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ACETAL MIGRATION DURING GLYCOSYLATIONS CATALYZED BY MERCURIC CYANIDE: SYNTHESIS OF *p*-NITROPHENYL 6-*O*-β-D-GALACTOPYRANOSYL-β-D-GALACTOPYRANOSIDE*

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ABSTRACT

Isopropylidenation of *p*-nitrophenyl β-D-galactopyranoside under kinetic control afforded the 4,6-acetal (**2**). The structure of **2** was established by ^{1}H- and ^{13}C-n.m.r. spectroscopy, and confirmed by methylation, and hydrolysis to 2,3-di-*O*-methyl-D-galactose. Selective benzoylation of **2** with benzoyl chloride in pyridine afforded the 3-*O*-benzoyl derivative **3**. Isomerization of **3** in a phase-transfer type of reaction gave a mixture of **3** and its 2-*O*-benzoyl isomer **4**. Glycosidation of **4** (catalyzed by mercuric cyanide) with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**7**) gave a crystalline disaccharide derivative (**8**). *O*-Deacylation of **8** furnished the disaccharide acetal (**9**). The ^{13}C-n.m.r. spectrum of **9** revealed the presence of a 3,4-acetal, indicating that neither **9** nor **8** was a (1→3)-linked disaccharide derivative. Deacetylation of **9** afforded *p*-nitrophenyl 6-*O*-β-D-galactopyranosyl-β-D-galactopyranoside (**10**). The (1→6) linkage in **10** was established by permethylation, and hydrolysis to 2,3,4-tri-*O*-methyl-D-galactose. Compound **10** was converted into the crystalline 2-benzoate (**11**) of its 3,4,2′,3′,4′,6′-hexaacetate, and **11** afforded the amorphous 2,3,4,2′,3′,4′,6′-heptaacetate. The disaccharide **10** was also synthesized by condensation of the bromide **7** with *p*-nitrophenyl 2,3-di-*O*-acetyl-β-D-galactopyranoside (**6**), followed by *O*-deacetylation; compound **6** was obtained from **2** by way of the crystalline 4,6-acetal 2,3-diacetate.

INTRODUCTION

In recent years, endoglycosidases have proved to be excellent tools for the structural study of various complex saccharides, including glycoproteins[2] and glycolipids[3]. Recently, we reported that *p*-nitrophenyl 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-α-D-galactopyranoside is a suitable substrate for endo-α-*N*-acetyl-D-galactosaminidases of *Diplococcus pneumoniae*[4] and *Clostridium perfringens*[5]. It

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has also been observed that *p*-nitrophenyl 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-β-D-galactopyranoside acts as an inhibitor for endo-α-*N*-acetyl-D-galactosaminidase of *D. pneumoniae*[4]. Thus, for further substrate specificity, and particularly for inhibition studies of such endo-enzymes, we became interested in the synthesis of *p*-nitrophenyl 3-*O*-β-D-galactopyranosyl-β-D-galactopyranoside and related compounds. It is obvious that the availability of such inhibitors would also facilitate the purification of these enzymes by affinity chromatography. We describe here an attempted synthesis of *p*-nitrophenyl 3-*O*-β-D-galactopyranosyl-β-D-galactopyranoside which resulted, instead, in the synthesis of its (1→6)-linked isomer, because of acetal migration.

RESULTS AND DISCUSSION

Two approaches for the synthesis of *p*-nitrophenyl 3-*O*-β-D-galactopyranosyl-β-D-galactopyranoside seemed equally feasible. It was envisaged that the free disaccharide could first be prepared, and then the *p*-nitrophenyl aglycon could subsequently be attached *via* the acylated glycosyl halide, in a manner analogous to that already achieved in this laboratory[6]. Alternatively, a suitably protected *p*-nitrophenyl galactopyranoside derivative could be glycosylated, to afford the desired disaccharide. A few syntheses of 3-*O*-β-D-galactopyranosyl-D-galactose had been described[7–10]. Two of these procedures[9,10], which utilize the less readily accessible 1,2:5,6-di-*O*-isopropylidene-α-D-galactofuranose[11], were examined by us. In our hands, the purification of the reaction mixtures was cumbersome, and the isolated yields of the desired disaccharide were discouragingly low[12]. For this reason, coupled with the fact that the starting 1,2:5,6-diacetal was, at best, obtained in only ~22% yield, we did not pursue this approach any further. Instead, we attempted to utilize *p*-nitrophenyl 2-*O*-benzoyl-4,6-*O*-isopropylidene-β-D-galactopyranoside as an intermediate.

