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# Note

## Synthesis of methyl *O*-(2-*O*-methyl-β-D-galactopyranosyl)-(1→4)-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-β-D-galactopyranoside and 4-nitrophenyl *O*-β-D-galactopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-β-D-galactopyranoside*

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Recent years have seen a remarkable surge of interest in the study of α-L-fucosyltransferases. Of these L-fucosyltransferases, the enzyme (1→3)-α-L-fucosyltransferase has attracted a great deal of clinical interest as a potential tumor marker. This enzyme catalyzes the transfer of an L-fucosyl group from GDP-L-fucose to O-3 of 2-acetamido-2-deoxy-D-glucose or D-glucose, and exhibits a strict specificity for acceptors having the nonreducing terminal sequence β-D-Gal*p*-(1→4)-D-Glc*p*NAc or -D-(Glc*p*)[2]. In recent studies, it was suggested that this enzyme is responsible for the unusually high accumulation of polyfucosylated, repeated N-acetyllactosamine chains that are found as part of glycolipids in a variety of human cancers[3].

Based on specificity for different acceptor-substrates and differences in biochemical properties between enzymes from different sources, at least seven (1→3)-α-L-fucosyltransferases are known[4]. To simplify the assay procedures for the specific quantitative determination of individual L-fucosyltransferases, we have embarked on a synthetic program to obtain compounds capable of acting as acceptors for a single enzyme, even in the presence of other, related enzymes. We have recently successfully applied this approach to the assay of (1→3)-α-L-fucosyltransferase from human serum[5] by use of our synthetic acceptor substrate, 2-acetamido-2-deoxy-4-*O*-(2-*O*-methyl-β-D-galactopyranosyl)-D-glucopyranoside (2′-*O*-methyl-*N*-acetyllactosamine)[5,6]. This same compound has also proven to be an excellent instrument in a number of clinical

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investigations for the specific assay of this enzyme activity in the sera and saliva of patients with various cancers[7–10]. As a further contribution to these investigations and in order to conduct further substrate specificity studies on (1→3)-α-L-fucosyltransferases, we report the synthesis of methyl *O*-(2-*O*-methyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-β-D-galactopyranoside (**7**). In addition, we also report the synthesis of a related trisaccharide, 4-nitrophenyl *O*-β-D-galactopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-β-D-galactopyranoside (**13**), which is primarily required for use in immunological studies. As we have shown in our previous publications[11], such compounds can be employed as synthetic or artificial antigens, after reduction of their nitro groups and subsequent coupling of the resulting amino groups (as their diazonium salts) to a protein. To a large extent our interest in procuring such compounds is enhanced by the increasing number of carbohydrate structures of glycoproteins and glycolipids that are being recognized as tumor-associated antigens[12–15].

Methyl *O*-β-D-galactopyranosyl-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside[16] (**1**) was treated with *tert*-butylchlorodiphenylsilane in *N,N*-dimethylformamide in the presence of imidazole to afford the 6″-*O*-silylated derivative **2** in 75% yield. This was converted, in 63% yield, into the 3″,4″-*O*-isopropylidene derivative **3** by treatment with 2,2-dimethoxypropane in acetone. Trisaccharide **3** was methylated with methyl iodide silver oxide[17] in 1:1 dichloromethane-*N,N*-dimethylformamide to afford, in 68% yield, after column chromatographic purification, the 2″-*O*-methylated derivative **4**, the $^1$H-n.m.r. spectrum of which exhibited the appropriate signals diagnostic of its identity. The use of a mixed-solvent system and silver oxide for this methylation reaction appeared to be far superior to the use of *N,N*-dimethylformamide as solvent and sodium hydride as base. The proportion of faster-migrating (t.l.c., solvent *B*) contaminants (presumably due to *N*- and *O*-methylation of the acetamido group) previously encountered in an analogous reaction[18] was substantially reduced.

Removal of the *tert*-butyldiphenylsilyl group of **4** was readily accomplished by treatment with tetrabutylammonium fluoride in oxolane to give **5**. Cleavage of the acetal group of **5** gave compound **6** which was in turn converted into **7** in 80% yield by catalytic hydrogenolysis. The $^{13}$C-n.m.r. spectrum of **7** was in accord with the structure assigned (see Table I).

