26**, as described for the preparation of **16** from **11**. Hydrogenolysis of the benzyl groups of **31** furnished amorphous **32**, the ^{13}C-n.m.r. spectrum of which was in accordance with the structure assigned (see Table I).



25 R = H, R^1 = OBn, R^2 = Bn, R^3 = CMe_2, R^4 = R^5 = R^6 = Ac
26 R = R^4 = R^5 = R^6 = H, R^1 = OBn, R^2 = Bn, R^3 = CMe_2
27 R = R^4 = R^5 = H, R^1 = OBn, R^2 = Bn, R^3 = CMe_2, R^6 = Bu^tPh_2Si
28 R = R^4 = H, R^1 = OBn, R^2 = Bn, R^3 = R^5 = CMe_2, R^6 = Bu^tPh_2Si
29 R = H, R^1 = OBn, R^2 = Bn, R^3 = R^5 = CMe_2, R^4 = Me, R^6 = Bu^tPh_2Si
30 R = R^6 = H, R^1 = OBn, R^2 = Bn, R^3 = R^5 = CMe_2, R^4 = Me
31 R = R^3 = R^5 = R^6 = H, R^1 = OBn, R^2 = Bn, R^4 = Me
32 R, R^1 = H, OH, R^2 = R^3 = R^5 = R^6 = H, R^4 = Me

In the ^{13}C-n.m.r. spectra of the disaccharide **7**, trisaccharide **17**, and tetrasaccharide **32**, the resonance for interglycosidic linkages were all in the region normally expected for *β*-D-glycosidic linkages. However, in the ^{13}C-n.m.r. spectra of **7**, **17**, and **32**, the resonance for C-2 of the terminal, nonreducing D-galactosyl group underwent a downfield shift, confirming the site of methylation. On the other hand, the D-glucosyl reducing residue in **32** appeared to exist more in the *β* configuration, as evidenced by the intensity of the signal for C-1*β* (δ 98.55), which was two times greater than that of the signal at δ 94.62 for C-1*α*.

Compound **7** was successfully shown to be an effective acceptor for (1→4)-*α*-L-fucosyltransferase and efficiently applied in our clinical investigation[18]. Further studies on other acceptors will be described elsewhere.

EXPERIMENTAL

General methods. — Melting points were determined with a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured at 25° with a Perkin–Elmer 241 polarimeter. All n.m.r. spectra were recorded at 25°, ^{1}H-n.m.r. with a Varian EM-390 instrument operating at 90 MHz, and ^{13}C-n.m.r. spectra with a Varian XL-100 instrument at 25.2 MHz; the possitions of the peaks (δ) are expressed from the tetramethylsilane signal. T.l.c. was conducted on aluminum sheets, precoated with 0.2-mm layers of Silica Gel 60F-254 (Merck, Darmstadt, Germany); the components were located by exposure to u.v. light or spraying the plates with 5% H_2SO_4 in ethanol and heating, or both. Silica gel used for column chromatography was Baker Analyzed (60–200 mesh). Organic solutions were generally dried with anhydrous Na_2SO_4. Acetonitrile was distilled from P_2O_5 immediately before use; 1,2-dichloroethane, *N*,*N*-dimethylformamide, and oxolane were dried over 4A molecular sieves. Elemental analyses were performed by Robertson Laboratory, 29 Samson Ave., Madison, New Jersey, 07940, U.S.A.

Benzyl O-*β*-D-*galactopyranosyl*-(1→3)-*2-acetamido-2-deoxy-4,6*-O-*isopropylidene*-*β*-D-*glucopyranoside* (**2**). — A solution of benzyl 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-3-*O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl)-*β*-D-glucopyranoside[13] (**1**; 8.5 g, 12.5 mmol) in 25mM sodium methoxide (200 mL) was stirred for 4 h at room temperature. The base was neutralized with IR 120 (H^+) resin, and the solution was filtered and concentrated. The residue was dissolved in methanol, and the addition of ether and hexane resulted in the precipitation of pure compound **2** (6.0 g, 94%), $[\alpha]_D$ −60° (*c* 0.95, methanol); ^{1}H-n.m.r. [$CDCl_3$ + Me_2SO-d_6]: δ 7.37–7.27 (m, 5 H, arom.), 1.93 (s, 3 H, NAc), 1.53 and 1.37 (2 s, 2 × 3 H, CMe_2).

