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STUDIES ON SYNTHESIS OF 1,2-*cis*-D-GALACTOSIDES WITH PARTIALLY BENZYLATED INTERMEDIATES*

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ABSTRACT

The 1-bromides of *p*-nitrobenzoylated 2-*O*-benzyl- (**1**) and 2,3- (**8**) and 2,6-di-*O*-benzyl-D-galactose (**14**) and 2-*O*-benzyl-D-glucose (**20**) were treated under Koenigs–Knorr conditions with 6-(trifluoroacetamido)-1-hexanol. Examination of the products by p.m.r. spectroscopy, g.l.c., and mass spectrometry revealed that, whereas the major product derived from **14** was the pyranoside (**19**), the glycosides derived from both **1** and **8** were mainly furanosides. The corresponding glycoside from **20** was entirely the pyranoside (**23**).

INTRODUCTION

We prepared the 2-benzyl, 2,3-dibenzyl, and 2,6-dibenzyl ethers of D-galactose earlier[1] for the ultimate purpose of synthesis of α-D-galactopyranosides. In the current study, these benzyl ethers were *p*-nitrobenzoylated, and the esters converted into their respective 1-bromides, which were condensed with 6-(trifluoroacetamido)-1-hexanol under Koenigs–Knorr conditions. The aglycon chosen for this study was designed for eventual linkage of the glycosides thus synthesized to macromolecules or solid matrices for biological investigations on the roles of carbohydrates[2,3].

RESULTS AND DISCUSSION

p-Nitrobenzoylation of 2-*O*-benzyl-D-galactose (**1**) was performed as described for the corresponding *gluco* analog by Ishikawa and Fletcher[4]. Thin-layer chromatography (t.l.c.) of the reaction mixture revealed two closely-migrating components (**2**) which could be separated and purified by fractional recrystallization from ethyl acetate. On subsequent treatment with hydrogen bromide, only the faster-moving component (minor product) appeared to have reacted.

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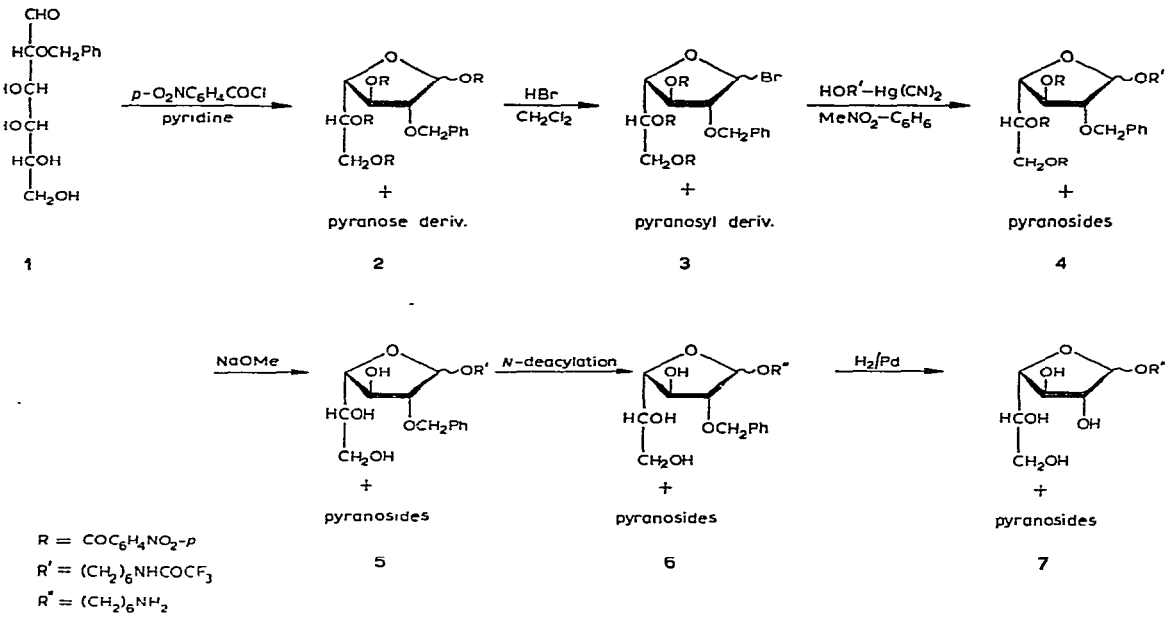
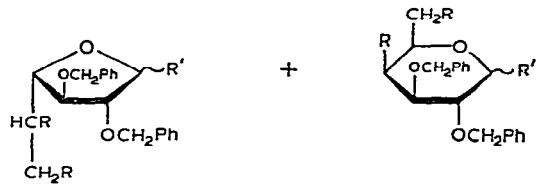


