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SYNTHESIS OF *p*-NITROPHENYL 2-*O*-β- AND 2-*O*-α-D-GALACTOPYRANOSYL-β-D-GALACTOPYRANOSIDE*

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ABSTRACT

Cleavage of the isopropylidene group of *p*-nitrophenyl 3-*O*-benzoyl-4,6-*O*-isopropylidene-β-D-galactopyranoside afforded *p*-nitrophenyl 3-*O*-benzoyl-β-D-galactopyranoside (**1**). Compound **1** was converted into its 4,6-*O*-benzylidene derivative (**2**) by reaction with the benzaldehyde–zinc chloride complex. Compound **2** was also prepared by selective benzoylation of *p*-nitrophenyl 4,6-*O*-benzylidene-β-D-galactopyranoside (**3**), obtained by benzylidenation of *p*-nitrophenyl β-D-galactopyranoside. The structures of **1**, **2**, and **3** were established by ^{1}H- and ^{13}C-n.m.r. spectroscopy. Glycosylation of **2** with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide, catalyzed by mercuric cyanide, afforded the protected disaccharide derivatives **4** and **6**, which, on deacetalation followed by deacylation, gave the β- and the α-(1→2)-linked disaccharides **8** and **10**, respectively. The structures of **4**, **6**, **8**, and **10** were established by n.m.r. spectroscopy. Additionally, the structures of **8** and **10** were confirmed by permethylation, and hydrolysis to 3,4,6-tri-*O*-methyl-D-galactose. Compounds **8** and **10** were also converted into their fully acetylated derivatives. Compounds **4** and **6** were deacylated, to furnish the corresponding benzylidenated derivatives **5** and **7**. The ^{13}C-n.m.r. spectra of **5** and **7** are discussed, together with those of the isomeric α- and β-(1→3)-linked disaccharides, and also with that of the β-(1→6)-linked isomer.

INTRODUCTION

In two previous papers in this series, we described the synthesis of *p*-nitrophenyl 6-*O*-β-D-galactopyranosyl-β-D-galactopyranoside[2] and of both the *p*-nitrophenyl 3-*O*-α- and -β-D-galactopyranosyl-β-D-galactopyranosides[3]. These disaccharides, as well as various related compounds, were needed in a study of the substrate specificity of some endoglycosidases. In furtherance of this work, we now describe the synthesis of *p*-nitrophenyl 2-*O*-β-D-galactopyranosyl-β-D-galactopyranoside and its α-(1→2)-

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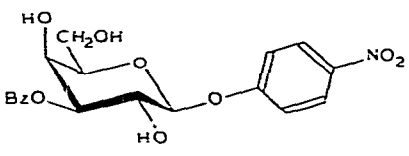
linked isomer. A route similar to that already described[3] was adopted, and the anomeric disaccharides were obtained in a ratio reminiscent of that found for the (1→3)-linked isomers[3]. As some of these disaccharides became available, it was considered of interest to record and discuss their respective, ^{13}C-n.m.r. spectra.

RESULTS AND DISCUSSION

Deacetalation of *p*-nitrophenyl 3-*O*-benzoyl-4,6-*O*-isopropylidene-β-D-galactopyranoside[2], and benzylidenation of the resulting triol **1** by treatment with the benzaldehyde–zinc chloride complex[4], afforded *p*-nitrophenyl 3-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside (**2**). Alternatively, *p*-nitrophenyl β-D-galactopyranoside was first subjected to the aforementioned benzylidenation procedure, and the resulting diol **3** was then selectively benzoylated at HO-3 with benzoyl chloride–pyridine, to give **2**. The ^{1}H-n.m.r. spectra of **1**, **2**, and **3** were all in agreement with the structures assigned (see Experimental section).



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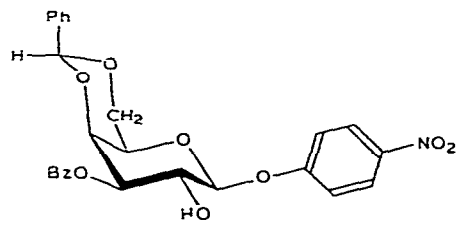

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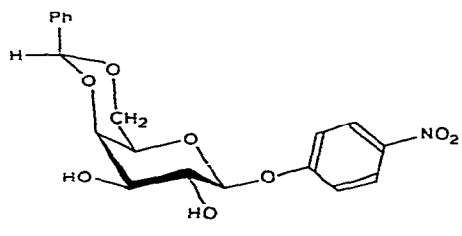


3

In the ^{13}C-n.m.r. spectrum of **1** (see Table I), the signals for C-2 and C-4 were shifted upfield by 2.9 and 3 p.p.m., respectively, whereas that of C-3 was shifted downfield by ~3.3 p.p.m., with respect to *p*-nitrophenyl β-D-galactopyranoside, as a result of substituting O-3 with a benzoyl group. A relatively small (0.8 p.p.m.) upfield shift was observed for C-1, C-5, and C-6.