Anal. Calc. for $C_{24}H_{35}NO_{11}$: C, 56.12; H, 6.88; N, 2.73. Found: C, 55.83; H, 6.85; N, 2.68.

Benzyl O-*(6*-O-tert-*butyldiphenylsilyl*-*β*-D-*galactopyranosyl)*-*(1→3)*-*2-acetamido-2-deoxy-4,6*-O-*isopropylidene*-*β*-D-*glucopyranoside* (**3**). — To an ice-cold and stirred solution of **2** (8.0 g, 15.6 mmol) and imidazole (4.0 g, 59 mmol) in dry *N*,*N*-dimethylformamide (150 mL) was added *tert*-butylchlorodiphenylsilane (8.0 mL; 31 mmol), and the stirring was continued for 1.5 h at 0°. After processing in the usual manner, the crude product was dissolved in dichloromethane. Addition of ether and hexane yielded **3** as an amorphous solid (10.0 g, 85.4%), $[\alpha]_D$ − 53° (*c* 1.7, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.77–7.17 (m, 15 H, arom.), 1.70 (s, 3 H, NAc), 1.30 and 1.40 (2 s, 2 × 3 H, CMe_2), and 1.03 (s, 9 H, CMe_3).

Anal. Calc. for $C_{40}H_{53}NO_{11}Si$: C, 63.86; H, 7.12; N, 1.86. Found: C, 63.67; H, 7.18; N, 2.10.

Benzyl O-*(6*-O-tert-*butyldiphenylsilyl-3,4*-O-*isopropylidene*-*β*-D-*galactopyranosyl)*-*(1→3)*-*2-acetamido-2-deoxy-4,6*-O-*isopropylidene*-*β*-D-*glucopyranoside* (**4**). — To a stirred solution of **3** (12.5 g; 16.62 mmol) in *N*,*N*-dimethylformamide (190 mL) were added 4-toluenesulfonic acid monohydrate (0.6 g) and 2,2-dimethoxypropane (112 mL), and the stirring was continued for 16 h at room temperature. The acid was

neutralized with a little triethylamine, and the solution concentrated to dryness. The residue was dissolved in chloroform, and the organic layer was washed with water, dried, and evaporated to dryness. The residue was purified in a column of silica gel with 5% acetone in chloroform as the eluent to give **4** (9.0 g, 68%), a white solid, $[\alpha]_D$ −24° (*c* 0.9, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.80–7.17 (m, 15 H, arom.), 1.77–1.40 (cluster of s. 15 H, 2 CMe_2, NAc), and 1.00 (s, 9 H, CMe_3).

Anal. Calc. for $C_{43}H_{57}NO_{11}Si$: C, 65.20; H, 7.27; N, 1.77. Found: C, 64.96; H, 7.06; N, 1.72.

Benzyl O-*(6*-O-tert-*butyldiphenylsilyl-3,4*-O-*isopropylidene-2*-O-*methyl-β*-D-*galactopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6*-O-*isopropylidene-β*-D-*glucopyranoside* (**5**). — A mixture of **4** (1.5 g, 1.9 mmol), methyl iodide (3.0 mL), and freshly prepared Ag_2O (3.0 g) in 1:1 *N*,*N*-dimethylformamide–dichloromethane (100 mL) was stirred for 16 h at room temperature. The solids were removed by filtration (Celite bed) and thoroughly washed with dichloromethane. Filtrate and washings were combined and concentrated 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 solid. The crude product mixture was applied to a column of silica gel. Elution with 3% acetone in chloroform gave **5** as an amorphous powder (1.0 g, 65.4%), $[\alpha]_D$ −14° (*c* 0.98, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.77–7.20 (m, 15 H, arom.), 3.17 (s, 3 H, OCH_3), 1.87–1.23 (cluster of s., 15 H, 2 CMe_2 and NAc), and 1.00 (s, 9 H, CMe_3).

Anal. Calc. for $C_{44}H_{59}NO_{11}Si$: C, 65.55; H, 7.39; N, 1.74. Found: C, 65.44; H, 7.07; N, 1.85.

