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SYNTHESIS OF 2-ACETAMIDO-2-DEOXY-4-*O*-(2-*O*-METHYL-β-D-GALACTOPYRANOSYL)-D-GLUCOPYRANOSE (*N*-ACETYL-2′-*O*-METHYLLACTOSAMINE)*

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ABSTRACT

1,3,4,6-Tetra-*O*-acetyl-2-*O*-methyl-D-galactopyranose, prepared from known methyl 6-*O*-acetyl-3,4-*O*-isopropylidene-β-D-galactopyranoside, was treated with hydrogen bromide in dichloromethane to afford 3,4,6-tri-*O*-acetyl-2-*O*-methyl-α-D-galactopyranosyl bromide. Condensation with benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside in acetonitrile in the presence of mercuric cyanide gave an approximately 1:1 mixture of benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-methyl-β- (**8**) and -α-D-galactopyranosyl)-α-D-glucopyranoside. *O*-Deacetylation and catalytic hydrogenolysis of the benzyl group furnished 2-acetamido-2-deoxy-4-*O*-(2-*O*-methyl-β- and α-D-galactopyranosyl)-D-glucopyranose. Alternatively, benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-β-D-galactopyranosyl-α-D-glucopyranoside was treated with *tert*-butyldiphenylchlorosilane in *N,N*-dimethylformamide, in the presence of imidazole, to give a 6′-*O*-*tert*-butyldiphenylsilyl intermediate that was in turn converted into its 3′,4′-*O*-isopropylidene acetal. Methylation with methyl iodide–silver oxide in *N,N*-dimethylformamide, followed by removal of the silyl and isopropylidene groups gave benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(2-*O*-methyl-β-D-galactopyranosyl)-α-D-glucopyranoside, which was further characterized as its triacetate **8**.

INTRODUCTION

α-L-Fucosyltransferases are responsible for the transfer of L-fucose from GDP-L-fucose. Initially, the study of this class of enzymes was largely related to the biosynthesis of blood-group active substances[2–4]. More recently this interest was

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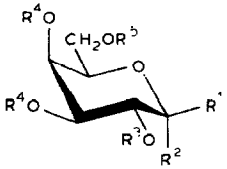
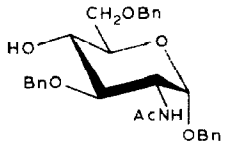
enhanced because these enzymes may be involved in the process of premalignant transformations[5,6]. α-L-(1→3)-Fucosyltransferase, which transfers L-fucose from GDP-L-fucose to 2-acetamido-2-deoxy-D-glucose or D-glucose, has been detected in various sources[3,7–10]. It has been suggested that this enzyme is being responsible for the accumulation of highly fucosylated polylactosamine compounds that are found in various human cancers[11–14]. Thus, specific quantitative determination of α-L-(1→3)-fucosyltransferase would greatly facilitate the monitoring of differentiation-dependent alteration in cell surfaces[15]. However, most of the substrates commonly employed for this determination acts as acceptors for more than one fucosyltransferase. Therefore, the availability of compounds capable of acting as acceptors for a single enzyme, even in the presence of other, related enzymes, would be of particular importance. One such compound is 2-acetamido-2-deoxy-4-*O*-(2-*O*-methyl-β-D-galactopyranosyl)-D-glucopyranose (*N*-acetyl-2′-*O*-methyllactosamine) (**12**). Its use as a specific acceptor for α-L-(1→3)-fucosyltransferase was reported and its synthesis briefly described[15]. However, in this synthetic procedure, a substantial proportion of the undesired α-D-(1→4)-linked disaccharide was produced and, for this reason, an alternative synthesis that would circumvent this drawback was investigated. We describe herein, in more detail, the aforementioned synthesis, and an alternative route to *N*-acetyl-2′-*O*-methyllactosamine (**12**).