In the ^{13}C-n.m.r. spectrum of **2** (see Table I), the signals for C-4 and C-6 exhibited downfield shifts of 1.8 and 6.3 p.p.m., respectively, by comparison to those observed for **1**, as would be expected from substitution at O-4 and O-6. In the spectrum of **3**, the signals for C-6, C-4, and C-3 were shifted upfield by 0.3, 1.1, and 2.6 p.p.m., respectively.

TABLE I

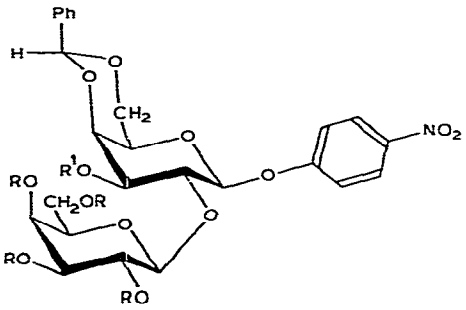
^{13}C-N.M.R. CHEMICAL SHIFTS[a] FOR SOME D-GALACTOPYRANOSIDES[b]

Compound	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*Ph-CH or OCH$_3$*
Methyl β-D-galactopyranoside	104.22	70.29	73.17	67.96	74.93	60.28	55.61
Methyl α-D-galactopyranoside	99.80	68.29	69.49	68.70	70.89	60.52	54.27
p-Nitrophenyl β-D-galactopyranoside[c]	100.73	70.25	73.30	68.20	75.90	60.43	
p-Nitrophenyl 4,6-*O*-benzylidene-β-D-galactopyranoside (**3**)[c]	99.51	69.32	71.39	68.09	75.49	66.21	99.85
p-Nitrophenyl 2-*O*-benzoyl-β-D-galactopyranoside[c]	97.91	72.33	70.77	68.09	75.95	60.00	
p-Nitrophenyl 3-*O*-benzoyl-β-D-galactopyranoside (**1**)[c]	99.95	67.36	76.56	65.18	75.15	59.64	
p-Nitrophenyl 2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside[c]	97.49	71.58	67.96	69.15	75.52	66.62	99.75
p-Nitrophenyl 3-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside (**2**)[c]	99.26	67.96	73.99	66.98	72.99	65.91	99.35

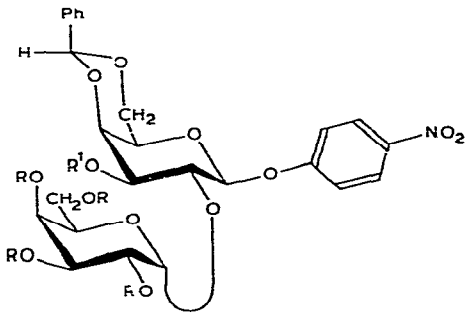
[a]In Me_2SO-d_6, with Me_4Si as the internal standard. [b]The values for *p*-nitrophenyl 2-*O*-benzoyl-β-D-galactopyranoside and its 4,6-*O*-benzylidene derivative are recorded for comparison. The values for methyl α- and β-D-galactopyranoside were used for assignments of the disaccharides in Table II. [c]Carbonyl and/or aromatic carbon resonances are not shown.

On glycosylation of compound **2** with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide for 8 h at room temperature, in acetonitrile and in the presence of mercuric cyanide, examination (after the customary processing[3]) of the crude mixture by thin-layer chromatography (t.l.c.) revealed the presence of a major product, slower-migrating than **2**, and a small proportion of a marginally slower-migrating compound; a trace of **2** was also present. Chromatographic separation on silica gel afforded the β- and the α-(1→2)-linked disaccharides (**4** and **6**, see later) in the ratio of 9:2. We had previously observed a similar ratio for the anomeric disaccharides on glycosylating the isomeric *p*-nitrophenyl 2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide under similar reaction-conditions[3].

