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SYNTHESIS OF *p*-NITROPHENYL 3-*O*-β-D-GALACTOPYRANOSYL-β-D-GALACTOPYRANOSIDE AND *p*-NITROPHENYL 3-*O*-α-D-GALACTOPYRANOSYL-β-D-GALACTOPYRANOSIDE*

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ABSTRACT

Glycosylation (catalyzed by mercuric cyanide) of *p*-nitrophenyl 2,4,6-tri-*O*-acetyl-β-D-galactopyranoside (**2**) with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide in acetonitrile afforded the α-(1→3)- and β-(1→3)-linked disaccharide heptaacetates (**4** and **6**, respectively) in almost equal proportions. Similar glycosylation of *p*-nitrophenyl 2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside (**3**) gave the β-(1→3)- and the α-(1→3)-linked, fully protected, disaccharide derivatives (**8** and **10**, respectively) in the ratio of 3 : 1. The structures of **4**, **6**, **8**, and **10** were evidenced by their respective ^{1}H-n.m.r. spectra. *O*-Deacetylation of **4** and **6** afforded, respectively, *p*-nitrophenyl 3-*O*-α-D-galactopyranosyl-β-D-galactopyranoside (**5**) and *p*-nitrophenyl 3-*O*-β-D-galactopyranosyl-β-D-galactopyranoside (**7**). *O*-Deacylation of **8** and **10** furnished the disaccharide derivatives (**9** and **11**). Cleavage of the benzylidene groups of **9** and **11** gave the disaccharides **7** and **5**, respectively. The structures of **5**, **7**, **9**, and **11** were established by ^{13}C-n.m.r. spectroscopy. Additionally, the structures of **5** and **7** were confirmed by permethylation, and acid hydrolysis to 2,4,6-tri-*O*-methyl-D-galactose. The synthesis of triacetate **2**, starting from *p*-nitrophenyl 3,4-*O*-isopropylidene-β-D-galactopyranoside (**1**), is also described. Compound **1** was obtained as the major product of the isopropylidenation of *p*-nitrophenyl β-D-galactopyranoside under thermodynamic control, and its structure was likewise established by ^{13}C-n.m.r. spectroscopy, and confirmed by methylation, and acid hydrolysis to 2,6-di-*O*-methyl-D-galactose. Compound **3** was obtained from *p*-nitrophenyl 2-*O*-benzoyl-4,6-*O*-isopropylidene-β-D-galactopyranoside by cleavage of the isopropylidene group, and acetalation of the resulting triol with benzaldehyde–zinc chloride.

INTRODUCTION

In a preceding paper in this series[1], we outlined our interest in the synthesis of

*Synthetic Studies in Carbohydrates, Part XXI. For Part XX, see ref. 1.
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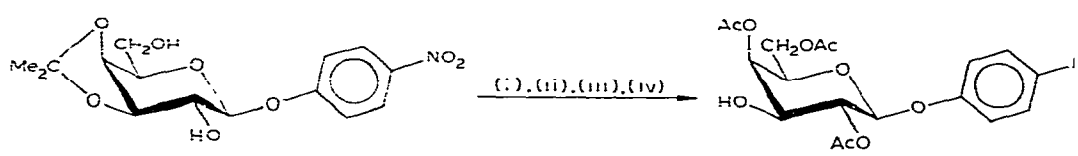
p-nitrophenyl 3-*O*-β-D-galactopyranosyl-β-D-galactopyranoside and related compounds. This interest mainly stemmed from a desire to conduct further substrate-specificity studies on some endogalactosidases. We have also demonstrated[1] how attempted synthesis of the β-(1→3)-linked disaccharide afforded, instead, the isomeric β-(1→6)-linked disaccharide, as a result of concomitant 4,6- to 3,4-acetal migration during glycosylation in acetonitrile under catalysis by mercuric cyanide. We now report successful syntheses of *p*-nitrophenyl 3-*O*-β-D-galactopyranosyl-β-D-galactopyranoside and its α-(1→3)-linked isomer by two different routes.

RESULTS AND DISCUSSION

Isopropylidenation of *p*-nitrophenyl β-D-galactopyranoside[2] in *N*,*N*-dimethylformamide for 40 min at 80° with 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid, and purification of the crude product in a column of neutral alumina, afforded the 3,4-acetal **1** in 43% yield. The ^{1}H-n.m.r. spectrum of **1** was in accord with the structure proposed; the *p*-nitrophenyl protons resonated as two doublets at δ 8.27 and 7.27, and H-1 was observed as a doublet (δ 5.08), with a spacing of 8 Hz, consistent with an equatorial disposition for the *p*-nitrophenyl group. The acetal methyl protons occurred as singlets at δ 1.54 and 1.38.

The ^{13}C-n.m.r. spectrum of **1** showed a resonance for the acetal carbon atom at 111.11 p.p.m., and the chemical shifts for the acetal methyl groups were separated by ~2 p.p.m., in agreement with the presence of a 3,4-acetal ring[3]. In the ^{13}C-n.m.r. spectrum of the isomeric 4,6-acetal[1], the acetal carbon atom resonated at 100.4 p.p.m., and the chemical shifts for the methyl groups were separated by 10.7 p.p.m. The structure of **1** was further confirmed by methylation, and acid hydrolysis of the product to 2,6-di-*O*-methyl-D-galactose.