RESULTS AND DISCUSSIONS

Methyl 6-*O*-acetyl-3,4-*O*-isopropylidene-β-D-galactopyranoside[16] (**1**) was treated with methyl iodide–silver oxide in *N*,*N*-dimethylformamide to afford a 2-methyl ether intermediate, the acetal group of which was cleaved in hot, 80% aqueous acetic acid to give crude methyl 6-*O*-acetyl-2-*O*-methyl-β-D-galactopyranoside (**2**). A small portion of **2** was *O*-deacetylated in methanolic sodium methoxide to furnish known[17] methyl 2-*O*-methyl-β-D-galactopyranoside (**3**). The remainder was converted, without prior purification, into the corresponding triacetate which was subjected to acetolysis in acetic anhydride containing ~1% (v/v) conc. sulfuric acid





1 R^1 = OMe, R^2 = R^3 = H, R^4, R^4 = CMe_2, R^5 = Ac
2 R^1 = OMe, R^2 = R^4 = H, R^3 = Me, R^5 = Ac
3 R^1 = OMe, R^2 = R^4 = R^5 = H, R^3 = Me
4 R^1 = OMe, R^2 = H, R^3 = Me, R^4 = R^5 = Ac
5 R^1, R^2 = H, OAc, R^3 = Me, R^4 = R^5 = Ac
6 R^1 = H, R^2 = Br, R^3 = Me, R^4 = R^5 = Ac

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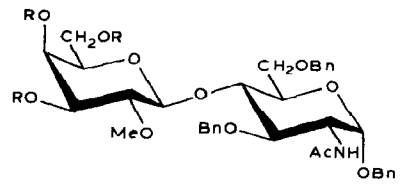
TABLE I

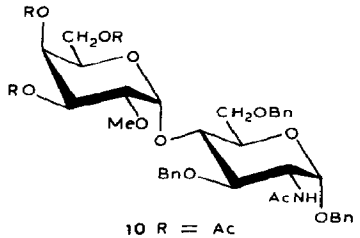
PROPOSED ATTRIBUTION OF ^{13}C-N.M.R.-CHEMICAL SHIFTS[a]

Compound	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*C-1'*	*C-2'*	*C-3'*	*C-4'*	*C-5'*	*C-6'*	*NAc*	*OMe*
GlcNAc[b]	90.33	54.12	70.96	70.27	71.80	60.96							22.52	
3,6-*O*-Bn-α-D-Glc*p*NAcOBn[c]	96.20	52.26	79.73	68.03	73.56	69.25							22.40	
9	96.11	52.06	77.52	76.59	70.64	67.97	102.63	81.55	72.08	67.56	74.80	60.07	22.37	59.58
11	96.02	52.07	72.68	77.71	69.02	69.36	96.50	79.82	68.23	68.66	69.97	60.33	22.40	58.61
12	90.10	53.59	70.01	80.83	68.14	60.18	102.97	81.24	72.34	68.14	75.18	59.95	22.45	59.79
13	90.10[d]	53.73	68.91	77.34	71.40	60.52	96.99[d]	78.58	68.53	69.94	70.49	60.52	22.28	58.09

[a]For solutions in di($^2H_3$)methyl sulfoxide, with Me_4Si as the internal standard. The resonances for benzyl, carbonyl, and aromatic carbon atoms are not shown. [b]2-Acetamido-2-deoxy-α-D-glucopyranose. [c]Benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside. [d]Two additional resonances with substantially reduced intensities, at δ 96.53 and 95.06, apparently due to the portion of the compound having the β configuration at the 2-acetamido-2-deoxy-D-glucopyranose residue.



8 R = Ac
9 R = H



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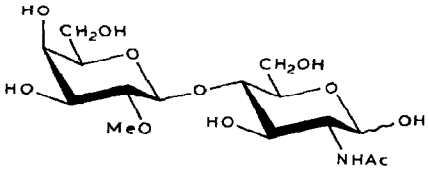
11 R = H



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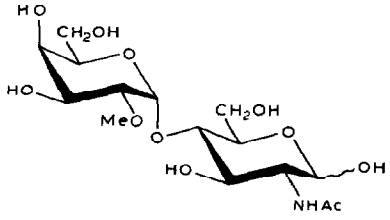

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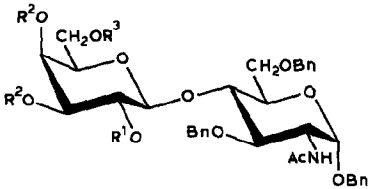
to afford 1,3,4,6-tetra-*O*-acetyl-2-*O*-methyl-D-galactopyranose (**5**). The α-D anomer of **5** was previously obtained by methylation of 1,3,4,6-tetra-*O*-acetyl-α-D-galactopyranose with diazomethane–borontrifluoride etherate[18]. Treatment of **5** with a saturated solution of hydrogen bromide in dichloromethane gave 3,4,6-tri-*O*-acetyl-2-*O*-methyl-α-D-galactopyranosyl bromide (**6**) as a crystalline solid. The ^{1}H-n.m.r. spectrum of **6** showed a lower-field doublet at δ 6.55 (1 H, $J_{1,2}$ 4 Hz), strongly suggesting that it existed almost exclusively as the α-D anomer.