Benzyl O-*(3,4*-O-*isopropylidene-2*-O-*methyl-β*-D-*galactopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6*-O-*isopropylidene-β*-D-*glucopyranoside* (**6**). — A solution of **5** (0.7 g; 0.9 mmol) in anhydrous oxolane (15 mL) was treated with a molar solution of tetrabutylammonium fluoride in oxolane (0.75 mL), and the stirring was continued for 1 h at room temperature. The mixture was concentrated to dryness and the residue was purified in a column of silica gel with 2% methanol in chloroform as the eluent to give **6** (0.35 g, 71%) as a white amorphous solid, $[\alpha]_D$ −7.6° (*c* 0.8, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.30–7.20 (m, 5 H, arom.), 3.20 (s, 3 H, OCH_3), 1.90 (s, 3 H, NAc), 1.50 and 1.30 (cluster of singlets, 4 × 3 H, 2 CMe_2).

Anal. Calc. for $C_{28}H_{41}NO_{11}$: C, 59.23; H, 7.29; N, 2.47. Found: C, 59.54; H, 6.93; H, 2.29.

Benzyl O-*(2*-O-*methyl-β*-D-*galactopyranosyl)-(1→3)-2-acetamido-2-deoxy-β*-D-*glucopyranoside* (**7**). — Compound **6** (0.32 g, 0.6 mmol) in 60% aqueous acetic acid was heated for 1 h at 60°. The acetic acid was evaporated under diminished pressure, the last traces being removed by coevaporation with several added portions of toluene. The residue was dissolved in methanol and addition of ether gave **7** (0.25 g, 91%) as a white amorphous powder, $[\alpha]_D$ −35° (*c* 0.6, methanol); for ^{13}C-n.m.r. data, see Table I.

Anal. Calc. for $C_{22}H_{33}NO_{11}$: C, 54.19; H, 6.84; N, 2.87. Found: C, 53.96; H, 6.78; N, 2.79.

Methyl O-*(2-acetamido-2-deoxy-4,6*-O-*isopropylidene-β*-D-*glucopyranosyl)-(1→3)-*

2,4,6-tri-O-benzyl-β-D-galactopyranoside (**9**). — A mixture of methyl 3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside[15] (**8**; 2.0 g, 3 mmol) and 4-toluenesulfonic acid monohydrate (0.2 g) in *N,N*-dimethylformamide (20 mL) and 2,2-dimethoxypropane (2 mL) was stirred overnight at room temperature. After the usual processing, the crude product was purified in a column of silica gel with 2% hexane in ethyl acetate as the eluent to give **9** (1.9 g, 85%), $[a]_D$ +15° (*c* 1, chloroform); ^{1}H-n.m.r. data ($CDCl_3$): δ 7.30–7.10 (m, 15 H, arom.), 3.48 (s, 3 H, OCH_3), 1.75 (s, 3 H, NAc), 1.48 and 1.40 (2 s, 2 × 3 H, CMe_2).

Anal. Calc. for $C_{39}H_{49}NO_{11}$: C, 66.18; H, 6.98; N, 1.98. Found: C, 65.87; H, 7.12; N, 1.82.

Methyl O-(β-D-galactopyranosyl)-(1→3)-O-(2-acetamido-2-deoxy-4,6-O-isopropylidene-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-O-benzyl-β-D-galactopyranoside (**11**). — A mixture of **9** (1.9 g, 2.7 mmol), 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (2.5 g, 6.08 mmol), mercury(II) cyanide (1.3 g, 5.15 mmol), and dry acetonitrile (50 mL) was stirred for 8 h at room temperature, and then evaporated to dryness. A solution of the solid residue in chloroform was successively washed with saturated aqueous $NaHCO_3$, 10% aqueous KI, and water, dried, and evaporated to dryness. Examination by t.l.c. (4:1 chloroform–acetone) showed the disappearance of **9** and the presence of a major product, faster-migrating than **9**, and several slower-migrating, minor contaminants. The crude mixture (5.5 g, containing **10**) was dissolved in 0.05M sodium methoxide in methanol (100 mL) and stirred for 3 h at room temperature. The base was neutralized by IR-120 (H^+) resin, the suspension filtered, the solution concentrated to dryness, and the residue applied to a column of silica gel. Elution with 10% methanol in ethyl acetate and evaporation of the fractions corresponding to the product gave amorphous **11** (1.45 g, 62%), $[a]_D$ −23° (*c* 1, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.40–7.20 (m, 15 H, arom.), 3.48 (s, 3 H, OCH_3), 1.68 (s, 3 H, NAc), 1.40 and 1.30 (2 s, 2 × 3 H, CMe_2).