Acetylation of **1** gave, in 94% yield, *p*-nitrophenyl 2,6-di-*O*-acetyl-3,4-*O*-isopropylidene-β-D-galactopyranoside which, on deacetalation in chloroform solution, in the presence of aqueous trifluoroacetic acid, afforded crystalline *p*-nitrophenyl 2,6-di-*O*-acetyl-β-D-galactopyranoside, and this was converted into *p*-nitrophenyl 2,4,6-tri-*O*-acetyl-β-D-galactopyranoside (**2**) by way of its 3,4-orthoester, according to literature procedures[4,5], without isolation of the intermediate. The structures of both *p*-nitrophenyl 2,6-di-*O*-acetyl-β-D-galactopyranoside and triacetate **2** were supported by their respective ^{1}H-n.m.r. spectra. Thus, in the spectrum of the diacetate,



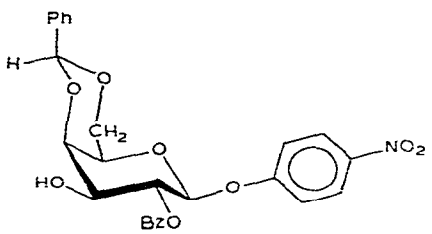
1 2

(i) Ac_2O–C_5H_5N ; (ii) F_3CCO_2H . H_2O . $CHCl_3$; (iii) $CH_3C(OEt)_3$. TsOH ; (iv) 80% F_3CCO_2H

a triplet at low field (δ 5.17, J 8 Hz), attributable to H-2, clearly indicated that O-2 was substituted with an acetyl group. The presence of two doublets (superimposed on the H-2 signal), which disappeared on addition of D_2O, suggested that two secondary hydroxyl groups were unsubstituted. Moreover, the absence of a triplet for OH-6 was indicative of its being substituted with the other acetyl group. Interestingly, the signal for the two acetyl groups, in contrast to those in the spectrum of the parent *p*-nitrophenyl 2,6-di-*O*-acetyl-3,4-*O*-isopropylidene-β-D-galactopyranoside (see the Experimental section), was observed as a singlet at δ 2.04.

In the ^{1}H-n.m.r. spectrum of **2**, the presence of three acetyl groups was accounted for by three high-field singlets (δ 2.22, 2.14, and 2.08). A one-proton multiplet (δ 3.98), which simplified to a doublet of doublets (J 4 and 10 Hz)* on D_2O exchange of the doublet at δ 2.82 (OH), could reasonably be assigned to H-3, confirming that HO-3 was not substituted by an acetyl group. An ill-resolved doublet of doublets at δ 5.43 was attributed to H-4, whereas H-1 resonated as a doublet at δ 5.14 with a spacing of 8 Hz.

p-Nitrophenyl 2-*O*-benzoyl-4,6-*O*-isopropylidene-β-D-galactopyranoside[1] was readily deacetalated by stirring in 60% aqueous acetic acid at ~80°, and the resulting triol was converted into *p*-nitrophenyl 2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside (**3**) by treatment with the benzaldehyde–zinc chloride complex[6]. The



3

structures of *p*-nitrophenyl 2-*O*-benzoyl-β-D-galactopyranoside and its 4,6-*O*-benzylidene derivative (**3**) were both supported by their ^{1}H- and ^{13}C-n.m.r. spectra. In the ^{1}H-n.m.r. spectrum of *p*-nitrophenyl 2-*O*-benzoyl-β-D-galactopyranoside, a doublet of doublets at δ 5.50 (J 13 and 7 Hz), superimposed on a doublet (J 7 Hz), was assigned to H-2, and the doublet was attributed to H-1. This assignment is substantiated by the fact that H-2 is the only proton on the ring (assuming the $^4C_1$ conformation) to possess an axial–axial–axial disposition with respect to two other protons on adjacent carbon atoms, *i.e.*, H-1 and H-3. The presence of two doublets (δ 5.30 and 5.02) and a triplet (δ 4.85), which disappeared on addition of D_2O, confirmed that two secondary hydroxyl groups, in addition to the primary hydroxyl group, were unsubstituted.

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*In the spectrum of the corresponding 2,2,2-trichloroethyl D-galactoside[5], the H-3 signal was observed as a doublet of doublets at δ 3.88 superimposed on the H-5 signal, and $J_{3,4}$ was assumed to be either 1.2 or 3.5 Hz.

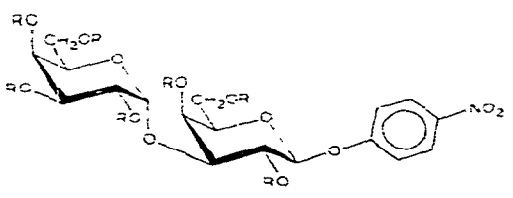
In the ^{1}H-n.m.r. spectrum of **3**, H-1 resonated as a doublet (δ 5.82), superimposed on a singlet (δ 5.72) for the benzylidene proton. The signal for H-2 was observed as a doublet of doublets (δ 5.50), superimposed on a doublet, which disappeared on addition of D_2O. The occurrence of the H-2 signal at low field confirms its being attached to a carbon atom carrying an acyloxy group.

In the ^{13}C-n.m.r. spectrum of *p*-nitrophenyl 2-*O*-benzoyl-β-D-galactopyranoside, the signals for C-1 and C-3 were shifted upfield by 2.8 and 2.5 p.p.m., respectively, whereas C-2 was shifted downfield by 2.2 p.p.m. with respect to the parent *p*-nitrophenyl β-D-galactopyranoside as a result of the substitution of O-2 with a benzoyl group. The signals for C-4 and C-5 were virtually unaffected, but a small (0.4 p.p.m.) upfield shift for the C-6 signal was observed.

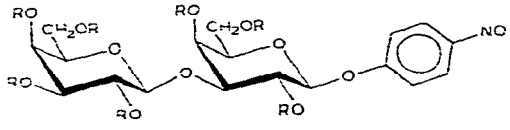
In the ^{13}C-n.m.r. spectrum of **3**, the signals for C-4 and C-6 exhibited downfield shifts of 1.1 and 6.6 p.p.m., respectively, and that of C-3 was shifted upfield by 2.8 p.p.m. (with respect to those observed for *p*-nitrophenyl 2-*O*-benzoyl-β-D-galactopyranoside) as would be expected for substitution at O-4 and O-6.