The lower specific rotation (+1.8°) of **4** compared to that (+91.4°) of **5** suggested that **4** and **6** were the β and the α anomer, respectively, and the ^{1}H-n.m.r. spectra of the two compounds were in accord with this supposition. Thus, in the ^{1}H-n.m.r. spectrum of **4**, both H-1 and H-1′ resonated as doublets, with spacings of 8 Hz, at δ 5.32 and 4.75, respectively, in support of the β configuration at both glycosidic linkages. In the spectrum of **6**, however, H-1 was observed as a doublet at δ 5.34 (*J* 8 Hz), whereas H-1′ resonated as a doublet, with spacings of ~2 Hz, at δ 5.64, indicating a β linkage for the *p*-nitrophenyl aglycon and an α linkage for the glycosyl moiety.



4 R = Ac, R¹ = Bz
5 R = R¹ = H

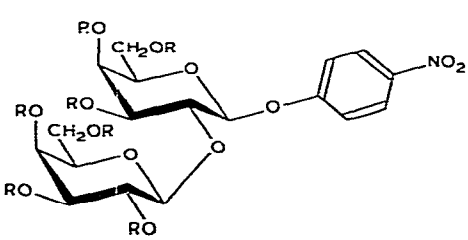


6 R = Ac, R¹ = Bz
7 R = R¹ = H

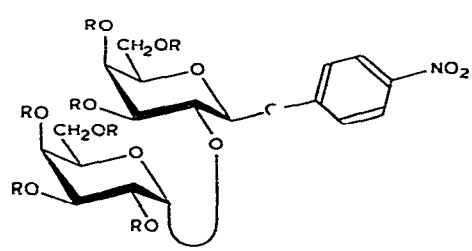
Deacetalation of either **4** or **6**, in hot, 60% aqueous acetic acid, was, apparently, accompanied by some acyl-group migration, or deacylation; a slower-migrating (t.l.c.) contaminant was observed in both cases. However, this problem was irrelevant to the operation that followed, as this consisted of complete deacylation of **4** and **6** to afford the crystalline α- and β-linked disaccharides (**8** and **10**, respectively). The anomeric configurations of both **8** and **10** could be inferred from their respective specific rotations, which had the same trends as those observed for **4** and **6**. Support for these assignments was available from ^{13}C-n.m.r. spectroscopy. Thus, in the ^{13}C-n.m.r. spectrum of **8**, the C-1′ signal was observed at 105.61 p.p.m., and that of C-1 at 98.90 p.p.m., in support of a β configuration at both anomeric centers. In the ^{13}C-n.m.r. spectrum of **10**, however, C-1′ and C-1 occurred at 100.03 and 98.21 p.p.m., respectively, indicating an α and a β configuration at the anomeric centers.

Permethylation, according to Kuhn *et al.*[5], of an α,β mixture of the (1→2)-linked disaccharides, followed by acid hydrolysis, gave 3,4,6-tri-*O*-methyl-D-galactose[6], which was clearly distinguishable (t.l.c.) from both 2,4,6-tri-*O*-methyl-D-galactose[7] and 2,3,4-tri-*O*-methyl-D-galactose[7] in three solvent systems previously utilized for this purpose[3,7].

Acetylation of disaccharide **8** with an excess of acetic anhydride in pyridine afforded an analytically pure, amorphous heptaacetate (**9**), the ^{1}H-n.m.r. spectrum of which was in agreement with the structure assigned (see Experimental section).



8 R = H
9 R = Ac



10 R = H
11 R = Ac

TABLE II

PROPOSED ^{13}C-N.M.R. CHEMICAL SHIFTS FOR SOME DISACCHARIDES[a,b]

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′*	*Ph-CH*
5	98.31	80.27	71.89	68.02	74.96	66.07	105.48	70.94	72.84	67.71	74.73	59.84	99.61
c	99.28	67.92	80.43	68.50	75.20	66.09	105.59	70.80	72.96	67.92	75.20	60.36	99.72
7	99.52	75.55	69.70	67.95	74.45	66.03	98.17	68.21	69.20	68.65	70.44	60.17	99.63
d	99.18	67.67	74.69	68.21	70.96	66.10	94.81	68.21	69.31	68.54	70.77	60.00	99.49
8	98.90	80.93	72.57	67.21	75.45	59.82	105.61	71.93	72.94	67.72	75.06	60.04	—
e	99.54	67.90	82.56	67.15	75.29	59.96	104.91	71.01	72.86	68.99	75.13	60.22	—
10	98.21	75.39	70.43	68.00	75.15	60.01	100.03	68.27	69.28	68.73	71.28	66.52	—
f	97.46	67.89	76.51	64.35	75.13	59.81	96.62	68.39	69.39	69.39	71.61	59.81	—
g	100.53	70.51	73.26	68.38	74.30	68.05	104.01	69.98	72.94	68.92	75.04	60.40	—