On isopropylidenation of the readily accessible *p*-nitrophenyl β-D-galactopyranoside[13] (**1**) in *N*,*N*-dimethylformamide with 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid for 3 h at room temperature, examination of the reaction mixture by thin-layer chromatography (t.l.c.) revealed the presence of the 4,6-acetal as the major product. Only a trace of the isomeric 3,4-acetal[14] was produced, along with some faster-migrating, unidentified products. After the customary processing, amorphous **2**, sufficiently pure for subsequent transformations, was obtained in ~93% yield. Further purification by chromatography on a column of silica gel afforded acetal **2** in ~84% yield. The ^{1}H-n.m.r. spectrum of purified **2** showed signals for the *p*-nitrophenyl protons at δ 8.27 and 7.27, and H-1 resonated as a doublet (δ 5.11) with a spacing of 8 Hz, consistent with an equatorial disposition for the *p*-nitrophenyl group. The acetal methyl groups resonated as two singlets at δ 1.5 and 1.43. In the spectrum of the 3,4-acetal[14], however, the resonance for the acetal methyl groups occurred at δ 1.54 and 1.38. Interestingly, similar differences in the chemical shifts of the isopropylidene groups of the isomeric 4′,6′- and 3′,4′-acetals of lactose have also been observed[15]. It is also noteworthy that the acetal methyl

groups of methyl 3,4-*O*-isopropylidene-α-D-galactopyranoside[16] and its 2,6-dimethyl ether[15,16] resonate as distinguishable singlets at δ 1.50 and 1.37, and δ 1.53 and 1.35, respectively, whereas those of the isomeric 4,6-acetal and its 2,3-dimethyl ether[15,16] were observed as singlets at δ 1.49 and 1.50, respectively.

The ^{13}C-n.m.r. spectrum of **2** showed a resonance for the acetal carbon atom at 100.4 p.p.m., and the chemical shifts for the acetal methyl groups were separated by ~10.7 p.p.m.*, in agreement with the presence of a 4,6-acetal ring[17]. The structure of **2** was further confirmed by converting it into 2,3-di-*O*-methyl-D-galactose. Thus, methylation of **2** according to Kuhn *et al.*[18], followed by acid hydrolysis, and comparison (t.l.c.) of the resulting dimethyl ether with authentic 2,3-di-*O*-methyl-D-galactose and its 2,6-isomer[15], revealed the presence of the former. The latter diether would have formed had the acetal group of **2** been situated at O-3 and O-4.

Benzoylation of **2** with ~1.2-molar equivalents of benzoyl chloride in pyridine for 2 h at −25° afforded a crystalline monobenzoyl derivative of **2** (78.5%) which was identified as *p*-nitrophenyl 3-*O*-benzoyl-4,6-*O*-isopropylidene-β-D-galactopyranoside (**3**) on the basis of its ^{1}H-n.m.r. spectrum. Thus, in the spectrum of **3**, a five-proton complex (δ 7.45–8.24) clearly indicated that only one benzoyl group had been introduced. A one-proton doublet of doublets (δ 5.28), with spacings of 10.5 and 4 Hz, assigned to H-3, was in accord with O-3 carrying an acyl group, as H-3 is the only proton (assuming that **3** adopts the $^4C_1$ conformation) having an

TABLE I

PARTIAL ASSIGNMENTS OF ^{13}C-N.M.R. RESONANCES FOR MONOSACCHARIDES **1–4** AND DISACCHARIDES **9** AND **10**[a]

Compound	*C-1*	*C-1′*	*C-5*	*C-5′*	*C-6*	*C-6′*	*O* / *(H₃C)₂C* / *O*	*C(CH₃)₂*
1[b]	100.73	—	75.90	—	60.43	[b]	—	—
2[c]	101.54	—	73.22	—	63.49	—	100.04	18.80 29.47
3[d]	101.22	—	75.37	—	62.98	—	99.20	18.99 29.80
4[d]	99.36	—	73.06	—	62.85	—	99.02	18.94 29.62
9[c]	101.13	105.74	76.66	80.56	70.14	62.55	111.25	26.48 28.31
10[e]	100.85	104.24	75.43	76.10	69.51	61.99	—	—

[a]p.p.m. from Me_4Si. [b]In Me_2SO-d_6. [c]In CD_3OD. [d]In acetone-d_6. [e]In D_2O.

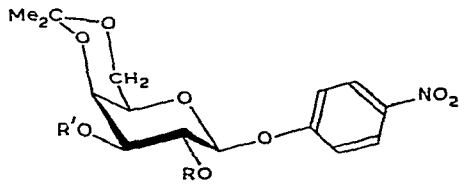
*In the ^{13}C-n.m.r. spectrum of the isomeric 3,4-acetal[14] in CD_3OD, the acetal carbon atom resonated at 111.11 p.p.m., and the chemical shifts for the methyl groups were separated by 2 p.p.m.

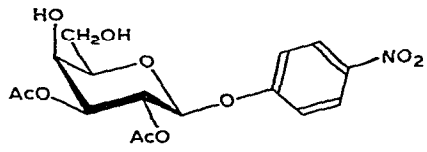
axial–axial and an axial–equatorial disposition with respect to two neighboring protons. The β configuration at the anomeric center was evidenced by a low-field, one-proton signal (δ 5.46), with a spacing of 8 Hz, attributable to H-1. The ^{13}C-n.m.r. spectrum of **3** (see Table I) also supported the β configuration, as C-1 occurred at 101.22 p.p.m., as well as the overall structure of **3**.