Glycosylation of benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**7**) with bromide **6** in acetonitrile, in the presence of powdered mercuric cyanide, and separation of the mixture by column chromatography on silica gel, gave, in almost equal proportions, benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-methyl-β-D-galactopyranosyl)-α-D-glucopyranoside (**8**) and benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-methyl-α-D-galactopyranosyl)-α-D-glucopyranoside (**10**). Compounds **8** and **10** were identified as the β-D and α-D anomers of the fully protected disaccharide derivatives, respectively, after *O*-deacetylation and comparison of the ^{13}C-n.m.r. spectra of the resulting compounds. Thus, *O*-deacetylation of **8** in 0.1M methanolic sodium methoxide gave, in 85% yield, the *O*-deacetylated derivative **9**, the ^{1}H-n.m.r. spectrum of which contained signals in support of the overall structure expected. In the ^{13}C-n.m.r. spectrum of **9** (see Table I), the signals for C-1 (δ 96.11), and for C-1′ (δ 102.63) were indicative of an α-D and a β-D configuration at the glycosidic linkages, respectively. On similar *O*-deacetylation, compound **10** furnished, in ~84% yield, the correspoding disaccharide derivative **11**, the ^{1}H-n.m.r. spectrum of which was, also, in accord with the overall structure expected. However, in contrast to that of **9**, the ^{13}C-n.m.r. spectrum of **11** contained signals

at δ 96.02 and 96.50 for C-1 and C-1′, respectively, concurring with α-D configurations at both of the glycosidic linkages.

Hydrogenolytic cleavage of the benzyl groups of **9** and **11** furnished, respectively, 2-acetamido-2-deoxy-4-*O*-(2-*O*-methyl-β-D-galactopyranosyl)-D-glucopyranose (*N*-acetyl-2′-*O*-methyllactosamine; **12**), and 2-acetamido-2-deoxy-4-*O*-(2-*O*-methyl-α-D-galactopyranosyl)-D-glucopyranose (**13**). The ^{13}C-n.m.r. spectra of both **12** and **13** were consistent with the structures assigned. In the ^{13}C-n.m.r. spectrum of **12**, the resonance for C-1′ was observed at δ 102.97, in agreement with a β-D configuration at the interglycosidic linkage. By contrast, the resonance for C-1′ in the ^{13}C-n.m.r. of **13** occurred at δ 96.99, indicating an α-D configuration at the interglycosidic linkage. In the spectra of both **12** and **13**, C-1 resonated at δ 90.10, suggesting a preponderantly α-D configuration at C-1 of the 2-acetamido-2-deoxy-D-glucopyranose residue. However, in the spectrum of **13**, the two C-1 resonances were observed at δ 96.53 and 95.06, with substantially reduced intensity, apparently accounting for the portion of the compound having a β-D configuration at C-1 of the 2-acetamido-2-deoxy-D-glucopyranose residue.



14 $R^1 = R^2 = R^3 = H$
15 $R^1 = R^2 = H, R^3 = Bu^tPh_2Si$
16 $R^1 = H, R^2, R^2 = CMe_2, R^3 = Bu^tPh_2Si$
17 $R^1 = Me, R^2, R^2 = CMe_2, R^3 = Bu^tPh_2Si$
18 $R^1 = Me, R^2, R^2 = CMe_2, R^3 = H$

In the aforementioned synthesis of *N*-acetyl-2′-*O*-methyllactosamine, the use of a glycosyl donor having a "non-participating" group at O-2 produced a substantial amount of the undesired α-D-linked disaccharide. Therefore, it seemed appropriate to attempt an alternative synthesis that would alleviate this problem. It was envisaged, for example, that a methyl group could be introduced at O-2′ of a suitably protected derivative of 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-D-glucopyranose. Thus, when benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-β-D-galactopyranosyl-α-D-glucopyranoside[20] (**14**) was allowed to react with *tert*-butyldiphenylchlorosilane in *N*,*N*-dimethylformamide, in the presence of imidazole, the 6′-*O*-silylated derivative **15** was obtained, in 89% yield, as an analytically pure foam. Compound **15** was converted, in ~76% yield, into its 3′,4′-*O*-isopropylidene derivative **16** by treatment with 2,2-dimethoxypropane in acetone, in the presence of *p*-toluenesulfonic acid monohydrate. Methylation of **16**, in a manner analogous to that described for **1** (to give **3**), followed by column chromatography on silica gel