Glycosylation of the triacetate **2** with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide in acetonitrile in the presence of mercuric cyanide for 4 h at room temperature, and examination of the reaction mixture by thin-layer chromatography (t.l.c.), showed the formation of two products, both moving faster than **2**. Chromatographic separation on a column of silica gel with 1 : 1 (v/v) benzene–ether as the eluant afforded the disaccharides **4** and **6** in almost equal proportions. The higher specific rotation of **4**, compared to that of **6**, suggested that **4** and **6** were, respectively, the α- and β-(1→3)-linked disaccharides, and this was readily verified by ^{1}H-n.m.r. spectroscopy. The ^{1}H-n.m.r. spectra of **4** and **6** both contained the resonances expected for the *p*-nitrophenyl group, as well as those for the acetyl groups. In the spectrum of **6**, a doublet at δ 5.10 with a spacing of 8 Hz, attributable to H-1, had its counterpart in the spectrum of **4** at δ 5.15, in conformity with a β configuration for the *p*-nitrophenyl aglycon in both **4** and **6**. However, a doublet at δ 4.62 (J 8 Hz), assigned to H-1′ in the spectrum of **6**, was absent from that of **4**. Instead, a doublet at δ 5.28 with a spacing of ~3.5 Hz was observed, in agreement with the β and α configuration at the interglycosidic linkage of **6** and **4**, respectively.

Deacetylation of **4** and **6** in methanolic sodium methoxide respectively furnished amorphous *p*-nitrophenyl 3-*O*-α-D-galactopyranosyl-β-D-galactopyranoside (**5**) and crystalline *p*-nitrophenyl 3-*O*-β-D-galactopyranosyl-β-D-galactopyranoside (**7**). The



4 R = Ac
5 R = –



6 R = Ac
7 R = H

anomeric configurations at the interglycosidic linkages were assigned by comparison of their respective ^{13}C-n.m.r. spectra; in that of **5**, the signal for C-1′ occurred at 96.62 p.p.m., whereas, in that of **7**, C-1′ resonated at 104.91 p.p.m. The signal for the anomeric carbon atom of a glycopyranoside that has the aglycon in equatorial orientation tends to occur to lower field than that of the corresponding anomer[7,8]. That the linkage was (1→3) in both disaccharides was evidenced by the noticeable, downfield shifts for the C-3 resonances (9.26 and 3.21 p.p.m. for **7** and **5**, respectively) compared to that observed for the parent *p*-nitrophenyl β-D-galactopyranoside.

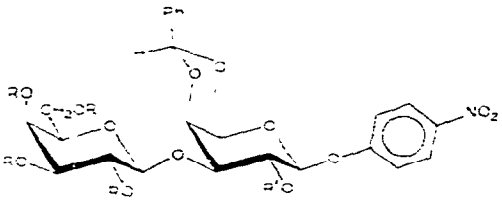
In view of the poor selectivity toward formation of the 1,2-*trans*-glycoside (the β anomer) exhibited by the reaction of the triacetate **2** with 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl bromide, and as it was our aim to obtain relatively large quantities of the β-(1→3)-linked disaccharide, an attempt to improve the selectivity was made. It seemed possible that a better yield of the β anomer could be achieved either by changing the reaction conditions (for example, by using a less-polar solvent-system or a different catalyst), or by altering the structure of the glycosyl moiety. It had been suggested[9] that, with highly polar solvents (*e.g.*, acetonitrile or nitromethane) and catalysts having strongly complexing anions [$Hg(CN_2)$ or $HgBr_2$], the glycosylation proceeds *via* a glycosyl cation rather than an acyloxonium ion, thus leading to a mixture of the *cis*- and *trans*-glycosides, in a ratio depending on the structure of the sugar and the reaction conditions.

For the present study, we chose to adopt the latter approach, *i.e.*, change in the structure of the glycosyl moiety, by changing the substituents in the vicinity of the hydroxyl group to be glycosylated. Hence, we utilized *p*-nitrophenyl 2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside (**3**) for an alternative synthesis of the desired β-(1→3)-linked disaccharide. Compound **3** was readily accessible from *p*-nitrophenyl 2-*O*-benzoyl-4,6-*O*-isopropylidene-β-D-galactopyranoside[1]. Additionally, the 4,6-benzylidene acetal group on the galactopyranosyl ring was not susceptible to acetal migration (or deacetalation) under similar conditions of glycosylation[10].

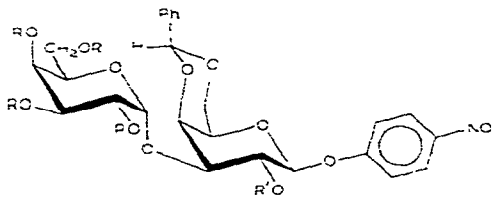
Reaction of **3** with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide, under conditions similar to those already described for **2**, proceeded readily, and no acetal migration was observed; however, the ratio of the (1→3)-linked glycosides was noticeably different from that observed for **2**. In a typical glycosylation of **3**, the disaccharide mixture obtained was subjected to preparative-layer chromatography (p.l.c.) on silica gel, to give crystalline **8** and **10** in the ratio* of 3:1. This was a clear indication that the substituents on the glycosyl moiety have a marked influence on the ratio of the anomeric glycosides under otherwise similar reaction-conditions.