T.l.c. of the crude reaction-mixture indicated the presence, also, of traces of a faster-migrating contaminant (presumably the 2,3-diester), as well as a slower-moving contaminant. Neither of these compounds was separated or identified.

Treatment of a solution of **3** in dichloromethane with a low concentration of aqueous sodium hydroxide, in a phase-transfer type of reaction, led to its partial isomerization into the corresponding 2-benzoate (**4**). The isomerization equilibrium appeared to be shifted slightly in favor of **4**. Under these isomerization-reaction conditions, de-esterification** of the monobenzoates was negligible, and only a trace of **1** was revealed by t.l.c. Column-chromatographic separation gave crystalline **3** and amorphous **4** in the ratio of 5:7. The ^{1}H-n.m.r. spectrum of **4** showed signals in support of the structure assigned. Thus, the presence of a benzoyl group and a *p*-nitrophenyl group was evident from the nine-proton signals in the aromatic region. The signals for H-1 and H-2 were superimposed on each other; a double doublet (δ 5.70, J 13 and 8 Hz) could reasonably be assigned to H-2, whereas a doublet (δ 5.68, J 8 Hz) was attributable to H-1. The acetal methyl protons resonated as two singlets, at δ 1.52 and 1.45. In the ^{13}C-n.m.r. spectrum of **4**, the acetal methyl groups were located at 18.94 and 29.62 p.p.m., and the acetal carbon atom occurred at 99.02 p.p.m., in conformity with a 4,6-acetal. The C-1 resonance was shifted ~2.2 p.p.m. upfield from that of the parent compound **2**, and ~1.9 p.p.m. upfield from that of the isomeric benzoate **3**, a further indication that C-2 was substituted with a benzoyloxy group. The C-1 resonances of 2-*O*-substituted β-D-galactose derivatives have generally been found upfield of those of the corresponding 2-*O*-unsubstituted compounds[20].

Acetylation of **2** with an excess of acetic anhydride in pyridine afforded the diacetate **5**. Crude **5** was sufficiently pure (t.l.c.) to be converted into **6**. However,





2 R = R′ = H
3 R = H, R′ = Bz
4 R = Bz, R′ = H
5 R = R′ = Ac

6

An attempt[12] to conduct the isomerization as described for the corresponding benzyl galactoside[19] resulted in extensive debenzoylation of **3.

analytically pure **5** was obtained by crystallization from absolute alcohol. Deacetalation of crude **5** with aqueous trifluoroacetic acid in chloroform afforded the known 2,3-diacetate (**6**) in ~72% yield. On reacetalation as described for **2**, a sample of **6** regenerated **5** (82%), indicating that no acetyl migration had intervened.

Condensation of **4** with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**7**) in acetonitrile, in the presence of mercuric cyanide, for 4 h at room temperature gave one major product (t.l.c.), faster-moving than **4**; a trace of another product, slower-moving than **4**, was also present. Column-chromatographic purification of the reaction mixture, and recrystallization of the product isolated, afforded the disaccharide **8** (~82%). In the ^{1}H-n.m.r. spectrum of **8**, the presence of a benzoyl group and a *p*-nitrophenyl group was accounted for by the nine-proton signals (δ 7.0–8.4). Two doublets, with a spacing of 8 Hz each, appeared within the region δ 5.65–3.80, which also contained some unresolved signals. The lower-field signal (δ 5.18) was tentatively assigned to H-1, whereas its higher-field counterpart was assigned to H-1′, as these are the only protons in the spectrum of **8** that would be expected to give rise to doublets. The large coupling constants are indicative of the β configuration at both anomeric centers. The four acetyl-group resonances were observed as distinct singlets at δ 2.20, 2.08, 2.00, and 1.76, and the isopropylidene methyl groups occurred as well-separated singlets at δ 1.66 and 1.38.

The slow-moving (t.l.c.) minor product, which was presumably the (1→3)-linked disaccharide, was neither isolated nor characterized.

Deacylation of **8** with sodium methoxide in methanol gave amorphous **9**, which had an analysis corresponding to the dihydrate. The ^{1}H-n.m.r. spectrum of **9** contained two doublets (δ 8.33 and 7.37, *J* 10 Hz each) for the *p*-nitrophenyl group, and two singlets (δ 1.53 and 1.37) for the isopropylidene methyl groups. The signal for H-1 was resolved as a doublet (δ 5.04, *J* 8 Hz), and H-1′ resonated as a doublet (δ 4.40, *J* 8 Hz). In the ^{13}C-n.m.r. spectrum of **9**, the signal for the acetal carbon atom occurred at 111.25 p.p.m., and the acetal methyl resonances were observed at 26.48 and 28.31 p.p.m. The large (~11 p.p.m.) downfield shift for the resonance of the acetal carbon atom of **9** (compared to that of **2**), and the small (~1.8 p.p.m.) difference in the chemical shift of the acetal methyl groups, strongly suggested the presence of a five-membered acetal ring[17]. These values agree well with those observed for the 3,4-acetal (see footnote*). It is thus apparent that acetal migration did occur during the glycosylation reaction. On considering the crude condensation-product, it seems reasonable to assume that the rate of acetal migration exceeded that of glycosylation by far, as little or no (1→3′)-linked disaccharide was produced. Acetal migration had been observed several times during attempts to glycosylate 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose under the Koenigs–Knorr conditions[21–23] or by the orthoester method[24], leading mainly to the formation of the (1→6′)-linked disaccharide instead of the (1→3)-linked isomer expected. Such migration has been rationalized as being caused by the presence of $HgBr_2$, formed during the Koenigs–