Anal. Calc. for $C_{45}H_{59}NO_{16}$: C, 62.13; H, 6.84; N, 1.61. Found: C, 61.85; H, 6.89; N, 1.59.

Methyl O-(6-O-tert-butyldiphenylsilyl-β-D-galactopyranosyl)-(1→3)-O-(2-acetamido-2-deoxy-4,6-O-isopropylidene-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-O-benzyl-β-D-galactopyranoside (**12**). — To a cold (0°, bath), stirred solution of **11** (0.72 g, 0.83 mmol) in anhydrous *N,N*-dimethylformamide (15 mL) containing imidazole (0.25 g, 3.7 mmol) was added *tert*-butylchlorodiphenylsilane (0.4 mL, 1.54 mmol), and the stirring continued for 1.5 h at 0°. After processing as described for **2** (to give **3**), followed by column-chromatographic purification with 2% methanol in chloroform as the eluent, **12** (0.64 g, 70%) was obtained as an amorphous white solid, $[a]_D$ −24° (*c* 1.1, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.70–7.10 (m, 25 H, arom.), 3.40 (s, 3 H, OCH_3), 1.60–1.40 (cluster of singlets, 9 H, CMe_2, NAc), and 1.05 (s, 9 H, CMe_3).

Anal. Calc. for $C_{61}H_{77}NO_{16}Si$: C, 66.10; H, 7.00; N, 1.26. Found: C, 66.39; H, 7.16; N, 1.40.

Methyl O-(6-O-tert-butyldiphenylsilyl-3,4-O-isopropylidene-β-D-galactopyranosyl)-(1→3)-O-(2-acetamido-2-deoxy-4,6-O-isopropylidene-β-D-glucopyranosyl)-

*(1→3)-2,4,6-tri-*O*-benzyl-β-*D*-galactopyranoside* (**13**). — Compound **12** (0.4 g, 0.36 mmol) in *N*,*N*-dimethylformamide (10 mL) and 2,2-dimethoxypropane (1 mL) containing 4-toluenesulfonic acid monohydrate (0.08 g) were stirred at room temperature as described for **3** (to give **4**). After processing as described above, the product was purified in a column of silica gel with 10% acetone in chloroform as the eluent to give **13** (0.3 g, 72%), $[\alpha]_D$ −12° (*c* 0.8, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.70–7.15 (m, 25 H, arom.), 3.45 (s, 3 H, OCH_3), 1.60–1.20 (cluster of s., 15 H, NAc, 2 CMe_2), and 1.00 (s 9 H, CMe_3).

Anal. Calc. for $C_{64}H_{81}NO_{16}Si$: C, 66.93; H, 7.11; N, 1.22. Found: C, 66.81; H, 7.13; N, 1.34.

Methyl O-*(6-*O*-tert-butyldiphenylsilyl-3,4-*O*-isopropylidene-2-*O*-methyl-β-*D*-galactopyranosyl)-(1→3)-*O*-(2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-*D*-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-*D*-galactopyranoside* (**14**). — Compound **13** (0.2 g, 0.17 mmol) was *O*-methylated in a manner analogous to that described for **4** (to give **5**) and the product mixture was purified in a column of silica gel with 5% acetone in chloroform to afford **14** (0.12 g, 55%), $[\alpha]_D$ −10° (*c* 0.9, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.70–7.25 (m, 25 H, arom.), 3.40 and 3.37 (2 s, 2 × 3 H, 2 OCH_3), 1.50–1.18 (cluster of s., 15 H, NAc and 2 CMe_2), and 1.00 (s, 9 H, CMe_3).

Anal. Calc. for $C_{65}H_{83}NO_{16}Si$: C, 67.16; H, 7.20; N, 1.20. Found: C, 66.83; H, 6.86; N, 1.29.