gave, in ~71% yield, the intermediate **17**, which was slightly contaminated (t.l.c. solvent *E*) with unchanged **16**. Removal of the *tert*-butyldiphenylsilyl group of **17** with M tetrabutylammonium fluoride in oxolane, followed by cleavage of the acetal group of the resulting intermediate (**18**) in hot, 80% aqueous acetic acid afforded **9**, which was identical (specific rotation and chromatographic mobility in solvent *C*) with an authentic sample obtained by *O*-deacetylation of **8**. Acetylation of this compound with 1:2 acetic anhydride–pyridine produced a crystalline material which had a m.p. and chromatographic mobility (solvent *A*) identical with those of a sample of **8**, obtained by the condensation of **7** with bromide **6**. Also, the specific rotations of both compounds were in good agreement.

EXPERIMENTAL

General methods. — Melting points were determined with a Fisher–Johns apparatus and are uncorrected. Optical rotation was measured at 25–27° with a Perkin–Elmer 241 polarimeter. N.m.r. spectra were recorded at ~25°, unless otherwise indicated, with a Varian XL-100 instrument, ^{1}H-n.m.r. spectra at 100 MHz, and ^{13}C-n.m.r. spectra at 25.2 MHz in the F.t. mode; the positions of the peaks 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. The silica gel used for column chromatography was Baker Analyzed (60–200 mesh). The following solvent systems (v/v) were used for chromatography: (*A*) 10:1 ether–hexane, (*B*) 4:1 chloroform–acetone, (*C*), 1:1 chloroform–acetone, (*D*) 6:1 chloroform–acetone, and (*E*) 2:3 ethyl acetate–hexane. Organic solutions were generally dried with anhydrous Na_2SO_4. Pyridine was distilled and stored over KOH. Ag_2O was prepared by the method of Helferich and Klein[20]. Elemental analyses were performed by Robertson Laboratory, Florham Park, New Jersey, U.S.A.

Methyl 2-O-methyl-β-D-galactopyranoside (**3**). — A mixture of methyl 6-*O*-acetyl-3,4-*O*-isopropylidene-β-D-galactopyranoside[16] (**1**; 6.9 g), freshly prepared[20] Ag_2O (8 g), and methyl iodide (8 mL), in *N*,*N*-dimethylformamide (40 mL) was stirred for 16 h at room temperature. T.l.c. (1:1 ethyl acetate–hexane) then showed the presence of a product faster-migrating than **1** and a trace of **1**. The *N*,*N*-dimethylformamide was evaporated under diminished pressure and the residue taken up in chloroform. The suspended solid was filtered off and thoroughly washed with chloroform. The filtrate and washings were combined and successively washed with water, dilute $Na_2S_2O_3$ solution, and water, dried, and evaporated to a syrup (~6.2 g), which was dissolved in 80% aqueous acetic acid and heated for 0.5 h at ~98°. The acetic acid was evaporated under reduced pressure, the last traces being removed by co-evaporation with several added portions of toluene to give crude methyl 6-*O*-acetyl-2-*O*-methyl-β-D-galactopyranoside (**2**) as a solid which was used without purification in the next step.

A portion of crude **2** (0.28) was *O*-deacetylated in 0.1M sodium methoxide in methanol (10 mL) to afford, after recrystallization from ethyl acetate, known[17] methyl 2-*O*-methyl-β-D-galactopyranoside (**3**), needles, m.p. 132–134°, $[\alpha]_D^{27}$ +0.3° (*c* 7.25, water); lit.[17] m.p. 131–132°, $[\alpha]_D^{17}$ +1.69° (*c* 11.25, water).