Knorr reaction[21], but no mechanism has yet been suggested. A similar explanation may be envisaged for the case at hand. However, we are unaware of an example of 4,6- to 3,4-acetal migration on the galactopyranose ring during similar glycosylation reactions. This appears to be the first instance of such a migration.

The acetal group of **9** was readily cleaved by treatment with 60% aqueous acetic acid for 2 h at ~80°, to afford crystalline **10** as the monohydrate. The ^{13}C-n.m.r. spectrum of **10** showed resonances for C-1 and C-1′ at 100.85 and 104.24 p.p.m. respectively, in agreement with the β configuration at both anomeric centers.

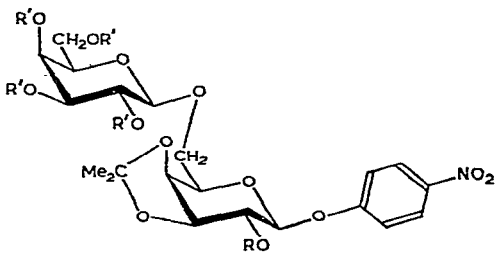
However, when a sample of the aforementioned, crude condensation-product (column-purified, but not crystallized) was deblocked, as already described, the resulting disaccharide was chromatographically (solvent *F*) identical to **10**, but had a substantially lower optical rotation, indicating that the crude condensation product was, presumably, contaminated with some of the α anomer.

Deacetalation of the fully protected disaccharide **8** with aqueous trifluoroacetic acid in chloroform, and acetylation of the resulting compound with 2:1 pyridine–acetic anhydride afforded crystalline **11**. The ^{1}H-n.m.r. spectrum of **11** contained signals in agreement with its assigned structure (see Experimental section).

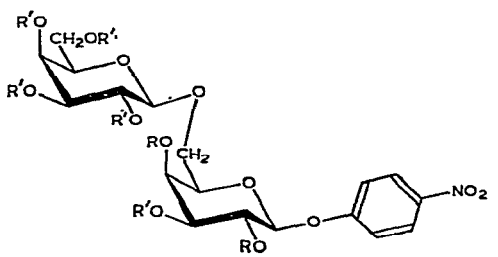
Acetylation of **10** with an excess of acetic anhydride in pyridine afforded the peracetylated disaccharide **12** as an analytically pure, white powder. The chromatographic mobility of **12** was identical with that of a sample of the fully acetylated disaccharide prepared by an independent route (see later).

Condensation of the 2,3-diacetate **6** with bromide **7**, in acetonitrile in the presence of mercuric cyanide, for 4 h at room temperature, followed by deacetylation with methanolic sodium methoxide of the crude product so obtained, yielded a free disaccharide having a specific rotation substantially lower than that of **10**. On peracetylation as described for **10**, however, examination of the product by t.l.c. (solvent *C*) showed one major compound (mobility identical to that of **12**), contaminated with a slower-migrating compound.

Purification of the crude, deacetylated product on a column of silica gel with 4:1 chloroform–methanol as the eluant, and recrystallization from chloroform–methanol, furnished pure disaccharide having physical constants similar to those of **10**.



8 R = Bz, R′ = Ac
9 R = R′ = H



10 R = R′ = H
11 R = Bz, R′ = Ac
12 R = R′ = Ac

Mercuric cyanide-catalyzed glycosylation of **6** with bromide **7** in 1:1 benzene–nitromethane afforded a disaccharide derivative of purity comparable to that obtained when the condensation was conducted in acetonitrile. In both cases, the major product was accompanied (t.l.c. solvent *G*) by some faster- and some slower-migrating contaminants.

Purification of a portion of the crude condensation-product by preparative-layer chromatography (p.l.c.) using solvent *G* as the irrigant, followed by acetylation, afforded a fully acetylated disaccharide which had a mobility (t.l.c. solvent *C*) identical to that of **12**. However, a small proportion of a slower-migrating contaminant was also present.

Deacetylation of the condensation product (purified by p.l.c.) with methanolic sodium methoxide furnished a disaccharide having a specific rotation close to that of **10**.

Permethylation of **10**, followed by acid hydrolysis, gave 2,3,4-tri-*O*-methyl-D-galactose, whereas no 2,4,6-tri-*O*-methyl-D-galactose was obtained. The isomeric trimethyl ethers were prepared according to literature procedure[15], and were clearly distinguishable in t.l.c.