[a]In Me_2SO-d_6, with Me_4Si as the internal standard. [b]Aromatic carbon resonances are not shown. [c]*p*-Nitrophenyl 4,6-*O*-benzylidene-3-*O*-β-D-galactopyranosyl-β-D-galactopyranoside. [d]*p*-Nitrophenyl 4,6-*O*-benzylidene-3-*O*-α-D-galactopyranosyl-β-D-galactopyranoside. [e]*p*-Nitrophenyl 3-*O*-β-D-galactopyranosyl-β-D-galactopyranoside. [f]*p*-Nitrophenyl 3-*O*-α-D-galactopyranosyl-β-D-galactopyranoside. [g]*p*-Nitrophenyl 6-*O*-β-D-galactopyranosyl-β-D-galactopyranoside.

Similar acetylation of **10** furnished the heptaacetate **11**, whose ^{1}H-n.m.r. spectrum was, also, in accord with the structure assigned. Interestingly, one of the acetyl groups was observed at a noticeably higher field (δ 1.46) than that of the other (δ 1.96–2.18) resonances.

In order to compare the benzylidenated, (1→2)-linked disaccharides with their (1→3)-linked counterparts[3], compounds **4** and **6** were subjected to Zemplén deacylation, to afford **5** and **7**, respectively. As evidenced by their ^{13}C-n.m.r. spectra (see Table II), **5** was the β-, whereas **7** was the α-, (1→2)-linked disaccharide.

Comments on the ^{13}C-n.m.r. assignments. — In order to attain a reasonable degree of uniformity in assigning the ^{13}C-n.m.r. resonances of the compounds described herein and elsewhere[2,3], it was necessary to record the spectra under similar conditions. Therefore, all of the spectra were recorded for samples in Me_2SO-d_6, with Me_4Si as the internal standard, and the spectra of methyl α- and β-D-galactopyranoside were recorded under the same conditions. The assignments for the carbon atoms of methyl α-D-galactopyranoside follow the same pattern as those reported by Gorin and Mazurek[8]*.

In the ^{13}C-n.m.r. spectrum of *p*-nitrophenyl β-D-galactopyranoside, the C-1 signal was observed at 100.73 p.p.m.; this value is appreciably lower than that observed for the corresponding methyl β-glycoside (see Table II), indicating the effect of the aglycon on the signal of the anomeric carbon atom. A similar trend was observed for the disaccharide derivatives (see Table II), which, with the exception of the β-(1→6)-linked disaccharide, had values for C-1 of less than 100 p.p.m.

Examination of Table II reveals how the availability of the isomeric glycosides greatly facilitates correlation of the ^{13}C-chemical shift of various carbon atoms and, hence, the identification of the sites of glycosylation. Thus, in the ^{13}C-n.m.r. spectrum of the β-(1→6)-linked disaccharide, the appreciable downfield shift (~9.6 p.p.m.) of the resonance for C-6, compared to that of the parent *p*-nitrophenyl β-D-galactopyranoside, clearly indicates that C-6 is substituted. An upfield shift of ~1.6 p.p.m. for C-5, and the fact that the resonances of the remaining carbon atoms are virtually unaffected, are also in conformity with this contention.

On comparing the spectra of the isomeric β-(1→2) and β-(1→3) disaccharides, the sites of substitution are readily identified by the occurrence of ~10.7- and ~9.3-p.p.m. downfield shifts for C-2 and C-3, respectively, in comparison to *p*-nitrophenyl β-D-galactopyranoside. These sites of substitution are further distinguished on examination of the spectra of the benzylidenated disaccharides. Thus, whereas the downfield shift for C-2 (10 p.p.m., compared to *p*-nitrophenyl β-D-galactopyranoside) in the (1→2)-linked disaccharide remains close to that of the unsubstituted disaccharide glycoside, that for C-3 (7.1 p.p.m.) in the (1→3)-linked disaccharide is shifted upfield by ~2.2 p.p.m., because of the presence of a substituent on the β-carbon atom (*i.e.*, C-4).