Methyl O-*(2-*O*-methyl-β-*D*-galactopyranosyl)-(1→3)-*O*-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-*D*-galactopyranoside* (**16**). — Treatment of a solution of **14** (0.1 g, 0.09 mmol) in oxolane (5 mL) with M tetrabutylammonium fluoride in oxolane (0.1 mL) as described for **5** (to give **6**) yielded crude **15** which was dissolved, without purification, in 80% aqueous acetic acid (10 mL) and stirred for 1.5 h at 75°. The acetic acid was evaporated under diminished pressure, the last traces being removed by coevaporation with several added portions of toluene. The residue was applied to a column of silica gel and eluted with 8% methanol in chloroform to give **16** (0.06 g, 82%), $[\alpha]_D$ −9° (*c* 0.7, methanol); ^{1}H-n.m.r. ($CDCl_3$): δ 7.35–7.25 (m, 15 H, arom.), 3.44 and 3.40 (2 s, 2 × 3 H, 2 × OCH_3), and 1.8 (s, 3 H, NAc).

Anal. Calc. for $C_{43}H_{57}NO_{16}$: C, 66.19, H, 6.81; N, 1.66. Found: C, 66.09; H, 7.14; N, 1.29.

Methyl O-*(2-*O*-methyl-β-*D*-galactopyranosyl)-(1→3)-*O*-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-(1→3)-β-*D*-galactopyranoside* (**17**). — A mixture of **16** (0.05 g, 0.06 mmol) and 10% Pd–C (0.1 g) in glacial acetic acid (2 mL) was shaken under H_2 at 345 kPa for 2 days at room temperature. The suspension was filtered through a Celite bed and the solid was thoroughly washed with glacial acetic acid. The filtrate and washings were combined and evaporated under diminished pressure. The crude product was applied to a column of silica gel. Elution with 5:4:1 (v/v) chloroform–methanol–water and evaporation of the fractions corresponding to the product gave **17** (0.02 g, 78%), $[\alpha]_D$ −2° (*c* 0.5, water); ^{13}C-n.m.r., see Table I.

Anal. Calc. for $C_{22}H_{39}NO_{16} \cdot 2.5H_2O$: C, 42.70; H, 7.22; N, 2.26. Found: C, 42.44; H, 6.93; N, 2.43.

Benzyl O-*(3-*O*-allyl-β-*D*-galactopyranosyl)-(1→4)-β-*D*-glucopyranoside* (**19**). —

A mixture of benzyl β-D-lactopyranoside (**18**; 10 g, 23 mmol) and dibutyltin oxide (7.5 g, 30 mmol) in benzene (200 mL) was heated for 20 h at reflux temperature with azeotropic distillation of water. The mixture was cooled, and allyl bromide (5.5 mL, 63.5 mmol), and tetrabutylammonium bromide (4 g, 12.4 mmol) were added. The mixture was refluxed with stirring for 3 h, and then concentrated, and the syrup was dissolved in water and washed with ethyl acetate to remove tin byproducts. The aqueous layer was concentrated to dryness to give a crude compound which was purified in a column of silica gel with 20% methanol in ethyl acetate to give **19** (7.1 g, 65%), white solid, $[\alpha]_D$ +11° (*c* 0.8, water); ^{1}H-n.m.r. (Me_2SO-d_6): δ 7.40–7.20 (m, 5 H, arom), and 6.02–5.80 (m, 1 H, —C*H*=CH_2).

Anal. Calc. for $C_{22}H_{32}O_{11}\cdot 0.5H_2O$: C, 54.87; H, 6.91. Found: C, 55.08; H, 6.83.

Benzyl O-*(3-O-allyl-2,4,6-tri-*O-*benzyl-β-*D-*galactopyranosyl)-(1→4)-2,3,6-tri-*O-*benzyl-β-*D-*glucopyranoside* (**20**). — To a stirred mixture of **19** (7 g, 14.8 mmol), powdered KOH (20 g; 360 mmol), and 18-Crown-6 (0.4 g, 1.5 mmol) in oxolane (100 mL) was added benzyl bromide (25 mL, 210 mmol) dropwise, and stirring was continued for 5 h at room temperature. The mixture was then diluted with chloroform (200 mL) and washed with water, dried, and evaporated. The residue was applied to a column of silica gel. Elution with 2:1 (v/v) hexane–ethyl acetate and evaporation of the fractions corresponding to the product yielded **20** (11.5 g, 80%), $[\alpha]_D$ −8° (*c* 1, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.30–7.10 (m, 35 H, arom.), and 6.00–5.80 (m, 1 H, —C*H*=CH_2).