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 a 0.25-mm layer 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*, 10:1 chloroform–methanol; *B*, 4:1 benzene–ethyl acetate; *C*, 2:1 benzene–ethyl acetate; *D*, 3:1 benzene–ethyl acetate; *E*, 5:1 chloroform–methanol; *F*, 3:1 chloroform–methanol; and *G*, 14:14:1 benzene–chloroform–methanol. Organic solutions were generally dried with anhydrous magnesium sulfate. Elemental analyses were performed by the 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 (Ft) mode; the positions of the peaks are expressed in p.p.m. from the Me_4Si signal.

p-*Nitrophenyl 4,6-O-isopropylidene-β-*D-*galactopyranoside* (**2**). — To a solution of *p*-nitrophenyl β-D-galactopyranoside (**1**) (3 g) in *N*,*N*-dimethylformamide (60 mL) were added *p*-toluenesulfonic acid (~30 mg) and 2,2-dimethoxypropane (7 mL). The mixture was stirred for 3 h at room temperature; t.l.c. (ethyl acetate) then showed the presence of a major product moving faster than **1**; there was also a small proportion of a faster-moving product (identical in mobility to the 3,4-isopropylidene acetal[14]), negligible proportions of still faster-moving contaminants, and a trace of unchanged **1**. Triethylamine (1 mL) was added, and the solution was evaporated under diminished

pressure at ~35°. The residue was taken up in a mixture of acetone and dichloromethane, the suspension filtered to remove some turbidity, the filtrate reevaporated, and the residue dissolved in a fresh portion of acetone. Precipitation by the addition of ether–pentane afforded acetal **2** (3.3 g) as a white, amorphous material, $[\alpha]_D$ −68.1° (*c* 1.3, acetone), which was sufficiently pure (t.l.c.) for subsequent acylations (see later). Chromatographically pure **2** was obtained by subjecting the crude product so obtained to chromatography on a column of silica gel. Elution with ethyl acetate removed the faster-migrating (t.l.c.) contaminants, and on evaporation, and reprecipitation from acetone solution as already described, fractions corresponding to **2** afforded the title-compound **2** (3 g, 84.3%), amorphous, $[\alpha]_D$ −72.2° (*c* 1.1, acetone); n.m.r. data (CD_3OD): δ 8.27 and 7.27 (d, 2 × 2 H, *J* 10 Hz, $C_6H_4NO_2$), 5.11 (d, 1 H, *J* 8 Hz, H-1), 1.50 and 1.43 (s, 2 × 3 H, CMe_2), 4.85 (broad s, superimposed on H_2O peak, 1 OH), and 4.50–3.60 (unresolved signals, 6 H).

Methylation of **2** *and hydrolysis of the product.* — A solution of **2** (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[15], 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. (solvent *A*) showed the presence of one compound identical in mobility with authentic 2,3-di-*O*-methyl-D-galactose, and clearly distinguishable from authentic, faster-migrating 2,6-di-*O*-methyl-D-galactose[15].

p-*Nitrophenyl 3-O-benzoyl-4,6-O-isopropylidene-β-D-galactopyranoside* (**3**). — To a cold (−25°), stirred solution of crude acetal **2** (10 g) in dry pyridine (150 mL) was added, dropwise, a solution of benzoyl chloride (5 g) in pyridine (50 mL) during 0.5 h. After being stirred for 1.5 h at −25°, the mixture was allowed to warm to room temperature (~1 h). T.l.c. (solvent *B*) then indicated the formation of one major product, faster-moving than **2**. A slightly faster-moving compound (presumably the 2,3-diester) was also present, together with traces of slower-migrating contaminants. The mixture was diluted with chloroform (150 mL), successively washed with ice-cold water, ice-cold 10% aqueous hydrochloric acid, cold water, cold saturated sodium hydrogencarbonate, and water, then dried, and evaporated. On crystallization from methanol, the residue gave compound **3** (10.2 g, 78.5%), m.p. 220–222°, $[\alpha]_D$ +20.8° (*c* 0.6, chloroform), +31.65° (*c* 1.4, acetone); n.m.r. data (CD_3COCD_3): δ 8.30 and 7.38 (s, 2 × 2 H, *J* 10 Hz, $C_6H_4NO_2$), 8.24–8.05 (complex, 2 H, C_6H_5CO), 7.80–7.45 (complex, 3 H, C_6H_5CO), 5.46 (d, 1 H, *J* 8 Hz, H-1), 5.38 (dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 4 Hz, H-3), 2.89 (s, OH), 1.41 and 1.37 (s, 2 × 3 H, CMe_2), and 4.70–3.80 (unresolved signals, 5 H).