4-Nitrophenyl *O*-β-D-galactopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-β-D-galactopyranoside (**13**) was obtained in five, good-yielding steps from methyl *O*-β-D-galactopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-β-D-galactopyranoside[16] (**8**). Thus, acetylation of **8** afforded in high yield the trisaccharide peracetate **9** as an amorphous solid, the $^1$H-n.m.r. spectrum of which was in agreement with its overall structure. Acetolysis of **9** furnished, in 77% yield, *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-1,2,4,6-tetra-*O*-acetyl-D-galactopyranose (**10**). In its $^1$H-n.m.r. spectrum a low-field doublet at $\delta$ 6.23 ($\sim$1 H$^*$, J$\sim$5 Hz) suggested that it existed almost exclusively as the α-D anomer. A similar observation was

TABLE I

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

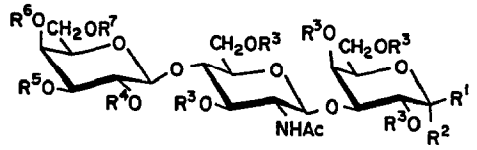
| *Residue or group* | *Compd.* | *Solvent* | *C-1* | *C-2* | *C-3* | *C-4* | *C-5* | *C-6* | *$OCH_3$* | *$CH_3CO$* |
|---|---|---|---|---|---|---|---|---|---|---|
| β-D-Gal*p*OMe | **8** | $D_2O$ | 106.69 | 72.52 | 85.10 | 71.16 | 77.39 | 62.74 | 60.00 | |
| β-D-Glc*p*NAc-(1→3) | | | 105.35 | 58.07 | 75.04 | 81.11 | 78.16 | 63.81 | | 24.99 |
| β-D-Gal*p*-(1→4) | | | 105.69 | 73.78 | 75.35 | 71.37 | 77.49 | 63.71 | | |
| β-D-Gal*p*OMe | **7** | $D_2O$ | 106.68 | 72.53 | 85.08 | 71.15 | 77.48 | 63.39 | 59.99 | |
| β-D-Glc*p*NAc-(1→3) | | | 105.32 | 58.11 | 75.04 | 81.11 | 78.02 | 63.77 | | 25.03 |
| 2-*O*-Me-β-D-Gal*p*-(1→4) | | | 105.39 | 83.71 | 74.97 | 71.42 | 77.56 | 63.70 | 62.73 | |
| β-D-Gal*p*$OC_6H_4NO_2$(4) | [b] | $(CD_3)_2SO$ | 99.90 | 68.80 | 81.55 | 66.88 | 75.25 | 60.03 | | |
| β-D-Glc*p*NAc-(1→3) | | | 101.89 | 56.17 | 74.01 | 70.25 | 76.56 | 60.57 | | 22.96 |
| β-D-Gal*p*OMe | **8**[c] | $(CD_3)_2SO$ | 104.00 | 69.27 | 82.39 | 67.12 | 74.75 | 60.30 | 55.70 | |
| β-D-Glc*p*NAc-(1→3) | | | 102.00 | 55.46 | 73.73 | 81.21 | 75.48 | 60.41 | | 23.02 |
| β-D-Gal*p*-(1→4) | | | 103.92 | 70.51 | 71.97 | 68.09 | 74.75 | 60.30 | | |
| β-D-Gal*p*$OC_6H_4NO_2$(4) | **13** | $(CD_3)_2SO$ | 100.13 | 68.84 | 81.86 | 67.00 | 75.35 | 60.11 | | |
| β-D-Glc*p*NAc-(1→3) | | | 102.06 | 55.41 | 73.12 | 81.25 | 75.47 | 60.40 | | 23.06 |
| β-D-Gal*p*-(1→4) | | | 103.92 | 70.50 | 71.93 | 68.07 | 74.76 | 60.40 | | |

[a] Carbonyl and aromatic resonances are not shown. [b] The chemical shifts for this compound[21] and those for compound **8** are included for comparison purposes. [c] Values taken from ref. 16.

made on acetolysis of a somewhat related compound[19]. Compound **10** was readily converted in high yield into *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-acetyl-α-D-galactopyranosyl bromide (**11**) by treatment with hydrogen bromide in dichloromethane at ~0°. The $^1$H-n.m.r. spectrum of crude **11** contained a doublet at δ 6.62 (~0.76 H*, J~4 Hz) that indicated the presence of the α-D anomer in 76%.