1,3,4,6-Tetra-O-*acetyl-2*-O-*methyl*-D-*galactopyranose* (**5**). — A solution of crude **2** (6 g) in pyridine (60 mL) and acetic anhydride (30 mL) was kept overnight at room temperature. The pyridine and acetic anhydride were evaporated under diminished pressure and several portions of toluene were added to, and evaporated from the residue, which was then thoroughly dried *in vacuo*, dissolved in acetic anhydride (100 mL) containing ~1% (v/v) concentrated H_2SO_4, and stirred for 6 h at room temperature. The mixture was then diluted with dichloromethane (400 mL), successively washed with cold water, cold saturated $NaHCO_3$, and water, dried, and evaporated to give **5** as a solid residue which was directly utilized in the next step.

A small portion of crude **5** was recrystallized from dichloromethane–ether–hexane to give a compound, m.p. 99–102°, $[\alpha]_D^{25}$ +121.4° (*c* 1.8, chloroform); lit.[18] (α-D anomer), m.p. 101–102°, $[\alpha]_D^{20}$ +98° (*c* 1, chloroform).

Anal. Calc. for $C_{15}H_{22}O_{10}$: C, 49.44; H, 6.10. Found: C, 49.59; H, 6.34.

3,4,6-Tri-O-*acetyl-2*-O-*methyl*-α-D-*galactopyranosyl bromide* (**6**). — To a cold (~0°) and stirred solution of crude **5** (6 g) in dichloromethane (100 mL) was added a saturated solution of HBr in dichloromethane (150 mL), dropwise, and the stirring continued for a total of 3 h. The mixture was then diluted with an equal volume of dichloromethane and successively washed with ice-cold water, cold saturated aqueous $NaHCO_3$, and water, dried, and evaporated. The residue crystallized from dichloromethane–ether–hexane to afford **6**, needles, m.p. 135–137°, $[\alpha]_D^{25}$ +206° (*c* 1.8, chloroform); ^{1}H-n.m.r. (90 MHz, $CDCl_3$): δ 6.55 (d, ~1 H, $J_{1,2}$ 4 Hz, H-1), 3.40 (s, 3 H, OMe), and 2.15–2.00 (3 × s, 9 H, OAc).

Benzyl 2-acetamido-3,6-di-O-*benzyl-2-deoxy-4*-O-[*3,4,6-tri*-O-*acetyl-2*-O-*methyl*-β- (**8**) *and* -α-D-*galactopyranosyl*]-α-D-*glucopyranoside* (**10**). — A mixture of **7** (3.43 g, 7 mmol), bromide **6** (4.03 g, 10.6 mmol), and powdered mercuric cyanide (1.78 g, 7 mmol) in acetonitrile (90 mL) was stirred for 5 h at room temperature. T.l.c. (solvent *A*) then showed the presence of two products in almost equal proportions and a trace of **7**. The acetonitrile was evaporated under diminished pressure, the residue taken up in chloroform, and the solids were filtered off through a bed of glass wool. The chloroform solution was successively washed with water, aqueous 10% KI solution, saturated aqueous $NaHCO_3$, and water, dried, and concentrated. The concentrate was applied to a column of silica gel and eluted first with 2:1 and then 4:1 (v/v) ether–hexane. The earlier fractions collected (1.3 g)* contained (t.l.c. solvent *A*) a little unchanged **7**, the faster-migrating α-D

*O-Deacetylation of this fraction (1.3 g) in methanolic sodium methoxide, followed by column chromatography with solvent *B*, and then with solvent *C* as the eluant, gave **7** (0.3 g) and **11** (see later) (0.4 g, equivalent to 0.5 g of **10** for a total yield of 37.9%).

anomer **10** (see later) and also some faster-migratin contaminants (presumably resulting from the decomposition of **6**). On evaporation of the solvent, the second fraction to be eluted gave **10** (1.6 g, 28.9%), amorphous, $[\alpha]_D^{25}$ +145.9° (*c* 0.62, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.50–7.20 (m, 15 H, arom.), 3.36 (s, 3 H, OMe), and 2.12, 2.03, 1.96, and 1.72 (s, 12 H, 3 OAc and NAc).

Anal. Calc. for $C_{42}H_{51}NO_{14}$: C, 63.53; H, 6.49; N, 1.76. Found: C, 63.22; H, 6.70; N, 1.80.

The last fraction that emerged from the column was pure (t.l.c. solvent *A*) **8** (2.2 g, 39.7%) which was crystallized from ethyl acetate–hexane, m.p. 136–138°, $[\alpha]_D^{25}$ +77.6° (*c* 0.67, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.50–7.20 (m, 15 H, arom.), 3.48 (s, 3 H, OMe), and 2.10, 2.04, 1.96, and 1.80 (s, 12 H, 3 OAc and NAc).