In the ^{13}C-n.m.r. spectra of the α-(1→2)- and α-(1→3)-linked disaccharides,

*Perlin *et al.*[9] reversed the values for C-2 and C-4.

downfield shifts of smaller magnitude for C-2 (2.09 p.p.m.) and C-3 (3.21 p.p.m.) are observed, in contrast to those for the β-linked counterparts, by comparison with the parent *p*-nitrophenyl glycoside.

In the ^{13}C-n.m.r. spectra of the benzylidenated disaccharides, the α-(1→3)-linked isomer showed a somewhat larger upfield shift (1.39 p.p.m.) for C-3, whereas, for the α-(1→2)-linked compound, the chemical shift value for C-2 (75.55 p.p.m.) remained close to that (75.39) of the unsubstituted disaccharide.

It is also noteworthy that C-5 in the benzylidenated, α-(1→3)-linked disaccharide resonated at an appreciably higher field (70.96 p.p.m.), in comparison to that (75.49 p.p.m.) for C-5 of *p*-nitrophenyl 4,6-*O*-benzylidene-β-D-galactopyranoside and of the unbenzylidenated disaccharide; this may be due to orientational changes at the glycosidic linkage.

EXPERIMENTAL

General methods. — Melting points were determined with a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured at room temperature with a Perkin–Elmer 241 polarimeter. T.l.c. was conducted on plates coated with 0.25-mm, and p.l.c. on plates coated with 0.75-mm, layers of silica gel 60 PF-254 (E. Merck, Darmstadt, Germany); the components were located either by exposure to u.v. light, or by spraying the plates with 5% sulfuric acid in ethanol and heating. The following solvent systems (v/v) were used for chromatography: *A*, 2:1 benzene–ethyl acetate; *B*, 4:1 benzene–ether; and *C*, 1:1 benzene–ethyl acetate. Organic solutions were generally dried with anhydrous magnesium sulfate. Elemental analyses were performed by Robertson Laboratory, Florham Park, New Jersey, U.S.A. N.m.r. spectra were recorded 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 Fourier-transform (F.-t.) mode; the positions of the peaks are expressed in p.p.m. from the Me_4Si signal.

p-*Nitrophenyl 3-O-benzoyl-β-D-galactopyranoside* (**1**). — *p*-Nitrophenyl 3-*O*-benzoyl-4,6-*O*-isopropylidene-β-D-galactopyranoside[2] (3 g) in 60% aqueous acetic acid (60 mL) was stirred for 2 h at 80°. T.l.c. (solvent *A*) then revealed the presence of a slower-migrating product. The acetic acid was evaporated under diminished pressure, the last traces being removed by co-evaporation with several portions of toluene. The solid residue was recrystallized from alcohol, and then from acetone–hexane, to afford compound **1** (2.3 g, 84%), m.p. 168–171°, $[\alpha]_D$ +46.2° (*c* 0.78, acetone); n.m.r. data (Me_2SO-d_6): δ 8.40–7.20 (2 d, *J* 10 Hz, and m, 9 H, aromatic), 5.72 (d, 1 H, *J* 6 Hz, exchangeable by D_2O, OH), 5.34 (d, 1 H, *J* 8 Hz, H-1), 5.18 (d, 1 H, *J* 6 Hz, exchangeable by D_2O, OH), 5.02 (dd, 1 H, *J* 3 and 10 Hz, H-3), 4.78 (t, 1 H, *J* 6 Hz, exchangeable by D_2O, OH), and 4.25–3.40 (m, 5 H, H-2,4,5,6,6′); for ^{13}C-n.m.r. data, see Table I.

Anal. Calc. for $C_{19}H_{19}NO_9$: C, 56.29; H, 4.73; N, 3.45. Found: C, 56.00, H, 4.46; N, 3.45.