As in the case of the disaccharide heptaacetates **4** and **6**, the high positive specific rotation (+103.5°) for **10** and the low negative specific rotation (−32.8°) for **8** were indicative of an α and a β configuration, respectively, at the interglycosidic

*A β:α ratio of 4.5:1 was observed in the reaction of *p*-nitrophenyl 3-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide under the same conditions[11].



8 R = Ac, R' = Bz
9 R = R' = H



10 R = Ac, R' = Bz
11 R = R' = H

linkage. The ^{1}H-n.m.r. spectra of both **8** and **10** were in agreement with this conclusion; thus, in the ^{1}H-n.m.r. spectrum of **10**, H-1′ was observed as a doublet (δ 5.53) with a spacing of 3 Hz, and H-1 resonated as a doublet (J 8 Hz) at δ 5.39, whereas, in the spectrum of **8**, H-1′ and H-1 were each observed as a doublet (J 8 Hz each), at δ 4.73 and 5.31, respectively.

Zemplén deacylation of **10** and **8** afforded *p*-nitrophenyl 4,6-*O*-benzylidene-3-*O*-α-D-galactopyranosyl-β-D-galactopyranoside (**11**) and *p*-nitrophenyl 4,6-*O*-benzylidene-3-*O*-β-D-galactopyranosyl-β-D-galactopyranoside (**9**), respectively.

That **9** was the β-(1→3)-linked disaccharide was evident on comparing its ^{13}C-n.m.r. spectrum with that of **11**. In the spectrum of **9**, the signal for C-1 occurred at 99.28 p.p.m., and that for C-1′, at 105.59 p.p.m. On the other hand, the spectrum of **11** contained signals at 94.81 p.p.m. (C-1′), and 99.18 (C-1), compatible with an α and a β configuration at the respective glycosidic linkage. In the spectra of both compounds, C-3 experienced a downfield shift, but this was much more noticeable for the β-(1→3)-linked compound **9**. Cleavage of the benzylidene groups of **9** and **11** with 60% aqueous acetic acid at ~80° afforded the title disaccharides **7** and **5**, respectively.

When only the β-(1→3)-linked disaccharide **7** was required, chromatographic separation of the reaction product (containing **8** and **10**) was unnecessary. Recrystallization of the crude mixture from methanol removed most of the α anomer **10**, and on deacylation followed by debenzylidenation, pure *p*-nitrophenyl 3-*O*-β-D-galactopyranosyl-β-D-galactopyranoside (**7**) was obtained in fairly good yield.

Permethylation of a portion of an α,β mixture of the (1→3)-linked disaccharide, followed by acid hydrolysis, gave 2,4,6-tri-*O*-methyl-D-galactose[12], clearly distinguishable in t.l.c. from both 2,3,4-tri-*O*-methyl-D-galactose[12] and 3,4,6-tri-*O*-methyl-D-galactose[13], in three solvent-systems previously utilized for this purpose[1,12].

Comments on the ^{13}C-n.m.r. assignments

The ^{13}C-n.m.r. spectra of the (1→3)-linked disaccharides **5** and **7**, as well as those of their 4,6-*O*-benzylidene derivatives **9** and **11**, have been recorded[11], in conjunction with those of the corresponding (1→2)- and (1→6)-linked isomers.

Assignments for the ^{13}C-n.m.r. signals of *p*-nitrophenyl β-D-galactopyranoside and some of its derivatives are listed in Table I. The assignments of the signals in the

TABLE I

PROPOSED ^{13}C-N.M.R. CHEMICAL-SHIFTS[a] OF *p*-NITROPHENYL β-D-GALACTOPYRANOSIDE AND SOME DERIVATIVES

Compound	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*Ph-CH or* $(CH_3)_2C$
p-Nitrophenyl β-D-galactopyranoside[b]	100.73	70.25	73.30	68.20	75.90	60.43	—
3,4-*O*-isopropylidene-[c]	101.21	73.69	74.80	74.67	80.47	62.30	111.11
2-*O*-benzoyl-	97.91	72.33	70.77	68.09	75.95	60.00	—
2-*O*-benzoyl-4,6-*O*-benzylidene- (3)	97.49	71.58	67.96	69.15	75.52	66.62	99.75

[a]In Me_2SO-d_6, with Me_4Si as the internal standard; but see footnote *c*. [b]The corresponding values in ref. 14 are: 103-35 (C-1), 73.15 (C-2), 71.20 (C-3), 70.45 (C-4), 72.05 (C-5), and 63.00 (C-6). [c]In CD_3OD; additional assignments: 28.34 and 26.48 p.p.m., acetal methyl groups.

spectra of the derivatives were based on the chemical shifts to be expected on substitution. The ^{13}C-n.m.r. spectrum of *p*-nitrophenyl β-D-galactopyranoside had a pattern similar to that reported[14]. However, in the spectrum we recorded, a downfield shift, ranging between 2.62 and 3.02 p.p.m., was observed for all of the signals. For example, the value reported[14] for C-1 was 103.35 p.p.m., whereas our value for the same carbon atom was 100.73 p.p.m. These differences may have arisen from the fact that our reference standard was internal Me_2SO-d_6, whereas, in ref. 14, Me_2SO-d_6 was used as an external standard.

The assignments of the ^{13}C signals for the disaccharides were made by comparison of their spectra with those of *p*-nitrophenyl β-D-galactopyranoside and of methyl α- and β-D-galactopyranoside, taking into consideration the expected shifts for the signals as a result of substitution at a particular hydroxyl group.