Bromide **11** was treated with Amberlyst A-26-(4-nitrophenoxide) resin[20] in 1:4 dichloromethane–2-propanol for 26 h at room temperature to give, after column-chromatographic purification, 4-nitrophenyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranoside (**12**), which was contaminated with some slightly faster- and slower-migrating impurities that were difficult to remove by column chromatography. It was, therefore, utilized in the next step without further purification.

Zemplén transesterification of **12** furnished trisaccharide **13** in 33% overall yield from **11**; the $^{13}$C-n.m.r. spectrum of **13** was consistent with the structure assigned (see Table I).




1 $R^1$ = OMe, $R^2$=$R^4$=$R^5$=$R^6$=$R^7$=H, $R^3$=Bn
2 $R^1$ = OMe, $R^2$=$R^4$=$R^5$=$R^6$=H, $R^3$=Bn, $R^7$=$Bu^tPh_2Si$
3 $R^1$ = OMe, $R^2$=$R^4$=H, $R^3$=Bn, $R^5$,$R^6$=$CMe_2$, $R^7$=$Bu^tPh_2Si$
4 $R^1$ = OMe, $R^2$=H, $R^3$=Bn, $R^4$=Me, $R^5$,$R^6$=$CMe_2$, $R^7$=$Bu^tPh_2Si$
5 $R^1$ = OMe, $R^2$= $R^7$=H, $R^3$=Bn, $R^4$=Me, $R^5$,$R^6$=$CMe_2$
6 $R^1$ = OMe, $R^2$=$R^5$=$R^6$=$R^7$=H, $R^3$=Bn, $R^4$=Me
7 $R^1$ = OMe, $R^2$=$R^3$=$R^5$=$R^6$=$R^7$=H, $R^4$=Me
8 $R^1$ = OMe, $R^2$=$R^3$=$R^4$=$R^5$=$R^6$=$R^7$=H
9 $R^1$ = OMe, $R^2$=$R^3$=$R^4$=$R^5$=$R^6$=$R^7$=Ac
10 $R^1$=$R^2$=H,OAc, $R^3$=$R^4$=$R^5$=$R^6$=$R^7$=Ac
11 $R^1$=H, $R^2$=Br, $R^3$=$R^4$=$R^5$=$R^6$=$R^7$=Ac
12 $R^1$=$OC_6H_4NO_2$(4), $R^2$=H, $R^3$=$R^4$=$R^5$=$R^6$=$R^7$=Ac
13 $R^1$=$OC_6H_4NO_2$(4), $R^2$=$R^3$=$R^4$=$R^5$=$R^6$=$R^7$=H

*$^{13}$C-N.m.r. assignments.* — The assignments of the $^{13}$C-n.m.r. resonances for **7** were made by comparing its spectrum with that of methyl *O*-β-D-galactopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-β-D-galactopyranoside[16] (**8**) and the $^{13}$C-n.m.r. assignments for **13** were made by comparing its spectrum with those of 4-nitrophenyl 3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-galactopyranoside[21] and **8**, as reported in Table 1. In the $^{13}$C-n.m.r. spectra of the two trisaccharides, the resonances for C-1, C-1′, and C-1″ were all in the region normally expected for β-D-glycosidic linkages. In the $^{13}$C-n.m.r. spectrum of **7**, the carbon atom resonances remained close to those of the respective carbon atoms in the nonmethylated trisaccha-

* Compared to the acetyl-group methyl protons.

ride **8**, except for those carbon atoms affected by alkylation. Thus, in the $^{13}C$-n.m.r. spectrum of **7**, the resonance for C-2″ showed a significant downfield shift of 9.93 p.p.m., by comparison to that of its counterpart in the spectrum of the parent trisaccharide **8**, evidencing that O-2″ was the site of methylation. Meanwhile, the signal for C-3″ was observed at a field higher than that of the respective carbon atom in the parent, nonmethylated trisaccharide **8** (see Table I) owing to the $\beta$ shift invariably observed with alkylation[22].