Anal. Calc. for $C_{22}H_{23}NO_9$: C, 59.32; H, 5.21; N, 3.14. Found: C, 59.54; H, 5.35; N, 3.24.

p-*Nitrophenyl 2-O-benzoyl-4,6-O-isopropylidene-β-D-galactopyranoside* (**4**). — To a stirred solution of 3-benzoate **3** (3.5 g) in dichloromethane (150 mL) were added

tetrabutylammonium chloride (0.1 g) and 0.05M aqueous sodium hydroxide (10 mL). The mixture was vigorously stirred for 0.5 h at room temperature; t.l.c. (solvent *B*) then indicated partial conversion of **3** into a slower-migrating, slightly preponderant compound. A trace of **1** (resulting from debenzoylation) was also present. The base was neutralized by the addition of a few drops of glacial acetic acid, and the aqueous layer was separated. The dichloromethane solution was washed three times with water (50 mL), dried, and concentrated to a small volume. The concentrate was applied to a column of silica gel, and eluted with solvent *B*. On evaporation, the first fractions yielded crystalline **3** (1.3 g); the fraction containing the slower-migrating compound afforded the 2-*O*-benzoyl derivative **4** (1.8 g), amorphous, $[\alpha]_D$ −31.5° (*c* 0.8, chloroform), −18.75° (*c* 0.6, acetone); n.m.r. data (CD_3COCD_3): δ 8.23 and 7.28 (d, 2 × 2 H, *J* 10 Hz, $C_6H_4NO_2$), 8.2–8.0 (complex, 2 H, C_6H_5CO), 7.7–7.4 (complex, 3 H, C_6H_5CO), 5.70 (dd, 1 H, $J_{1,2}$ 8, $J_{2,3}$ 13 Hz, H-2), 5.68 (d, 1 H, *J* 8 Hz, H-1), 2.98–2.86 (broad s, 1 H, OH), 1.52 and 1.45 (s, 2 × 3 H, CMe_2), and 4.6–3.8 (unresolved signals, 5 H).

Anal. Calc. for $C_{22}H_{23}NO_9$: C, 59.32; H, 5.21; N, 3.14. Found: C, 59.66; H, 5.31; N, 3.06.

p-*Nitrophenyl 2,3-di*-O-*acetyl-4,6*-O-*isopropylidene-β*-D-*galactopyranoside* (**5**).— A solution of the crude acetal **2** (see earlier) (2 g) in pyridine (20 mL) and acetic anhydride (10 mL) was kept overnight at room temperature. T.l.c. (solvent *C*) then revealed the presence of one faster-migrating product, together with traces of slower-migrating contaminants. The pyridine and acetic anhydride were evaporated under diminished pressure (~40°), the last traces being removed by co-evaporation with several portions of toluene. The solid residue (2.4 g, 96%) was sufficiently pure (t.l.c., solvent *C*) to be utilized in the next step. An analytical sample was recrystallized from absolute alcohol, m.p. 167–169°, $[\alpha]_D$ +1.6° (*c* 0.9, chloroform).

Anal. Calc. for $C_{19}H_{23}NO_{10}$: C, 53.63; H, 5.46; N, 3.29. Found: C, 53.55; H, 5.73; N, 3.14.

p-*Nitrophenyl 2,3-di*-O-*acetyl-β*-D-*galactopyranoside* (**6**). — A solution of crude **5** (2 g) in chloroform (90 mL) containing trifluoroacetic acid (9 mL) and water (1 mL) was kept for 20 min at room temperature. T.l.c. (ethyl acetate) then showed the presence of one slow-moving product. The solvents were evaporated, and several portions of toluene were added to, and evaporated from, the residue, to give crude **6** (1.6 g). Recrystallization from acetone–pentane gave pure **6** (1.3 g, 71.8%), m.p. 177–179° (undepressed on admixture with an authentic sample[25]), $[\alpha]_D$ −5.1° (*c* 0.8, chloroform); lit.[25] m.p. 177–178°, $[\alpha]_D$ −5.2° (*c* 0.67, chloroform).

A portion (0.2 g) of **6** was isopropylidenated as described for **2**. After processing, and recrystallization from absolute alcohol, it yielded **5** (0.18 g, 81.8%), m.p. 167–169°.

Condensation of **4** *with 2,3,4,6-tetra*-O-*acetyl*-α-D-*galactopyranosyl bromide* (**7**). — To a solution of **4** (1 g) in acetonitrile (25 mL) were added mercuric cyanide (0.75 g) and mercuric bromide **7** (1.6 g). The mixture, stirred for 4 h at room temperature then showed the disappearance (solvent *D*) of **4**, and the formation of one major

product, faster-moving than **4**. A small amount of another product, slower than **4**, was also present. The solvent was evaporated, the residue stirred with chloroform, and the suspension filtered through glass wool. The filtrate was successively washed with water, M aqueous potassium iodide, aqueous sodium hydrogencarbonate, and water, then dried, and evaporated. Purification of the product on a column of silica gel, with solvent *B* as the eluant, gave a solid (1.6 g), homogeneous in t.l.c. (solvent *D*), $[\alpha]_D$ −22.2° (*c* 0.6, chloroform). On recrystallization from ethyl acetate–hexane, it yielded disaccharide **8** (1.42 g, 81.6%), m.p. 151–153°, $[\alpha]_D$ −39.2° (*c* 0.7, chloroform); n.m.r. data ($CDCl_3$): δ 8.26 and 7.07 (d, 2 × 2 H, *J* 10 Hz, $C_6H_4NO_2$), 8.16–8.0 (complex, 2 H, C_6H_5CO), 7.7–7.35 (complex, 3 H, C_6H_5CO), 5.18 (d, 1 H, *J* 8 Hz, H-1), 4.60 (d, 1 H, *J* 8 Hz, H-1′), 2.20, 2.08, 2.00, 1.76 (s, 3 H, each, CH_3CO), 1.66 and 1.38 (s, 2 × 3 H, CMe_2), and 5.65–3.80 (unresolved signals, 12 H).