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. Solvent *A*, used for t.l.c. and p.l.c., was 20:10:1 benzene–ether–methanol. 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) were determined 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 3,4-O-isopropylidene-β-D-galactopyranoside* (**1**). — *p*-Nitrophenyl β-D-galactopyranoside (6.5 g) in *N,N*-dimethylformamide (60 mL) was stirred at ~80°, and *p*-toluenesulfonic acid (75 mg) was added, followed by 2,2-dimethoxypropane (3 mL). The mixture was stirred for 40 min at 80°, cooled, and the acid neutralized by the addition of triethylamine (3 mL). The solution was evaporated under diminished pressure, the syrupy residue dissolved in chloroform, and the solution successively washed with aqueous sodium hydrogencarbonate and water, dried, and evaporated. Examination of the residue by t.l.c. (ethyl acetate) revealed the presence of a major product faster-moving than *p*-nitrophenyl 4,6-*O*-isopropylidene-β-D-galactopyranoside[1]. A small proportion of the latter compound was also present, together with traces of some faster-migrating contaminants. The residue was stirred with ether, and the precipitate filtered off, washed with ether, dissolved in methanol (20 mL) plus acetone (4 mL), and the solution applied to a short column of neutral alumina (Sigma, Type WN-3, activity grade 1), and eluted with benzene. On evaporation, and recrystallization of the residue from chloroform–ether–hexane,

the fractions corresponding to the major product gave compound **1** (3.2 g, 43.2%), m.p. 173–175°, $[\alpha]_D$ −36.3° (*c* 1.2, acetone); n.m.r. data (CD_3OD): δ 8.27 and 7.27 (d, 2 × 2 H, *J* 10 Hz, $C_6H_4NO_2$), 5.08 (d, 1 H, *J* 8 Hz, H-1), 4.85 (bs, superimposed on H_2O peak, OH), 1.54 and 1.38 (s, 2 × 3 H, CMe_2), and 4.50–3.60 (unresolved signals, 6 H); for the ^{13}C-n.m.r. data, see Table I.

Anal. Calc. for $C_{15}H_{19}NO_8$: C, 52.78; H, 5.62; N, 4.10. Found: C, 52.58; H, 5.58; N, 3.95.

Methylation and hydrolysis of **1**. — A solution of acetal **1** (0.1 g) in *N,N*-dimethylformamide (5 mL) and methyl iodide (2 mL) was stirred overnight at room temperature in the presence of barium oxide (0.4 g) and barium hydroxide octahydrate (0.4 g). After the usual processing, the resulting dimethyl ether was directly hydrolyzed with 0.5M sulfuric acid (3 mL) for 5 h at ~98°. After being cooled, the hydrolyzate was made neutral with Amberlite IR-45 (OH^-) resin, and evaporated, and the residue was dissolved in a little methanol. T.l.c. with 10:1 chloroform–methanol showed the presence of one compound, identical in mobility to authentic 2,6-di-*O*-methyl-D-galactose, but different from the slower-migrating 2,3-di-*O*-methyl-D-galactose.

p-*Nitrophenyl 2,6-di*-O-*acetyl-3,4*-O-*isopropylidene-β*-D-*galactopyranoside*.— A solution of acetal **1** (4 g) in pyridine (40 mL) and acetic anhydride (20 mL) was kept for 24 h at room temperature, and then evaporated under diminished pressure, the last traces of the solvents being removed by co-evaporation with several portions of toluene. Recrystallization of the residue from ethanol gave *p*-nitrophenyl 2,6-di-*O*-acetyl-3,4-*O*-isopropylidene-β-D-galactopyranoside (4.7 g, 94%), m.p. 188–190°, $[\alpha]_D$ −2.1° (*c* 0.4, chloroform); n.m.r. data (Me_2SO-d_6): δ 8.27 and 7.16 (d, 2 × 2 H, *J* 10 Hz, $C_6H_4NO_2$), 5.49 (d, 1 H, *J* 8 Hz, H-1), 5.08 (t, 1 H, *J* 8 Hz, H-2), 2.08 and 2.04 (s, 6 H, 2 OAc), 1.47 and 1.31 (s, 2 × 3 H, CMe_2), and 4.70–4.00 (unresolved signals, 5 H).

Anal. Calc. for $C_{19}H_{23}NO_{10}$: C, 53.63; H, 5.46; N, 3.29. Found: C, 53.84; H, 5.45; N, 3.17.

p-*Nitrophenyl 2,6-di*-O-*acetyl-β*-D-*galactopyranoside*. — A solution of *p*-nitrophenyl 2,6-di-*O*-acetyl-4,6-*O*-isopropylidene-β-D-galactopyranoside (4.4 g) in chloroform (330 mL) containing trifluoroacetic acid (40 mL) and water (0.5 mL) was kept for 1 h at room temperature, and then evaporated, and several portions of toluene were added to, and evaporated from, the residue. Recrystallization from ethyl acetate–hexane afforded the title compound (3.5 g, 88.6%), m.p. 192–194°, $[\alpha]_D$ −6.0° (*c* 1, chloroform), $[\alpha]_D$ −21.25° (*c* 0.3, acetone); n.m.r. data (Me_2SO-d_6): δ 8.26 and 7.18 (d, 2 × 2 H, *J* 10 Hz, $C_6H_4NO_2$), 5.38 (d, 1 H, *J* 8 Hz, H-1), 5.17 (t*, 1 H, *J* 8 Hz, H-2), 5.10–5.30 (d, 2 H, superimposed on H-2, disappeared on addition of D_2O, 2 OH), and 4.30–3.60 (unresolved signals, 5 H).

Anal. Calc. for $C_{16}H_{19}NO_{10}$: C, 49.87; H, 4.98; N, 3.64. Found: C, 49.89; H, 5.00; N, 3.62.