## 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. T.l.c. was conducted on aluminum sheets, precoated with 0.2-mm layers of Silica Gel 60F-254 (E. Merck, Darmstadt, Germany); the components were located either by exposure to u.v. light or by spraying with 5% $H_2SO_4$ in ethanol (or both) and charring. Silica gel used for column chromatography was Baker Analyzed (600–200 mesh). The following solvent systems (v/v) were used for chromatography: (*A*) 7:3 chloroform–acetone, (*B*) 9:1 chloroform–acetone, (*C*) 5:4:1 chloroform–methanol-water, (*D*) 9:1 chloroform–methanol, and (*E*) 2:1 chloroform–acetone. N.m.r. spectra were recorded at ~25°; $^1H$-n.m.r. spectra either with a Varian EM-390 or Bruker WP-200, and $^{13}C$-n.m.r. spectra either with a Bruker WP-200 or a Bruker AM-400 instrument, at 90, 200, 50.3, and 100.6 MHz, respectively; the chemical shifts ($\delta$) are expressed from the tetramethylsilane signal. Solutions in organic solvents were generally dried with anhydrous $Na_2SO_4$. Dichloromethane and pyridine were dried over 4A molecular sieves and KOH, respectively. Elemental analyses were performed by Robertson Laboratory, 29 Samson Ave., Madison, New Jersey 07940, U.S.A.

*Methyl* O-(6-O-tert-*butyldiphenylsilyl*-β-D-*galactopyranosyl*)-(1→4)-O-(2-*acetamido*-3,6-*di*-O-*benzyl*-2-*deoxy*-β-D-*glucopyranosyl*)-(1→3)-2,4,6-*tri*-O-*benzyl*-β-D-*galactopyranoside* (**2**). — To a cold (0°, bath), stirred solution of **1** (ref. 16; 1.81 g, 1.79 mmol) and imidazole (0.63 g, 9.3 mmol) in *N,N*-dimethylformamide (35 mL) was added *tert*-butylchlorodiphenylsilane (1.1 g, 4 mmol), and stirring was continued for 4 h at ~0°. The mixture was then poured into ice–water and extracted with chloroform. The chloroform solution was successively washed with water, saturated $NaHCO_3$, and water, dried, and concentrated, and the residue applied to a column of silica gel and eluted with a solvent gradient consisting of 0–5% methanol in chloroform. Concentration of fractions corresponding to the product afforded **2** (1.67 g, 75%), amorphous, $[\alpha]_D^{21}$ −4° (*c* 1.1, chloroform), t.l.c. (*A*) $R_F$ 0.20; $^1H$-n.m.r. ($CDCl_3$): $\delta$ 7.82–7.15 (m, 35 H, arom.), 3.45 (s, 3 H, OMe), 1.42 (s, 3 H, NAc), and 1.02 (s, 9 H, $CMe_3$).

*Anal.* Calc. for $C_{72}H_{84}NO_{16}Si$: C, 69.32; H, 6.79; N, 1.12. Found: C, 68.94; H, 6.53; N, 1.07.

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

*4,6-tri-O-benzyl-β-D-galactopyranoside* (**3**). — To a solution of **2** (0.54 g) in dry acetone (30 mL) were added 2,2-dimethoxypropane (30 mL) and 4-toluenesulfonic acid monohydrate (0.1 g). The mixture was stirred for 6 h at room temperature, made neutral by the dropwise addition of triethylamine, and then concentrated. The residue was purified in a column of silica gel by use of a solvent gradient consisting of 0–10% acetone in chloroform to give **3** (0.35 g, 63%), amorphous, $[\alpha]_D^{21}$ + 1° (*c* 1.1, chloroform), t.l.c. (*B*), $R_F$ 0.19; $^1$H-n.m.r. ($CDCl_3$): δ 7.70–7.12 (m, 35 H, arom.), 3.46 (s, 3 H, OMe), 1.48 and 1.33 (s, 3 H each, $CMe_2$), 1.45 (s, 3 H, NAc), and 1.02 (s, 9 H, $CMe_3$).