Anal. Calc. for $C_{42}H_{51}NO_{14}$: C, 63.53; H, 6.49; N, 1.76. Found: C, 63.41; H, 6.75; N, 1.66.

Benzyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-4-O-(2-O-methyl-β-D-galactopyranosyl)-α-D-galactopyranoside (**9**). — A solution of **8** (1.2 g) in 0.1M methanolic sodium methoxide (40 mL) was stirred for 1 h at room temperature. T.l.c. (solvent *C*) showed the disappearance of **8** and the presence of a single, slower-migrating product. The base was neutralized with a few drops of glacial acetic acid, the solvent evaporated under diminished pressure, and then several portions of toluene were added to, and evaporated from the residue, which was dissolved in chloroform. The solution was washed with water, dried, and evaporated, and the residue dissolved in a small volume of dichloromethane. Addition of ether–hexane precipitated **9** (0.85 g, 85%), amorphous, $[\alpha]_D^{25}$ +102.4° (*c* 1.4, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.50–7.20 (m, 15 H, arom.), 3.56 (s, 3 H, OMe), and 1.80 (s, 3 H, NAc).

Anal. Calc. for $C_{36}H_{42}NO_{11}$: C, 65.04; H, 6.38; N, 2.11. Found: C, 65.24; H, 6.66; N, 2.13.

Benzyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-4-O-(2-O-methyl-α-D-galactopyranosyl)-α-D-glucopyranoside (**11**). — Compound **10** was *O*-deacetylated in methanolic sodium methoxide as described for **8** (to give **9**) to afford amorphous **11** (0.95 g, 84.1%), $[\alpha]_D^{25}$ +155.3° (*c* 1.1, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.50–7.20 (m, 15 H, arom.), 3.40 (s, 3 H, OMe), and 1.80 (s, 3 H, NAc).

Anal. Calc. for $C_{36}H_{42}NO_{11} \cdot 0.5\ H_2O$: C, 64.16; H, 6.45; N, 2.08. Found: C, 63.90; H, 6.77; N, 2.17.

2-Acetamido-2-deoxy-4-O-(2-O-methyl-β-D-galactopyranosyl)-D-glucopyranose (**12**). — A mixture of **9** (0.7 g) and 10% Pd–C (0.7 g), in glacial acetic acid (35 mL), was shaken under H_2 at ~345 kPa for 3 days at room temperature. The suspension was filtered off (a bed of Celite), the solids were thoroughly washed with glacial acetic acid, and the filtrate and washings combined and evaporated. The residue crystallized from aqueous alcohol to furnish **12** (0.35 g, 83.3%), m.p. 226–228°, $[\alpha]_D^{25}$ +47.9 (initial) →34.9° (16 h; *c* 1.1, water); for ^{13}C-n.m.r. data, see Table I.

Anal. Calc. for $C_{15}H_{27}NO_{11}$: C, 45.33; H, 6.86; N, 3.53. Found: C, 45.17; H, 6.91; N, 2.48.

*2-Acetamido-2-deoxy-4-*O*-(2-*O*-methyl-α-*D*-galactopyranosyl)-*D*-glucopyranose* (**13**). — Compound **11** (0.6 g) was hydrogenolyzed as described for **9** (to give **12**), to afford, after recrystallization from ethanol–ethyl acetate, **13** (0.32 g, 88.9%), m.p. 198–200°, $[\alpha]_D^{25}$ +147.9 (initial) →137.5° (16 h; *c* 1.4, water); for ^{13}C-n.m.r. data, see Table I.

Anal. Calc. for $C_{15}H_{27}NO_{11}$: C, 45.33; H, 6.86; N, 3.53. Found: C, 45.09; H, 6.95; N, 3.30.

*Benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(6-*O*-tert-butyldiphenylsilyl-β-*D*-galactopyranosyl)-α-*D*-glucopyranoside* (**15**). — A mixture of **14** (4 g, 6 mmol), imidazole (1.32 g, 19.2 mmol), and *tert*-butyldiphenylchlorosilane (2.64 g, 9.6 mmol) in *N,N*-dimethylformamide (35 mL) was stirred for 3 h at room temperature. T.l.c. (solvent *B*) showed the presence of a major product, faster-migrating than **14**, and a trace of a faster-migrating contaminant. The mixture was poured into ice–water and extracted with chloroform. The chloroform solution was washed with water, saturated aqueous $NaHCO_3$, and water, dried, and concentrated. The concentrate was applied to a column of silica gel, and elution with solvent *D* and evaporation of the fractions corresponding to the product afforded **15** (4.84 g, 89%), foam, $[\alpha]_D^{27}$ +76.6° (*c* 1.1, chloroform); ^{1}H-n.m.r. (90 MHz, $CDCl_3$): δ 7.80–7.10 (m, 25 H, aromatic), 1.70 (s, 3 H, NAc), and 1.03 (s, 9 H, CMe_3).

Anal. Calc. for $C_{51}H_{61}NO_{11}Si$: C, 68.65; H, 6.90; N, 1.57. Found: C, 68.51; H, 6.73; N, 1.34.

*Benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(6-*O*-tert-butyldiphenylsilyl-3,4-*O*-isopropylidene-β-*D*-galactopyranosyl)-α-*D*-glucopyranoside* (**16**). — A mixture of **15** (4.8 g) and *p*-toluenesulfonic acid monohydrate (1 g) in acetone (75 mL) and 2,2-dimethoxypropane (75 mL) was stirred for 1 h at room temperature. T.l.c. (solvent *E*) revealed the presence of a major product, faster-migrating than **15**, and a trace of a faster-migrating contaminant. The acid was neutralized by the dropwise addition of triethylamine, the solvents were evaporated *in vacuo*, and the residue was subjected to column chromatography on silica gel with solvent *E* as the eluant. On evaporation of the solvent, the first fraction to be eluted (0.98 g) contained (t.l.c., solvent *E*) mainly **16**, together with a small proportion of a faster-migrating contaminant. Continued elution with solvent *E* then gave chromatographically pure **16** (3.8 g, 76.2%), amorphous, $[\alpha]_D^{27}$ +81.6° (*c* 1.4, chloroform); ^{1}H-n.m.r. (90 MHz, $CDCl_3$): δ 7.80–7.10 (m, 25 H, arom.), 1.70 (s, 3 H, NAc), 1.43 and 1.26 (s, 6 H, CMe_2), and 1.03 (s, 9 H, CMe_3).

Anal. Calc. for $C_{54}H_{65}NO_{11}Si$: C, 69.56; H, 7.04; N, 1.50. Found: C, 69.61; H, 7.05; N, 1.54.

*Benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(3,4-*O*-isopropylidene-2-*O*-methyl-β-*D*-galactopyranosyl)-α-*D*-glucopyranoside* (**18**). — A mixture of **16** (0.9 g), Ag_2O (2 g), and methyl iodide (2 mL) in *N,N*-dimethylformamide (15 mL) was stirred for 16 h at room temperature. The solids were removed by filtration and

thoroughly washed with *N,N*-dimethylformamide, and the filtrate and washings combined and evaporated under reduced pressure. The residue was stirred in chloroform, and the precipitated silver salt filtered off and washed with chloroform. The chloroform solution was washed with water, aqueous $Na_2S_2O_3$ solution, and water, dried, and concentrated. T.l.c. (solvent *E*) showed the presence of a major product, faster-migrating than **16**, a trace of **16**, and a small proportion of a faster-migrating contaminant. The mixture was purified in a column of silica gel with 1:3 (v/v) ethyl acetate–hexane as the eluant to give **17** (0.65 g, 70.6%), which was slightly contaminated (t.l.c., solvent *E*) with unchanged **16**. A solution of crude **17** (0.65 g) in dry oxolane (10 mL) was stirred for 3 h at room temperature in the presence of M tetrabutylammonium fluoride (1 mL). The mixture was evaporated, the residue dissolved in chloroform, and the solution applied to a column of silica gel. Elution with solvent *D* and evaporation of the fractions corresponding to the product gave **18** (0.35 g, 71.4%), amorphous, $[\alpha]_D^{27}$ +103.9° (*c* 0.9, chloroform); ^{1}H-n.m.r. (90 MHz, $CDCl_3$): δ 7.20 (br. s, 15 H, arom.), 3.40 (s, 3 H, OMe), 1.66 (s, 3 H, NAc), and 1.40 and 1.30 (s, 6 H, CMe_2).