p-*Nitrophenyl 4,6-benzylidene-β-D-galactopyranoside* (**3**). — Zinc chloride (3 g)

was quickly added, with stirring, to benzaldehyde (10.5 mL). Stirring was continued for 0.5 h, and then *p*-nitrophenyl β-D-galactopyranoside (3 g) was added. The mixture was stirred for 4 h at room temperature, poured into stirred, ice-water–hexane mixture (1:1 v/v, 150 mL), and the precipitate filtered off, thoroughly washed with cold water and hexane, dried in the air, and recrystallized from acetone–hexane, to afford compound **3** (3.2 g, 82%). m.p. 236–238°, $[\alpha]_D$ −129.5° (*c* 0.38, acetone); n.m.r. data (Me_2SO-d_6): δ 8.30–7.20 (2 d, *J* 10 Hz, and m, 9 H, aromatic), 5.61 (s, 1 H, PhC*H*), 5.42 (d, 1 H, *J* 4 Hz, exchangeable by D_2O, OH), 5.21 (d, 1 H, *J* ~7.5 Hz, H-1), 5.13 (d, 1 H, *J* 5 Hz, exchangeable by D_2O, OH), and 4.30–3.40 (m, 6 H, H-2, 3,4,5,6,6'); for ^{13}C-n.m.r. data, see Table I.

Anal. Calc. for $C_{19}H_{19}NO_8$: C, 58.60; H, 4.93; N, 3.60. Found: C, 58.35; H, 5.09; N, 3.45.

p-*Nitrophenyl 3-O-benzoyl-4,6-O-benzylidene-β-D-galactopyranoside* (**2**). — *Method* (*a*). Compound **1** (2 g) was benzylidenated as described for preparation of **3**, to afford, after recrystallization from acetone–hexane, compound **2** (1.8 g, 74%). m.p. 258–260°, $[\alpha]_D$ +12.8° (*c* 0.72, acetone); n.m.r. data (Me_2SO-d_6): δ 8.40–7.20 (2 d, *J* 10 Hz, and complex m, 14 H, aromatic), 5.92 (d, 1 H, *J* 4 Hz, exchangeable by D_2O, OH), 5.66 (s, 1 H, PhC*H*), 5.53 (d, 1 H, *J* 8 Hz, H-1), 5.29 (dd, 1 H, *J* 4 and 10 Hz, H-3), 4.59 (d, 1 H, *J* 3 Hz, H-4), and 4.30–3.80 (m, 4 H, H-2,5,6,6'); for ^{13}C-n.m.r. data, see Table I.

Anal. Calc. for $C_{26}H_{23}NO_9$: C, 63.28; H, 4.71; N, 2.81. Found: C, 63.05; H, 4.76; N, 2.73.

Method (*b*). To a cold (−10°), stirred solution of compound **3** (1 g) in pyridine (15 mL) was added, dropwise, a solution of benzoyl chloride (0.43 g) in pyridine (5 mL). The mixture was stirred for 1 h at −10°, and then for 3 h at room temperature. The pyridine was evaporated under diminished pressure, and traces were removed by co-evaporation with several portions of toluene. T.l.c. (solvent *A*) of the crude product showed the presence of **2** as the major component. A trace of a faster-migrating compound (presumably, the 2,3-diester) and a negligible proportion of the slower-migrating, 2-*O*-benzoyl derivative[3] were also revealed by t.l.c. Recrystallization from acetone–hexane afforded pure **2** (0.88 g, 69.3%), m.p. 258–260°, undepressed by admixture with a sample from (*a*).

p-*Nitrophenyl 3-O-benzoyl-4,6-O-benzylidene-2-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-galctopyranoside* (**4**) *and* p-*nitrophenyl 3-O-benzoyl-4,6-O-benzylidene-2-O-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-β-D-galactopyranoside* (**6**). — To a solution of the 3-benzoate **2** (1.5 g) in acetonitrile (80 mL) were added mercuric cyanide (1.5 g) and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (2.4 g), and the mixture was stirred for 8 h at room temperature. After processing in the usual way[2,3], t.l.c. (solvent *B*, two irrigations) revealed the presence of a major product, slower-migrating than **2**, and a small proportion of a marginally slower-migrating compound; a trace of **2** was also present. The residue was taken up in benzene (~40 mL), and the suspension filtered to remove unchanged **2** (0.1 g), which crystallized. The filtrate was applied to a column of silica gel, and eluted with

10:1 benzene–ether. On evaporation, the first fractions gave starting **2** (0.1 g). Continued elution with the same solvent-system afforded **4** (1.65 g), amorphous, $[\alpha]_D$ +1.8° (*c* 0.8, chloroform); n.m.r. data ($CDCl_3$): δ 8.30–7.00 (2 d, *J* 10 Hz and complex m, 14 H, aromatic), 5.56 (s, 1 H, PhC*H*), 5.32 (d, 1 H, *J* 8 Hz, H-1), 4.75 (d, 1 H, *J* 8 Hz, H-1′), 2.08, 2.02, 1.88, and 1.64 (s, 3 H each, 4 OAc), and 5.45–3.70 (unresolved signals, 12 H).