*Anal.* Calc. for $C_{75}H_{88}NO_{16}Si$: C, 69.96; H, 6.89; N, 1.09. Found: C, 69.97; H, 7.10; N, 1.10.

*Methyl* O-(*6*-O-tert-*butyldiphenylsilyl-3,4*-O-*isopropylidene-2*-O-*methyl-β*-D-*galactopyranosyl*)-(*1→4*)-O-(*2-acetamido-3,6-di*-O-*benzyl-2-deoxy-β*-D-*glucopyranosyl*)-(*1→3*)-*2,4,6-tri*-O-*benzyl-β*-D-*galactopyranoside* (**4**). — A mixture of **3** (0.32 g), freshly prepared[17] $Ag_2O$ (0.64 g), and methyl iodide (0.8 mL) in 1:1 (v/v) *N,N*-dimethylformamide–dichloromethane (10 mL) was stirred in the dark for 16 h at room temperature. The solids were removed by filtration (Celite) and thoroughly washed with dichloromethane. The filtrate and washings were combined and concentrated under diminished pressure to give a residue which was taken up in chloroform. The chloroform solution was successively washed with water, aqueous $Na_2S_2O_3$ solution, and water, dried, and concentrated. T.l.c. (*B*) showed the presence of a major product, faster-migrating than **3**, and a small proportion of a faster-migrating contaminant. The mixture was purified in a column of silica gel with a solvent gradient consisting of 0–5% acetone in chloroform to afford **4** (0.22 g, 68%), amorphous, $[\alpha]_D^{21}$ +0.5° (*c* 0.8, chloroform), t.l.c. (*B*) $R_F$ 0.57; $^1$H-n.m.r. ($CDCl_3$): δ 7.69–7.04 (m, 35 H, arom.), 3.52 (s, 3 H, OMe-1), 3.50 (s, 3 H, OMe-2″), 1.52 and 1.36 (s, 3 H each, $CMe_2$), 1.46 (s, 3 H, NAc), and 1.01 (s, 9 H, $CMe_3$).

*Anal.* Calc. for $C_{76}H_{90}NO_{16}Si$: C, 70.13; H, 6.97; N, 1.08. Found: C, 70.09; H, 6.71; N, 0.94.

*Methyl* O-(*3,4*-O-*isopropylidene-2*-O-*methyl-β*-D-*galactopyranosyl*)-(*1→4*)-O-(*2-acetamido-3,6-di*-O-*benzyl-2-deoxy-β*-D-*glucopyranosyl*)-(*1→3*)-*2,4,6-tri*-O-*benzyl-β*-D-*galactopyranoside* (**5**). — A solution of **4** (0.2 g, 0.15 mmol) in dry oxolane (5 mL) was treated with a M solution of tetrabutylammonium fluoride in oxolane (0.25 mL), and the stirring was continued for 3 h at room temperature. The mixture was concentrated to dryness, and the residue was purified in a column of silica gel by use of a solvent gradient consisting of 0–15% acetone in chloroform to afford **5** (0.16 g, 98%), amorphous, $[\alpha]_D^{21}$ +10° (*c* 0.8, chloroform), t.l.c. (*B*) $R_F$ 0.24; $^1$H-n.m.r. ($CDCl_3$): δ 7.47–7.14 (m, 25 H, arom.), 3.52 (s, 3 H, OMe-1), 3.51 (s, 3 H, OMe-2″), 1.52 and 1.32 (s, 3 H each, $CMe_2$), and 1.49 (s, 3 H, NAc).

*Anal.* Calc. for $C_{60}H_{72}NO_{16}$: C, 67.78; H, 6.83; N, 1.32. Found: C, 67.53; H, 6.42; N, 1.17.