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*After addition of D_2O, to remove the OH signals.

p-*Nitrophenyl 2,4,6-tri-O-acetyl-β-D-galactopyranoside* (**2**). — A solution of *p*-nitrophenyl 2,6-di-*O*-acetyl-β-D-galactopyranoside (1.8 g) in triethyl orthoacetate (30 mL) containing *p*-toluenesulfonic acid monohydrate (90 mg) was stirred for 2 h at room temperature. Triethylamine (5 mL) was added, and the solution was poured into ice–water, and extracted with dichloromethane (200 mL). The extract was washed with water, dried, and evaporated to a syrup (~2 g) which was taken up in 80% aqueous acetic acid (50 mL), and the solution stirred for 20 min at room temperature. The solvent was then evaporated under diminished pressure, the last traces being removed by co-evaporation with water. Several portions of alcohol were now added to, and evaporated from, the residue, to give a residue which crystallized from ethyl acetate–ether–hexane, to afford **2**, m.p. 192–194°, $[\alpha]_D$ −17.2° (*c* 0.5, chloroform); n.m.r. data ($CDCl_3$): δ 8.23 and 7.10 (d, 2 × 2 H, *J* 10 Hz, $C_6H_4NO_2$), 5.43 (dd, 1 H, *J* ~3.5 and 1 Hz, H-4), 5.34 (dd, 1 H, *J* 10 and 8 Hz, H-2), 5.14 (d, 1 H, *J* 8 Hz, H-1), 4.28–4.08 (m, 3 H, H-5,6,6'), 3.98 (dd**, 1 H, *J* 10 and ~3.5 Hz, H-3), 2.82 (d, 1 H, *J* ~6 Hz, disappeared on addition of D_2O, HO-3), and 2.22, 2.14, and 2.08 (s, 3 × 3 H, 3 OAc).

Anal. Calc. for $C_{18}H_{21}NO_{11}$: C, 50.58; H, 4.96; N, 3.28. Found: C, 50.65; H, 4.83; N, 3.38.

p-*Nitrophenyl 2-O-benzoyl-β-D-galactopyranoside.* — *p*-Nitrophenyl 2-*O*-benzoyl-4,6-*O*-isopropylidene-β-D-galactopyranoside[1] (3 g) in 60% aqueous acetic acid (60 mL) was stirred for 2 h at ~80°; t.l.c. (ethyl acetate) then indicated the formation of a slower-migrating product. The acetic acid was evaporated under diminished pressure, and then several portions of toluene were added to, and evaporated from, the residue, which was recrystallized from absolute alcohol, to give *p*-nitrophenyl 2-*O*-benzoyl-β-D-galactopyranoside (2.45 g, 89.7%); m.p. 195–198°, $[\alpha]_D$ −17.8° (*c* 0.44, acetone); ^{1}H-n.m.r. data (Me_2SO-d_6): δ 8.20 and 7.20 (d, 2 × 2 H, *J* 10 Hz, $C_6H_4NO_2$), 8.10–7.40 (m, 5 H, C_6H_5CO), 5.50 (d, *J* 7 Hz, and dd, *J* 13 and 7 Hz, 2 H, H-1,2), 5.30 (d, 1 H, *J* 5 Hz, OH), 5.02 (d, 1 H, *J* ~4.5 Hz, OH), 4.85 (t, 1 H, *J* 6 Hz, HO-6), and 4.16–3.50 (m, 5 H, H-3,4,5,6,6'); for the ^{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, 56.32; H, 5.01; N, 3.18.

p-*Nitrophenyl 2-O-benzoyl-4,6-O-benzylidene-β-D-galactopyranoside* (**3**). — Zinc chloride (2 g) was quickly added, with stirring, to benzaldehyde (7 mL), and stirring was continued for 0.5 h. *p*-Nitrophenyl 2-*O*-benzoyl-β-D-galactopyranoside (2 g) was then added, and the mixture was stirred for 4 h at room temperature, and poured into a stirred, 1 : 1 mixture of ice–water and hexane (~100 mL). The precipitate was filtered off, thoroughly washed with cold water and hexane, air-dried, and recrystallized from absolute alcohol, to afford **3** (1.7 g, 70%); m.p. 176–178°, $[\alpha]_D$ −54.9° (*c* 0.83, acetone); ^{1}H-n.m.r. data (Me_2SO-d_6): δ 8.26 and 7.29 (d, 2 × 2 H,

**This signal appeared as a multiplet, but, on addition of D_2O, it was simplified to a doublet of doublets.

J 10 Hz, $C_6H_4NO_2$), 8.20–7.40 (m, 10 H, C_6H_5CO and C_6H_5CH), 5.82 (d, 1 H, *J* 8 Hz, H-1), 5.72 (s, 1 H, C_6H_5CH), 5.60 (d, 1 H, *J* 6 Hz, OH), 5.50 (dd, 1 H, *J* 10 and 8 Hz, H-2), and 4.50–4.00 (m, 5 H, H-3,4,5,6,6'); for the ^{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, 62.99; H, 4.50; N, 2.71.

p-*Nitrophenyl 2,4,6-tri*-O-*acetyl-3*-O-(*2,3,4,6-tetra*-O-*acetyl-α*-D-*galactopyranosyl*)-*β*-D-*galactopyranoside* (**4**) *and* p-*nitrophenyl 2,4,6-tri*-O-*acetyl-3*-O-(*2,3,4,6-tetra*-O-*acetyl-β*-D-*galactopyranosyl*)-*β*-D-*galactopyranoside* (**6**). — To a solution of the triacetate **2** (0.68 g) in acetonitrile (20 mL) were added mercuric cyanide (0.4 g) and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (1.3 g). The mixture was stirred for 4 h at room temperature; t.l.c. (ether) then showed the formation of two products, both faster-moving than **2**. A small proportion of unchanged **2** was also present. After processing in the usual way, the foamy residue (~1.3 g) was subjected to chromatography in a column of silica gel, using 1:1 benzene–ether as the eluant. On evaporation, fractions corresponding to the faster-migrating compound gave amorphous **4** (0.46 g); $[\alpha]_D$ +80° (*c* 0.4, chloroform); n.m.r. data ($CDCl_3$): δ 8.26 and 7.12 (d, 2 × 2 H, *J* 10 Hz, $C_6H_4NO_2$), 5.28 (d, 1 H, *J* ~3.5 Hz, H-1'), 5.15 (d, 1 H, *J* 8 Hz, H-1), 2.20, 2.16, 2.10, 2.07, 2.04, and 1.98 (s, 21 H, 7 OAc), and 5.70–5.20 and 4.40–3.90 (unresolved signals, 12 H).