Anal. Calc. for $C_{39}H_{49}NO_{11} \cdot 0.5\ H_2O$: C, 65.34; H, 7.04; N, 1.91. Found: C, 65.18; H, 6.95; N, 1.91.

Deacetalation and peracetylation of **18**. — Compound **18** (0.28 g) in 80% aqueous acetic acid (10 mL) was stirred for 1 h at ~98°. Acetic acid was evaporated under diminished pressure, the last traces being removed by co-evaporation with several portions of toluene to give a solid, which was dissolved in a small volume of dichloromethane. Addition of ether–hexane precipitated **9** (0.21 g, 87.5%), amorphous, $[\alpha]_D^{27}$ +102° (*c* 1.3, chloroform); it had identical chromatographic mobility (solvent *C*) with an authentic sample of **9** (obtained by *O*-deacetylation of **8**).

A portion (0.18 g) of this compound was dissolved in 2:1 pyridine–acetic anhydride (8 mL) and kept overnight at room temperature. The pyridine and acetic anhydride were evaporated under reduced pressure, and the residue was co-evaporated with several added portions of toluene. Crystallization from ethyl acetate–hexane gave **8** (0.17 g, 80.9%), m.p. 136–138°, $[\alpha]_D^{27}$ +74.5° (*c* 0.73, chloroform); it had a chromatographic mobility (solvent *A*) identical with that of an authentic sample obtained by the condensation of **7** with **6**.

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REFERENCES

1 R. DUBEY, R. K. JAIN, S. A. ABBAS, AND K. L. MATTA, *Carbohydr. Res.*, 165 (1987) 189–196.
2 T. H. BEYER, J. E. SADLER, J. J. REARICK, J. C. PAULSON, AND R. L. HILL, *Adv. Enzymol.*, 52 (1982) 23–176.

3 L. SHEN, E. J. GROLMAN, AND V. GINSBURG, *Proc. Natl. Acad. Sci. U.S.A.*, 59 (1968) 224–230.
4 M. A. CHESTER AND W. M. WATKINS, *Biochem. Biophys. Res. Commun.*, 34 (1969) 835–842.
5 E. H. HOLMES AND S. HAKOMORI, *J. Biol. Chem.*, 257 (1982) 7698–7703.
6 E. H. HOLMES AND S. HAKOMORI, *J. Biol. Chem.*, 258 (1983) 3706–3713.
7 H. SCHENKEL-BRUNNER, M. A. CHESTER, AND W. M. WATKINS, *Eur. J. Biochem.*, 30 (1972) 269–277.
8 T. PACUSZKA AND J. KOSCIELAK, *FEBS Lett.*, 41 (1974) 348–351.
9 S. YAZAWA AND K. FURUKAWA, *J. Immunogenet.*, 7 (1980) 137–148.
10 I. JABBAL AND H. SCHACHTER, *J. Biol. Chem.*, 246 (1971) 5154–6161.
11 S. HAKOMORI, *Annu. Rev. Immunol.*, 2 (1984) 103–126.
12 S. HAKOMORI, E. NUDELMAN, S. B. LEVERY, AND R. KANNAGI, *J. Biol. Chem.*, 259 (1984) 4672–4680.
13 Y. FUKUSHI, S. HAKOMORI, E. NUDELMAN, AND N. COCHRAN, *J. Biol. Chem.*, 259 (1984) 4681–4685.
14 S. B. LEVERY, E. D. NUDELMAN, N. H. ANDERSEN, AND S. HAKOMORI, *Carbohydr. Res.*, 151 (1986) 311–328.
15 R. MADIYALAKAN, S. YAZAWA, S. A. ABBAS, J. J. BARLOW, AND K. L. MATTA, *Anal. Biochem.*, 152 (1986) 22–28.
16 S. S. RANA, J. J. BARLOW, AND K. L. MATTA, *Tetrahedron Lett.*, 22 (1981) 5007–5010.
17 J. W. H. OLDHAM AND D. J. BELL, *J. Chem. Soc.*, 60 (1938) 323–325.
18 I. O. MASTRONARI, S. M. FLEMATTI, J. O. DEFERRARI, AND E. D. GROS, *Carbohydr. Res.*, 3 (1966) 177–183.
19 A. VEYRIÈRES, *J. Chem. Soc., Perkin Trans. 1*, (1981) 1626–1629.
20 B. HELFERICH AND W. KLEIN, *Justus Liebigs Ann. Chem.*, 450 (1926) 219–229.