*Methyl* O-(*2*-O-*methyl-β*-D-*galactopyranosyl*)-(*1→4*)-O-(*2-acetamido-3,6-di*-O-*benzyl-2-deoxy-β*-D-*glucopyranosyl*)-(*1→3*)-*2,4,6-tri*-O-*benzyl-β*-D-*galactopyranoside* (**6**). — Compound **5** (0.14 g) in 60% aqueous acetic acid (20 mL) was stirred for 2 h at

~65°. Acetic acid was evaporated under diminished pressure, the last traces being removed by coevaporation with several portions of toluene. The residue was purified in a column of silica gel by use of a solvent gradient consisting of 10–30% acetone in chloroform. On concentration, the fractions corresponding to the product gave a solid which was dissolved in a little dichloromethane. Addition of ether–hexane caused the precipitation of **6** (0.12 g, 89%), amorphous, $[\alpha]_D^{21}$ +5° (*c* 0.6, chloroform), t.l.c. (*A*) $R_F$ 0.11; $^1$H-n.m.r. ($CDCl_3$): $\delta$ 7.44–7.22 (m, 25 H, arom.), 3.55 (s, 3 H, OMe-1), 3.51 (s, 3 H, OMe-2″), and 1.50 (s, 3 H, NAc).

*Anal.* Calc. for $C_{57}H_{68}NO_{16}$: C, 66.91; H, 6.70; N, 1.37. Found: C, 66.53; H, 6.64; N, 1.32.

*Methyl* O-*(2-*O*-methyl-β-*D*-galactopyranosyl)-(1→4)*-O-*(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-(1→3)-β-*D*-galactopyranoside* (**7**). — A mixture of **6** (0.114 g) and 10% Pd–C (0.1 g) in glacial acetic acid (10 mL) was shaken under $H_2$ at ~345 kPa for 2 days at room temperature. The suspension was filtered through a bed of Celite, the solid thoroughly washed with glacial acetic acid, and the filtrate and washings were combined and concentrated under diminished pressure. The residue was applied to a column of silica gel and eluted with a solvent gradient consisting of 0–30% methanol in chloroform. Concentration of the fractions corresponding to the product gave a solid, which was dissolved in water (10 mL) and lyophilized to afford **7** (0.045 g, 80%), amorphous, $[\alpha]_D^{23}$ +6° (*c* 0.95, water), t.l.c. (*C*) $R_F$ 0.27; $^{13}$C-n.m.r. see Table I.

*Anal.* Calc. for $C_{57}H_{68}NO_{16}\cdot 1.5H_2O$: C, 43.99; H, 7.05; N, 2.33. Found: C, 44.02; H, 6.71; N, 1.94.

*Methyl* O-*(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-(1→4)*-O-*(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-acetyl-β-*D*-galactopyranoside* (**9**). — Compound **8** (ref. 16; 1.87 g) was stirred overnight in 1:2 acetic anhydride–pyridine (30 mL) 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 dissolved in a small volume of dichloromethane. Addition of ether–hexane caused the precipitation of **9** (2.74 g, 87%), amorphous, $[\alpha]_D^{26}$ +10° (*c* 1.1, chloroform), t.l.c. (*D*) $R_F$ 0.65; $^1$H-n.m.r. ($CDCl_3$): $\delta$ 3.43 (s, 3 H, OMe) and 2.13–1.87 (cluster of s, 30 H, 9 OAc and NAc).

*Anal.* Calc. for $C_{39}H_{55}NO_{25}$: C, 49.94; H, 5.91; N, 1.49. Found: C, 49.67; H, 5.64; N, 1.33.

O-*(2,3,4,6-Tetra-*O*-acetyl-β-*D*-galactopyranosyl)-(1→4)*-O-*(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-(1→3)-1,2,4,6-tetra-*O*-acetyl-α-*D*-galactopyranose* (**10**). — A solution of **9** (2.65 g) in acetic anhydride (45 mL) containing 0.8% by volume of concentrated $H_2SO_4$ was stirred for 6 h at room temperature. The mixture was then diluted with dichloromethane (300 mL), successively washed with water, saturated $NaHCO_3$, and water, dried, and concentrated to a syrup which was dissolved in a small volume of dichloromethane. Addition of ether–hexane caused the precipitation of **10** (2.09 g, 77%), white amorphous material, $[\alpha]_D^{26}$ +54° (*c* 1.0, chloroform), t.l.c. (*D*) $R_F$ 0.76; $^1$H-n.m.r. ($CDCl_3$): $\delta$ 6.23 (d, ~1 H, J~5 Hz, H-1) and 2.13–1.73 (cluster of s, 33 H, 10 OAc and NAc).