Anal. Calc. for $C_{36}H_{41}NO_{18}$: C, 55.73; H, 5.34; N, 1.80. Found: C, 55.93; H, 5.37; N, 2.04.

p-*Nitrophenyl 6-O-β-D-galactopyranosyl-3,4-O-isopropylidene-β-D-galactopyranoside* (**9**). — Compound **8** (0.8 g) in methanol (80 mL) containing 0.1M sodium methoxide in methanol (10 mL) was kept for 6 h at room temperature; t.l.c. (solvent *E*) then indicated the formation of one product, slower than **8**. After de-ionization with Amberlite IR-120 (H^+) resin, and evaporation, the slightly yellowish residue was dissolved in water, the solution treated with a little charcoal, the suspension filtered with the aid of Celite, and the filtrate freeze-dried, to give compound **9** (0.5 g, 96%), amorphous, $[\alpha]_D$ −40.6° (*c* 0.3, water); n.m.r. data (CD_3OD): δ 8.33 and 7.37 (d, 2 × 2 H, *J* 10 Hz, $C_6H_4NO_2$), 5.04 (d, 1 H, *J* 8 Hz, H-1), 4.40 (d, 1 H, *J* 8 Hz, H-1′), 1.53 and 1.37 (s, 2 × 3 H, CMe_2), and 4.7–3.2 (unresolved signals).

Anal. Calc. for $C_{21}H_{29}NO_{13} \cdot 2\ H_2O$: C, 46.74; H, 6.18; N, 2.59. Found: C, 47.01; H, 5.39; N, 2.41.

p-*Nitrophenyl 6-O-β-D-galactopyranosyl-β-D-galactopyranoside* (**10**). — The disaccharide acetal **9** (0.5 g) was stirred in 60% aqueous acetic acid (10 mL) for 2 h at 80°. T.l.c. (solvent *F*) then showed the formation of a single, slower-migrating product. The acetic acid was evaporated off under diminished pressure, the last traces being removed by co-evaporation with toluene. The slightly yellowish residue (0.46 g) was dissolved in methanol, the solution decolorized with a little charcoal, the suspension filtered through a mixed bed of Celite and silica gel, and the filtrate evaporated. Crystallization from chloroform–methanol gave disaccharide **10**, m.p. 224–226°, $[\alpha]_D$ −59.3° (*c* 0.6, water).

Anal. Calc. for $C_{18}H_{25}NO_{13} \cdot H_2O$: C, 44.90; H, 5.66; N, 2.91. Found: C, 44.88; H, 5.77; N, 2.67.

In another experiment, the column-purified, condensation product (without recrystallization) was deacylated, and the acetal groups cleaved exactly as described for crystalline **8**, to give a disaccharide, $[\alpha]_D$ −29.7° (*c* 0.8, water), which had identical mobility (t.l.c., solvent *F*) with **10**.

p-*Nitrophenyl 3,4-di-O-acetyl-2-O-benzoyl-6-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-galactopyranoside* (**11**). — A solution of the fully protected

disaccharide **8** (0.2 g) in chloroform (9 mL) was treated with trifluoroacetic acid (0.9 mL) and water (0.1 mL), and the solution kept for 0.5 h at room temperature, and evaporated under diminished pressure; several portions of toluene were added to, and evaporated from, the residue, which was then dried *in vacuo*, and directly acetylated in a mixture of acetic anhydride (2 mL) and pyridine (4 mL) overnight at room temperature. The solution was evaporated under diminished pressure, and the solid residue was recrystallized from ethyl acetate–ether–hexane, to yield compound **11** (0.16 g), m.p. 228–230°, $[\alpha]_D$ −14.3° (*c* 0.8, chloroform); n.m.r. data ($CDCl_3$): 8.25 and 7.22 (d, 2 × 2 H, *J* 10 Hz, $C_6H_4NO_2$), 8.1–7.4 (complex, 5 H, C_6H_5CO), 5.3 (d, 1 H, *J* 8 Hz, H-1), 4.54 (d, 1 H, *J* 8 Hz, H-1′), 2.23, 2.21, 2.08, 2.00, 1.95, and 1.88 (s, 6 × 3 H, CH_3CO), and 6.0–3.7 (unresolved signals, 12 H).