Anal. Calc. for $C_{64}H_{68}O_{11}$: C, 75.86; H, 6.76. Found: C, 76.16; H, 6.88.

Benzyl O-*(2,4,6-tri-*O-*benzyl-β-*D-*galactopyranosyl)-(1→4)-2,3,6-tri-*O-*benzyl-β-*D-*galactopyranoside* (**21**). — A mixture of the 3'-*O*-allyl derivative **20** (10 g; 9.8 mmol) and 10% Pd–C (1 g) in 2:1:1 ethanol–glacial acetic acid–water (100 mL) was stirred for 40 h at 75–80°. The suspension was filtered off through a Celite bed. Filtrate and washings were concentrated to a light-yellow syrup. The crude product was applied to a column of silica gel, and elution with 2:1 (v/v) hexane–ethyl acetate afforded **21** (6 g, 62%) as a colorless syrup, $[\alpha]_D$ −5° (*c* 1, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.30–7.10 (m, 35 H, arom.).

Anal. Calc. for $C_{61}H_{64}O_{11}$: C, 75.28; H, 6.63. Found: C, 75.09; H, 6.48.

Benzyl O-*(2-acetamido-2-deoxy-3,4,6-tri-*O-*acetyl-β-*D-*glucopyranosyl)-(1→3)-*O-*(2,4,6-tri-*O-*benzyl-β-*D-*galactopyranosyl)-(1→4)-2,3,6-tri-*O-*benzyl-β-*D-*glucopyranoside* (**22**). — A mixture of **21** (3 g; 3.1 mmol), 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline (1.8 g, 5.5 mmol), and 4-toluenesulfonic acid (0.76 g) in 1,2-dichloroethane (20 mL), protected from moisture, was heated overnight at 70° in an atmosphere of N_2. The mixture was cooled, the acid neutralized by the addition of a few drops of pyridine, and the solution concentrated to dryness. Examination of the crude product by t.l.c. with 2:1 (v/v) ethyl acetate–hexane revealed the presence of a major product migrating slower than the lactose derivative **21**, some unchanged **21**, and also some slower-migrating contaminants (presumably decomposition products of oxazoline). The crude material was purified by silica gel column chromatography. Elution with 2:1 (v/v) ethyl acetate–hexane afforded **22** (2.6 g,

65%) as a white amorphous solid, $[\alpha]_D$ −22° (*c* 1, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.40–7.10 (m, 35 H, arom.), 2.02 and 1.95 (2 s, 9 H, 3 × OAc), and 1.48 (s, 3 H, NAc).

Anal. Calc. for $C_{75}H_{83}NO_{19}$: C, 69.16; H, 6.42; N, 1.08. Found: C, 68.89; H, 6.35; N, 1.06.

Benzyl O-*(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-(1→3)*-O-*(2,4,6-tri*-O-*benzyl-β-*D*-galactopyranosyl)-(1→4)-β-*D*-glucopyranoside* (**23**). — *O*-Deacetylation of **22** (2.5 g, 1.9 mmol) in 0.05M methanolic sodium methoxide (100 mL) in a manner analogous to that described for **1** (to give **2**) afforded **23** (2.1 g, 93%), amorphous, $[\alpha]_D$ −16° (*c* 1.1, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.20–7.00 (m, 35 H, arom.), and 1.50 (s, 3 H, NAc).

Anal. Calc. for $C_{69}H_{77}NO_{16}$: C, 70.45; H, 6.60; N, 1.19. Found: C, 70.19; H, 6,61; N, 1.23.