*Anal.* Calc. for $C_{40}H_{55}NO_{26}$: C, 49.74; H, 5.74; N, 1.45. Found: C, 49.55; H, 5.59; N, 1.38.

*O-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-(1→4)-O-(2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-O-acetyl-α-D-galactopyranosyl bromide* (**11**). — To a cold (~0°, bath), stirred solution of **10** (1.3 g) in dry dichloromethane (50 mL) was added dropwise, during 40 min, a saturated solution of HBr in dry dichloromethane (55 mL), and stirring was continued for an additional 1.5 h at ~0°. The solution was then diluted with dichloromethane (200 mL), and successively washed with cold water, cold saturated $NaHCO_3$, and cold water, dried, and concentrated to a solid. The residue was dissolved in dichloromethane, and addition of ether–hexane caused the precipitation of **11** (1.17 g, 91%), amorphous, $[\alpha]_D^{26}$ +82° (*c* 1.5, chloroform), t.l.c. (*E*) $R_F$ 0.44; $^1$H-n.m.r. ($CDCl_3$): $\delta$ 6.56 (d, ~0.76 H, J ~4 Hz, H-1) and 2.12–1.86 (cluster of s·, 30 H, 9 OAc and NAc).

*4-Nitrophenyl O-β-D-galactopyranosyl-(1→4)-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-β-D-galactopyranoside* (**13**). — A mixture of bromide **11** (0.3 g) and Amberlyst A-26-(4-nitrophenoxide) resin (3 g) in 1:4 dichloromethane–2-propanol (20 mL) was stirred for 26 h at room temperature. T.l.c. (*D*) revealed the presence of an intensely u.v.-visible product slightly slower moving than **11**. Some slower- and some faster-migrating contaminants that were not detectable under u.v. light (presumably resulting from decomposition of **11**) were also revealed by t.l.c. After dilution with dichloromethane (30 mL), the resin was filtered off, thoroughly washed with dichloromethane, and the filtrate and washings were combined and concentrated. The crude product was subjected to column chromatography on silica gel and eluted with a solvent gradient consisting of 0–20% acetone in chloroform. On concentration, the fractions corresponding to the product gave a solid which was dissolved in dichloromethane. Addition of ether–hexane caused the precipitation of **12** (0.16 g) as a white amorphous material that was contaminated (t.l.c. *E*) with a slightly slower- and with faster-moving impurities (undetectable under u.v. light). This material was utilized, without purification, in the next step.

A suspension of peracetate **12** in 0.5M methanolic sodium methoxide (20 mL) was stirred overnight at room temperature. T.l.c. (*C*) then showed the disappearance of **12** and the presence of a predominantly u.v.-visible, slower-migrating product; a trace of a slower-migrating impurity (undetectable under u.v. light) was also revealed in t.l.c. The base was neutralized by the dropwise addition of glacial acetic acid, and the solution was de-ionized with Amberlite IR-120 ($H^+$) cation-exchange resin. The resin was filtered off through a bed of Celite and thoroughly washed with methanol, and the filtrate and washings were combined and concentrated. The residue was dissolved in a small volume of water and addition of ethanol caused the precipitation of **13** (0.07 g, 33%, based on **11**) which showed in t.l.c. (*C*) a trace of the slower-migrating contaminant. An analytically pure sample, homogeneous in t.l.c. (solvent *C*), was obtained after a second crystallization of **13** from aqueous ethanol containing a little methanol, m.p. 267–268°, $[\alpha]_D^{26}$ −21° (*c* 0.6, water), t.l.c. (*C*) $R_F$ 0.40; for $^{13}$C-n.m.r. data, see Table I.

*Anal.* Calc. for $C_{26}H_{38}N_2O_{18}\cdot 2H_2O$: C, 44.44; H, 6.03; N, 3.99. Found: C, 44.31; H, 6.09; N, 3.88.

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