Anal. Calc. for $C_{40}H_{41}NO_{18}$: C, 58.31; H, 5.03; N, 1.70. Found: C, 58.03; H, 5.24; N, 1.62.

Further elution of the column gave a fraction (0.25 g) which contained (t.l.c.) almost equal proportions of **4** and the slow-moving component.

The last fractions afforded **6** (0.31 g), amorphous, $[\alpha]_D$ +91.4° (*c* 0.35, chloroform); n.m.r. data ($CDCl_3$): δ 8.40–7.00 (2 d, *J* 10 Hz and complex m, 14 H, aromatic), 5.64 (d, 1 H, *J* ~2 Hz, H-1′), 5.56 (s, 1 H, PhC*H*), 5.43 (dd, 1 H, *J* 4 and 10 Hz, H-3), 5.34 (d, 1 H, *J* 8 Hz, H-1), 2.06, 1.92, 1.68, and 1.42 (s, 3 H each, OAc), and 5.26–3.50 (unresolved signals, 11 H).

Anal. Calc. for $C_{40}H_{41}NO_{18}$: C, 58.31; H, 5.03; N, 1.70. Found: C, 58.41; H, 5.30; N, 1.45.

The aforementioned, mixed fraction was subjected to p.l.c. (solvent *B*), to afford **4** (0.13 g) and **6** (0.09 g).

p-*Nitrophenyl 2*-O-β-D-*galactopyranosyl*-β-D-*galactopyranoside* (**8**). — Compound **4** (0.5 g) was stirred in 60% aqueous acetic acid (10 mL) for 1.5 h at 80–85°. The acetic acid was removed under diminished pressure and finally by co-evaporation with toluene, and the residue was dissolved in chloroform. Addition of ether–petroleum ether caused the precipitation of a white powder (0.42 g) for which t.l.c. (solvent *C*) showed the presence of a major product, slower-migrating than **4**, and also a slower-migrating contaminant (presumably due to acyl-group migration, or deacetylation). The crude, debenzylidenated compound was stirred in methanol (15 mL) containing 0.1M sodium methoxide in methanol (5 mL). The suspension rapidly dissolved, and, in ~10 min, crystallization ensued. The mixture was kept for 1 h at room temperature, cooled, the base neutralized by the addition of a few drops of glacial acetic acid, and the crystalline material filtered off, and thoroughly washed with cold methanol, to afford disaccharide **8** (0.28 g), m.p. 287° (dec.), $[\alpha]_D$ −58° (*c* 0.3, water); for ^{13}C-n.m.r. data, see Table II.

Anal. Calc. for $C_{18}H_{25}NO_{13} \cdot 0.5\ H_2O$: C, 45.76; H, 5.56; N, 2.96. Found: C, 45.66; H, 5.62; N, 2.59.

p-*Nitrophenyl 2*-O-α-D-*galactopyranosyl*-β-D-*galactopyranoside* (**10**). — Compound **6** (0.26 g) was debenzylidenated in 60% aqueous acetic acid (5 mL) exactly as described for **4** (to give **8**). On examination by t.l.c. (solvent *C*), the product (0.21 g) was found to be contaminated by a slower-migrating compound. The crude product (0.15 g) was therefore deacylated in methanolic sodium methoxide. After de-ionization with Amberlite IR-120 (H^+) ion-exchange resin, and evaporation of the methanol, the solid residue was recrystallized from alcohol, to afford **10** (0.09 g), m.p. 282° (dec.), $[\alpha]_D$ +35.4° (*c* 0.3, water); for ^{13}C-n.m.r. data, see Table II.

Anal. Calc. for $C_{18}H_{25}NO_{13} \cdot 0.5\ H_2O$: C, 45.76; H, 5.56; N, 2.96. Found: C, 45.48; H, 5.65; N, 2.79.

p-*Nitrophenyl 4,6-O-benzylidene-2-O-β-*D*-galactopyranosyl-β-*D*-galactopyranoside* (**5**). — Compound **4** (0.1 g) was taken up in methanol (5 mL) containing a catalytic amount of sodium methoxide in methanol; the suspended **4** rapidly dissolved and, in a few minutes, crystallization ensued. After being kept for 2 h at room temperature, the mixture was cooled, and the crystals were collected by filtration, and thoroughly washed with cold methanol. Recrystallization from methanol afforded **5** (0.05 g, 75%), m.p. 280–282° (dec.), $[\alpha]_D$ −84.8° (*c* 0.33, *N*,*N*-dimethylformamide); for ^{13}C-n.m.r. data, see Table II.