Anal. Calc. for $C_{32}H_{39}NO_{20}$: C, 50.72; H, 5.20; N, 1.85. Found: C, 50.27; H, 5.47; N, 1.91.

Continued elution of the column with the same solvent-system furnished the disaccharide derivative **6**; amorphous (0.52 g); $[\alpha]_D$ +1.0° (*c* 0.4, chloroform); n.m.r. data ($CDCl_3$): δ 8.24 and 7.10 (d, 2 × 2 H, *J* 10 Hz, $C_6H_4NO_2$), 5.10 (d, 1 H, *J* 8 Hz, H-1), 4.62 (d, 1 H, *J* 8 Hz, H-1'), 2.19, 2.18, 2.13, 2.10, 2.08, 2.06, and 1.99 (s, 21 H, 7 OAc), and 4.80–5.70 and 4.30–3.80 (unresolved signals, 12 H).

Anal. Calc. for $C_{32}H_{39}NO_{20}$: C, 50.72; H, 5.20; N, 1.85. Found: C, 50.41; H, 5.19; N, 1.95.

p-*Nitrophenyl 3*-O-*α*-D-*galactopyranosyl-β*-D-*galactopyranoside* (**5**). — The disaccharide heptaacetate **4** (0.35 g) was deacetylated overnight in methanol (20 mL) containing a catalytic amount of sodium methoxide. The solution was de-ionized with Amberlite TR-120 (H^+) resin, the suspension filtered, and the filtrate evaporated. The residue was mixed with a small volume of water, the suspension filtered through a pad of Celite, and the filtrate freeze-dried, to afford disaccharide **5** (0.17 g, 81%); $[\alpha]_D$ +85.9° (*c* 0.69, water).

Anal. Calc. for $C_{18}H_{25}NO_{13} \cdot 2H_2O$: C, 43.28; H, 5.86; N, 2.80. Found: C, 43.39; H, 5.42; N, 2.48.

p-*Nitrophenyl 3*-O-*β*-D-*galactopyranosyl-β*-D-*galactopyranoside* (**7**). — The disaccharide heptaacetate **6** (0.4 g) was stirred in methanol (20 mL) containing a catalytic amount of sodium methoxide and the clear solution was kept overnight at room temperature, by, when, crystals of **7** had appeared. The mixture was cooled, and the base neutralized with a few drops of glacial acetic acid. The crystals were

filtered off, thoroughly washed with cold methanol, and recrystallized from ethanol–methanol, to give **7** (0.18 g, 75%); m.p. 254–256° (dec.), $[\alpha]_D$ −35° (*c* 0.44, water).

Anal. Calc. for $C_{18}H_{25}NO_{13} \cdot H_2O$: C, 44.90; H, 5.66; N, 2.91. Found: C, 45.24; H, 5.61; N, 2.61.

p-*Nitrophenyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-β-*D*-galactopyranoside* (**8**) *and* p-*nitrophenyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-galactopyranosyl)-β-*D*-galactopyranoside* (**10**). — *Method a.* To a solution of the 2-benzoate **3** (0.9 g) in acetonitrile (30 mL) were added mercuric cyanide (0.75 g) and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (1.6 g); the mixture was stirred for 4 h at room temperature, and t.l.c. in 3:1 benzene–ether (3 developments) then showed the disappearance of **3** and the formation of one major product marginally slower-moving than **3**. After the customary processing, the residue crystallized from absolute alcohol, yielding material (1.1 g) that was homogeneous in t.l.c. (ethyl acetate) and had $[\alpha]_D$ +1.3° (*c* 0.7, chloroform). Evaporation of the mother liquors, and recrystallization of the residue from absolute alcohol, afforded a second crop (0.3 g), $[\alpha]_D$ +25° (*c* 1.0, chloroform). On re-examination by t.l.c. (solvent *A*) of the first crop ($[\alpha]_D$ +1.3°), the presence of a major, slower-migrating compound, and a small proportion of a slightly faster-migrating compound, was revealed. The second crop ($[\alpha]_D$ +25°) contained almost equal proportions of the two compounds. On recrystallization from methanol, the major compound contained only a trace of the faster-moving compound, and was therefore utilized in the next step without further purification.

Method b. In another experiment, the 2-benzoate **3** (0.5 g) was glycosylated exactly as described in (*a*), using the appropriate quantities of reagents. The crude reaction-product was recrystallized from absolute alcohol to afford 0.65 g of a mixture (t.l.c., solvent *A*) consisting, as in (*a*), of a major, slower-migrating and a minor, faster-migrating product. The disaccharide fraction was subjected to p.l.c. with solvent *A* as the irrigant. The faster-migrating compound (0.14 g) was recrystallized from alcohol, to afford **10**, m.p. 270–272°, $[\alpha]_D$ +103.5° (*c* 0.85, chloroform); ^{1}H-n.m.r. data ($CDCl_3$): δ 8.20–7.00 (d, 2 × 2 H, *J* 10 Hz, and complex, 10 H, aromatic), 5.94 (dd, 1 H, *J* 10 and 8 Hz, H-2), 5.55 (s, 1 H, C_6H_5CH), 5.53 (d, 1 H, *J* 3 Hz, H-1′), 5.39 (d, 1 H, *J* 8 Hz, H-1), 2.04, 1.90, 1.88, and 1.44 (s, 12 H, 4 OAc), and 5.20–3.70 (unresolved signals, 11 H).