Anal. Calc. for $C_{37}H_{41}NO_{20}$: C, 54.20; H, 5.05; N, 1.71. Found: C, 54.11; H, 5.30; N, 1.49.

p-*Nitrophenyl 2,3,4-tri*-O-*acetyl-6*-O-(*2,3,4,6-tetra*-O-*acetyl-β*-D-*galactopyranosyl*)-*β*-D-*galactopyranoside* (**12**). — A portion (50 mg) of **10** was kept overnight at room temperature in a mixture of pyridine (4 mL) and acetic anhydride (2 mL), whereupon t.l.c. (solvent *C*) showed its complete conversion into a single, faster-migrating compound. The pyridine and acetic anhydride were evaporated under diminished pressure, the last traces being removed by co-evaporation with toluene. Attempted crystallization of the residue afforded the peracetylated disaccharide **12** (70 mg) as a white powder, $[\alpha]_D$ −23.1° (*c* 0.3, chloroform).

Anal. Calc. for $C_{32}H_{39}NO_{20}$: C, 50.72; H, 5.20; N, 1.85. Found: C, 50.42; H, 5.45; N, 1.91.

Condensation of p-*nitrophenyl 2,3-di*-O-*acetyl-β*-D-*galactopyranoside* (**6**) *with bromide* **7** *in the presence of mercuric cyanide. — Method* (*a*). A mixture of the 2,3-diacetate **6** (0.8 g), the bromide **7** (1.6 g), and mercuric cyanide (1 g) in acetonitrile (25 mL) was stirred for 4 h at room temperature. After the usual processing, the foamy residue (2.2 g) was stirred with ethanol–ether–hexane, and the solvents were decanted. The solid residue weighed 1.6 g, and showed in t.l.c. (3:1 ethyl acetate–hexane, or solvent *G*), one major compound, faster than **6**. Traces of slightly slower-migrating and slightly faster-migrating contaminants were also present. The crude product was deacetylated for 4 h at room temperature in methanol (40 mL) containing 0.1M sodium methoxide in methanol (4 mL). After de-ionization with Amberlite IR-120 (H^+) resin, and evaporation of the solvent, a fresh portion of methanol was added, followed by chloroform–ether. The white solid that was precipitated was filtered off, and washed with ether; wt. 0.95 g, $[\alpha]_D$ −37.3° (*c* 1.3, water). Re-examination of this material by t.l.c. (solvent *F*) showed it to be contaminated with a small proportion of a slower-moving compound.

A portion (50 mg) of this crude compound was acetylated exactly as described for **10**, to give one major compound having chromatographic mobility (solvent *C*) identical to that of **12**, but it was contaminated with a trace of a slower-moving compound; it had $[\alpha]_D$ −16° (*c* 1.5, chloroform).

The rest of the crude, deacetylated product was purified by chromatography

on a column of silica gel with 4:1 chloroform–methanol as the eluant. Evaporation of the eluate, and crystallization of the residue from chloroform–methanol, afforded disaccharide **10**, m.p. 224–226°, $[\alpha]_D$ −60.3° (*c* 0.7, water).

Method (*b*). A stirred solution of **6** (0.8 g) in 1:1 benzene–nitromethane (120 mL) was boiled under reflux until 40 mL of the solvents had been distilled. It was then cooled to room temperature, mercuric cyanide (0.51 g) and bromide **7** (0.8 g) were added, and the mixture was stirred for 16 h at room temperature. Additional portions of mercuric cyanide (0.51 g) and bromide **7** (0.8 g) were added, and stirring was continued for a further 24 h. Processing in the usual way gave a foam which was stirred with benzene (10 mL) and ether (20 mL). Addition of a large excess of pentane, and filtration of the resulting precipitate, yielded 2.3 g of a powder, which, in t.l.c. (solvent *G*), showed one major product, accompanied by traces of some faster-migrating and some slower-migrating contaminants. Purification of this product by preparative-layer chromatography (p.l.c.) with solvent *G*, afforded a disaccharide derivative having $[\alpha]_D$ −12.6° (*c* 0.5, chloroform).

A portion (~50 mg) of this material was acetylated with 2:1 pyridine–acetic anhydride, as described in (a), to yield the fully acetylated disaccharide (70 mg) which had chromatographic mobility (solvent *C*) identical to that of **12**; $[\alpha]_D$ −18.2° (*c* 0.45, chloroform). A trace of a slower-moving contaminant was also revealed by t.l.c.

A sample of the p.l.c.-purified disaccharide derivative was deacetylated with sodium methoxide in methanol. Crystallization of the product from chloroform–methanol gave **10**, $[\alpha]_D$ −56.9° (*c* 0.4, water).

Permethylation and hydrolysis of **10**. — A solution of **10** (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[15], the resulting 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 15:1 chloroform–methanol, and examined by t.l.c., which 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*, at a rate the same as that of authentic 2,3,4-tri-*O*-methyl-D-galactose[15], and clearly different from that of the faster-moving 2,4,6-tri-*O*-methyl-D-galactose[15], and also from that of 3,4,6-tri-*O*-methyl-D-galactose[26] (which had an intermediate mobility).

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*This solvent system is especially recommended for distinguishing between the isomeric, trimethyl ethers of D-galactose[27]. See also ref. 15.

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