Benzyl O-*(2-acetamido-2-deoxy-4,6-*O-*isopropylidene-β-*D*-glucopyranosyl)-(1→3)*-O-*(2,4,6-tri*-O-*benzyl-β-*D*-galactopyranosyl)-(1→4)-2,3,6-tri*-O-*benzyl-β-*D*-galactopyranoside)* (**24**). — A solution of **23** (1.8 g; 1.5 mmol) in *N,N*-dimethylformamide (20 mL) was treated with 4-toluenesulfonic acid (0.2 g) and 2,2-dimethoxypropane (5 mL) in a manner similar to that described for **3** (to give **4**). It was then processed and the crude product mixture was purified in a column of silica gel with 5% methanol in ethyl acetate as the eluent to give **24** as an amorphous solid (1.5 g, 81%), $[\alpha]_D$ −24° (*c* 0.8, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.30–7.10 (m, 35 H, arom.), 1.50 (s, 3 H, NAc), 1.37 and 1.28 (2 s, 2 × 3 H, CMe_2).

Anal. Calc. for $C_{72}H_{81}NO_{16}$: C, 71.09; H, 6.71; N, 1.15. Found: C, 70.93; H, 6.65; N, 1.12.

Benzyl O-*β-*D*-galactopyranosyl-(1→3)*-O-*(2-acetamido-2-deoxy-4,6-*O-*isopropylidene-β-*D*-glucopyranosyl)-(1→3)*-O-*(2,4,6-tri*-O-*benzyl-β-*D*-galactopyranosyl)-(1→4)-2,3,6-tri*-O-*benzyl-β-*D*-glucopyranoside* (**26**). — Compound **24** (1.4 g; 1.15 mmol) was treated with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (3.2 g, 7.8 mmol) in the presence of $Hg(CN)_2$ (1.5 g, 6 mmol) in a manner analogous to that described for **9** (to give **10**). After the usual processing, the crude product (containing **25**) was *O*-deacetylated in 0.05M methanolic sodium methoxide (100 mL), and the mixture was purified in a column of silica gel. Elution with ethyl acetate afforded unchanged **24**, and continued elution with 10% methanol in ethyl acetate gave **26** (0.94 g, 59%), $[\alpha]_D$ −24° (*c* 1.0, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.30-7.08 (m, 35 H, arom.), 1.60 (s, 3 H, NAc), 1.48 and 1.38 (2 s, 2 × 3 H, CMe_2).

Anal. Calc. for $C_{78}H_{91}NO_{21}$: C, 67.96; H, 6.65; N, 1.02. Found: C, 67.72; H, 6.60; N, 1.06.

Benzyl O-*(6-*O-tert-*butyldiphenylsilyl-β-*D*-galactopyranosyl)-(1→3)*-O-*(2-acetamido-2-deoxy-4,6-*O-*isopropylidene-β-*D*-glucopyranosyl)-(1→3)*-O-*(2,4,6-tri*-O-*benzyl-β-*D*-galactopyranosyl)-(1→4)-2,3,6-tri*-O-*benzyl-β-*D*-glucopyranoside* (**27**). — To a cold (0°, bath), stirred solution of **26** (0.7 g, 0.51 mmol) in anhydrous *N,N*-dimethylformamide (5 mL) containing imidazole (0.09 g, 1.3 mmol) was added *tert*-butylchlorodiphenylsilane (0.21 g; 0.76 mmol), and the stirring was continued for 2 h at 0°. After processing as described for **2** (to give **3**), followed by column-chromatographic purification with ethyl

acetate as the eluent, **27** (0.61 g, 74%) was obtained as an amorphous white solid, $[\alpha]_D$ −24° (*c* 0.8, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.70–7.00 (m, 45 H, arom.), 1.65 (s, 3 H, NAc), 1.39 and 1.29 (2 s, 2 × 3 H, CMe_2), and 1.00 (s, 9 H, CMe_3).

Anal. Calc. for $C_{94}H_{109}NO_{21}Si$: C, 69.82; H, 6.80; N, 0.87. Found: C, 69.53; H, 6.69; N, 0.92.

Benzyl O-*(6*-O-tert-*butyldiphenylsilyl-3,4*-O-*isopropylidene-β*-D-*galactopyranosyl)-(1→3)*-O-*(2-acetamido-2-deoxy-4,6*-O-*isopropylidene-β*-D-*glucopyranosyl)-(1→3)*-O-*(2,4,6-tri*-O-*benzyl-β*-D-*galactopyranosyl)-(1→4)-2,3,6-tri*-O-*benzyl-β*-D-*glucopyranoside* (**28**). — A solution of **27** (0.30 g, 0.19 mmol) in *N,N*-dimethylformamide (10 mL) containing 4-toluenesulfonic acid monohydrate (0.05 g) was treated with 2,2-dimethoxypropane (1 mL) in a manner similar to that described for **3** (to give **4**), and the product mixture was purified in a column of silica gel with ethyl acetate to afford **28** (0.25 g, 68%), amorphous, $[\alpha]_D$ −7° (*c* 0.7, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.70–7.00 (m, 45 H, arom.), 1.80 (s, 3 H, NAc), 1.50–1.30 (cluster of s., 12 H, 2 CMe_2), and 1.00 (s, 9 H, CMe_3).