Anal. Calc. for $C_{40}H_{41}NO_{18}$: C, 58.31; H, 5.03; N, 1.70. Found: C, 58.26; H, 5.20; N, 1.63.

The slow-migrating, major product (0.42 g) was similarly recrystallized from alcohol, to afford **8**, m.p. 252–255°, $[\alpha]_D$ −32.8° (*c* 0.53, chloroform); ^{1}H-n.m.r. data ($CDCl_3$): δ 8.20–6.90 (d, 1 × 2 H, *J* 10 Hz, and complex, 10 H, aromatic), 5.92 (dd, 1 H, *J* 10 and 8 Hz, H-2), 5.63 (s, 1 H, C_6H_5CH), 5.35 (center of m*, 1 H, H-4?), 5.31 (d, 1 H, *J* 8 Hz, H-1), 5.20 (dd, 1 H, *J* 10 and 8 Hz, H-2′), 4.80 (dd,

*Couplings concealed as a result of partial superimposition on the H-1 signal.

1 H, *J* 10 and 3 Hz, H-3'), 4.73 (d, 1 H, *J* 8 Hz, H-1'), 2.12, 2.05, 1.90, and 1.60 (s, 12 H, 4 OAc), and 4.60–3.60 (unresolved signals, 8 H).

Anal. Calc. for $C_{40}H_{41}NO_{18}$: C, 58.31; H, 5.03; N, 1.70. Found: C, 58.34; H, 5.23; N, 1.73.

p-*Nitrophenyl 4,6-O-benzylidene-3-O-β-*D*-galactopyranosyl-β-*D*-galactopyranoside* (**9**). — *Method i.* The major compound from *a* (crystallized from methanol; 0.7 g) was taken up in methanol (60 mL) containing 0.1M sodium methoxide (3 mL). The suspension was stirred until complete dissolution had occurred, and then kept overnight at room temperature. After being cooled, some solid material precipitated, and the suspension was filtered. The filtrate was de-ionized with Amberlite TR-120 (H^+) resin, and evaporated. The residue was taken up in hot ethyl acetate, and the suspension filtered. Precipitation by addition of ether–hexane to the filtrate afforded **9** (0.34 g, 72.3%) as a powder; $[\alpha]_D$ −55° (*c* 0.3, methanol).

Anal. Calc. for $C_{25}H_{29}NO_{13} \cdot H_2O$: C, 52.71; H, 5.50; N, 2.46. Found: C, 52.71; H, 5.58; N, 2.13.

Method ii. A portion (0.2 g) of **8** from *b* was similarly deacylated with methanolic sodium methoxide, to afford **9** (0.12 g); $[\alpha]_D$ −59.3° (*c* 0.2, methanol).

p-*Nitrophenyl 4,6-O-benzylidene-3-O-α-*D*-galactopyranosyl-β-*D*-galactopyranoside* (**11**). — Compound **10** (0.1 g) in methanol (5 mL) containing a catalytic amount of sodium methoxide was kept overnight at room temperature. After de-ionization with Amberlite IR-120 (H^+) resin, the methanol was evaporated, and the residue was washed with ether–petroleum ether by decantation. Removal of the traces of solvents under diminished pressure afforded **11** as a slightly yellowish solid; $[\alpha]_D$ +22.1° (*c* 0.2, methanol).

Anal. Calc. for $C_{25}H_{29}NO_{13} \cdot 0.5\ H_2O$: C, 53.56; H, 5.41; N, 2.50. Found: C, 53.38; H, 5.34; N, 2.19.

Deacetalation of **9** *and* **11**. — A portion (0.2 g) of **9** (from *i*) in 60% aqueous acetic acid (5 mL) was stirred for 2.5 h at ~80°. Acetic acid was removed under diminished pressure by repeated co-evaporation with toluene. The residue crystallized from ethanol–methanol, to give **7** (0.1 g, 76.5%); m.p. 254–256°, $[\alpha]_D$ −33.4° (*c* 0.4, water).

A portion (0.05 g) of **11**, similarly deacetalated, gave, after freeze-drying of an aqueous solution, amorphous **5**, $[\alpha]_D$ +82.2° (*c* 0.33, water).

Permethylation and hydrolysis of a mixture of **9** *and* **11**. — A portion (0.1 g) of the second crop from *a* ($[\alpha]_D$ +25°) in *N,N*-dimethylformamide (5 mL) and methyl iodide (2 mL) was stirred overnight at room temperature in the presence of barium oxide (0.4 g) and barium hydroxide octahydrate (0.4 g). After the usual processing, the permethylated disaccharide was hydrolyzed with 0.5M sulfuric acid for 5 h at ~98°. The acid was neutralized with barium carbonate, the suspension filtered, the filtrate evaporated, and the residue dissolved in a small volume of methanol and examined by t.l.c. (15:1 chloroform–methanol); this showed two spots, attributable to 2,3,4,6-tetra-*O*-methylgalactose (fast), and a tri-*O*-methylgalactose. The latter compound moved in the same solvent-system, as well as in two other

systems especially recommended[15] for distinguishing methylated sugars (1 : 1 benzene–acetone, and 83 : 17 isopropyl ether–methanol), at a rate identical with that of authentic 2,4,6-tri-*O*-methyl-D-galactose[12], and clearly different from that of the slower-moving 2,3,4-tri-*O*-methyl-D-galactose[12], and also from that of 3,4,6-tri-*O*-methyl-D-galactose[13] (which had an intermediate mobility).

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