Anal. Calc. for $C_{25}H_{29}NO_{13} \cdot H_2O$: C, 52.71; H, 5.50; N, 2.46. Found: C, 52.74; H, 5.39; N, 2.24.

p-*Nitrophenyl 4,6-O-benzylidene-2-O-α-*D*-galactopyranosyl-β-*D*-galactopyranoside* (**7**). — Deacylation of compound **6** (0.07 g) as described for **4**, and recrystallization of the product from methanol, afforded disaccharide **7** (0.03 g, 63.8%), m.p. 259–262° (dec.), $[\alpha]_D$ −15.8° (*c* 0.2, *N*,*N*-dimethylformamide); for ^{13}C-n.m.r. data, see Table II.

Anal. Calc. for $C_{25}H_{29}NO_{13} \cdot H_2O$: C, 52.71; H, 5.50; N, 2.46. Found: C, 52.48; H, 5.46; N, 2.27.

p-*Nitrophenyl 3,4,6-tri-O-acetyl-2-O-(2,3,4,6-tetra-O-acetyl-β-*D*-galactopyranosyl)-β-*D*-galactopyranoside* (**9**). — The disaccharide **8** (0.03 g) was acetylated overnight in 1:2 acetic anhydride–pyridine, to afford, after the usual processing[3], amorphous heptaacetate **9** (0.04 g, 81.6%), $[\alpha]_D$ −27.5° (*c* 0.6, chloroform): ^{1}H-n.m.r. data ($CDCl_3$): δ 8.4–7.0 (2 d, 4 H, *J* 10 Hz, aromatic), 5.22 (d, 1 H, *J* 8 Hz, H-1), 4.74 (d, 1 H, *J* 8 Hz, H-1'), 2.20, 2.12, 2.10, 2.08, 2.04, 2.02, and 1.98 (s, 21 H, 7 OAc), and 5.6–3.9 (unresolved signals, 12 H).

Anal. Calc. for $C_{32}H_{39}NO_{20}$: C, 50.72; H, 5.20; N, 1.85. Found: C, 50.45; H, 5.19; N, 1.59.

p-*Nitrophenyl 3,4,6-tri-O-acetyl-2-O-(2,3,4,6-tetra-O-acetyl-α-*D*-galactopyranosyl)-β-*D*-galactopyranoside* (**11**). — Acetylation of **10** (0.025 g) as described for **8** furnished amorphous heptaacetate **11** (0.03 g, 73.2%), $[\alpha]_D$ +38.2° (*c* 0.6, chloroform); n.m.r. data ($CDCl_3$): δ 8.4–7.0 (2 d, 4 H, *J* 10 Hz, aromatic), 5.45 (ill-resolved d, 1 H, *J* ~2 Hz, H-1'), 5.22 (d, 1 H, *J* 8 Hz, H-1), 2.18, 2.15, 2.08, 2.06, 2.04, 1.96, and 1.46 (s, 21 H, 7 OAc), and 5.7–4.0 (unresolved signals, 12 H).

Anal. Calc. for $C_{32}H_{39}NO_{20}$: C, 50.72; H, 5.20; N, 1.85. Found: C, 50.56; H, 5.43; N, 1.56.

Permethylation of a mixture of **8** *and* **10**, *and hydrolysis.* — A sample (0.1 g; obtained by deacetalation, followed by deacylation, of a mixed fraction of **4** and **6** from a column; see earlier) containing almost equal proportions of **8** and **10** was permethylated[5], and the product was hydrolyzed as previously described[3]. Examination of the hydrolyzate by t.l.c. (15:1 chloroform–methanol) showed two spots, attributable to 2,3,4,6-tetra-*O*-methyl-D-galactose (fast) and a tri-*O*-methylgalactose. The latter compound moved in the same solvent system, as well as in 1:1 benzene-

acetone, and in 83 : 17 isopropyl ether–methanol, at a rate the same as that of authentic 3,4,6-tri-*O*-methyl-D-galactose[6], and clearly different from those of the faster-moving and the slower-moving 2,4,6-tri-*O*-methyl-D-galactose[7] and 2,3,4-tri-*O*-methyl-D-galactose[7], respectively.

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