Anal. Calc. for $C_{97}H_{113}NO_{21}Si$: C, 70.31; H, 6.87; N, 0.85. Found: C, 70.08; H, 6.78; N, 0.93.

Benzyl O-*(6*-O-tert-*butyldiphenylsilyl-3,4*-O-*isopropylidene-2*-O-*methyl-β*-D-*galactopyranosyl)-(1→3)*-O-*(2-acetamido-2-deoxy-4,6*-O-*isopropylidene-β*-D-*glucopyranosyl)-(1→3)*-O-*(2,4,6-tri*-O-*benzyl-β*-D-*galactopyranosyl)-(1→4)-2,3,6-tri*-O-*benzyl-β*-D-*glucopyranoside* (**29**). — Compound **28** (0.24 g, 0.14 mmol) was *O*-methylated exactly following the procedure described for the preparation of **5** (from **4**), and the product mixture was purified in a column of silica gel with 2:1 (v/v) ethyl acetate–hexane to afford **29** (0.14 g, 58%), amorphous, $[\alpha]_D$ −6.5° (*c* 0.82, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.71–7.05 (m, 45 H, arom.), 3.40 (s, 3 H, OCH_3), 1.90 (s, 3 H, NAc), 1.45–1.24 (cluster of s., 12 H, 2 CMe_2), and 1.01 (s, 9 H, CMe_3).

Anal. Calc. for $C_{98}H_{115}NO_{21}Si$: C, 70.44; H, 6.94; N, 0.84. Found: C, 70.16; H, 7.01; N, 0.94.

Benzyl O-*(2-O-methyl-β*-D-*galactopyranosyl)-(1→3)*-O-*(2-acetamido-2-deoxy-β*-D-*glucopyranosyl)-(1→3)*-O-*(2,4,6-tri*-O-*benzyl-β*-D-*galactopyranosyl)-(1→4)-2,3,6-tri*-O-*benzyl-β*-D-*glucopyranoside* (**31**). — Treatment of a stirred solution of **29** (0.1 g, 0.06 mmol) in dry oxolane (5 mL) with a M solution of tetrabutylammonium fluoride in oxolane (0.5 mL) yielded intermediate **30** which was dissolved without purification in 70% aqueous acetic acid (5 mL) and stirred for 2 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 applied to a column of silica gel and eluted with 10–20% methanol in ethyl acetate to give **31** (0.086 g, 85%), a white amorphous solid, $[\alpha]_D$ −12° (*c* 0.88, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.40–7.20 (m, 35 H, arom.), 3.48 (s, 3 H, OCH_3), and 1.70 (s, 3 H, NAc).

Anal. Calc. for $C_{76}H_{89}NO_{21}$: C, 67.49; H, 6.63; N, 1.04. Found: C, 67.20; H, 6.73; N, 1.14.

O-*(2*-O-*Methyl-β*-D-*galactopyranosyl)-(1→3)*-O-*(2-acetamido-2-deoxy-β*-D-*glucopyranosyl)-(1→3)*-O-*β*-D-*galactopyranosyl)-(1→4)*-D-*glucopyranose* (**32**). —

Compound **31** (0.08 g, 0.06 mmol) was hydrogenolyzed in glacial acetic acid (2 mL) as described for **16** (to give **17**) to afford **32** (0.032 g, 75%), amorphous, $[\alpha]_D$ +10° (*c* 0.5, water); ^{13}C-n.m.r., see Table I.

Anal. Calc. for $C_{27}H_{47}NO_{21}\cdot 3\,H_2O$: C, 41.80; H, 6.88; N, 1.81. Found: C, 42.02; H, 6.91; N, 2.11.

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