Similarly, the reaction mixtures from *p*-nitrobenzoylation of both 2,3- (**8**) and 2,6-di-*O*-benzyl-D-galactose (**14**) produced multiple spots in t.l.c., and for both, differences in the reactivity of these *p*-nitrobenzoates with hydrogen bromide were again observed. Therefore, the *p*-nitrobenzoylation reaction-conditions were modified for the 2-benzyl ether, as well as the 2,6-dibenzyl ether, to increase the relative amount of the reactive species. Such modification was not necessary for *p*-nitrobenzoylation of 2,3-di-*O*-benzyl-D-galactose, as the reactive *p*-nitrobenzoate in this case constituted the major fraction of material.

The differential reactivity of the *p*-nitrobenzoates towards hydrogen bromide was somewhat unexpected. No discernible differences in reactivity towards hydrogen bromide have been found for the α- and β-*p*-nitrobenzoates of 2-*O*-benzyl-D-glucopyranose and of 2,3-di-*O*-benzyl-D-glucopyranose[4]. In addition, it has been shown[5] that both anomers of 2,3,5-tri-*O*-benzyl-1-*O*-(*p*-nitrobenzoyl)-D-arabinofuranose react with hydrogen bromide to form the same 1-bromide. In view of the finding that both furanosides and pyranosides were present in the final 6-aminohexyl glycosides, it is possible that the difference observed in the reactivity of these *p*-nitrobenzoates towards hydrogen bromide may originate from differences in the ring forms and their respective anomers. However, the precise rates of reaction of these possible isomers towards hydrogen bromide were not determined.

Because of the known instability of some 1-bromides, the reaction products from the treatment with hydrogen bromide were only partially purified before they were treated with 6-(trifluoracetamido)-1-hexanol under Koenigs–Knorr conditions employing mercuric cyanide. The desired products were isolated by gel-filtration chromatography.



	R	R'
8	OH	OH
9	$OCOC_6H_4NO_2$-*p*	$OCOC_6H_4NO_2$-*p*
10	$OCOC_6H_4NO_2$-*p*	Br
11	$OCOC_6H_4NO_2$-*p*	$O(CH_2)_6NHCOCF_3$
12	OH	$O(CH_2)_6NHCOCF_3$
13	OH	$O(CH_2)_6NH_2$

P.m.r. spectra obtained at various stages of the sequence of deacylation and debenzylation were in general agreement with the structural assignments, although unambiguous anomeric assignment was not possible owing to the presence of signals of benzyl methylene groups appearing in the 4–5 p.p.m. range. After debenzylation, it was possible to examine anomeric proton resonances.

In the p.m.r. spectrum of the final glycoside derived from 2-*O*-benzyl-D-galactose, no signal was seen at the position corresponding to the anomeric proton of 6-aminohexyl β-D-galactopyranoside, indicating the absence of the β-pyranoside. Doublets at this position were, however, present in the spectra of the final glycosides originating from 2,3- and 2,6-di-*O*-benzyl-D-galactose, corresponding to 10 and 25 mole %, respectively.

In all three cases, a downfield signal appearing at δ 5.4–5.5, and corresponding to 75–100 mole % of the anomeric signal, was observed. However, the signal was not the clearly defined doublet expected from a simple α-anomeric proton, but a composite signal formed by two doublets (each having $J_{1,2}$ 2–3 Hz). Based on gas–liquid chromatography (g.l.c.) analyses (see Table I, discussed later), it may be concluded that the two downfield doublets are signals from the anomeric protons of the α-pyranoside and the two furanoside anomers. Indeed, the α-anomeric proton signal (δ 5.42, $J_{1,2}$ 2.0 Hz) of 6-aminohexyl α-D-galactopyranoside synthesized by another method* coincides with the upper-field doublet (δ 5.43) seen in these spectra.

The relative proportions of pyranosides and furanosides were analyzed by g.l.c. of their 1-*O*-acetyl-tetra-*O*-methyl derivatives. All four of the isomers possible, *viz.*, the α- and β-pyranosides and α- and β-furanosides, were separable from each other. The results of this analysis are shown in Table I.

Whereas furanosides were the preponderant products in Koenigs–Knorr reactions with intermediates derived from 2-*O*-benzyl-D-galactose and 2,3-di-*O*-

*J. Schneider and Y. C. Lee, unpublished results.

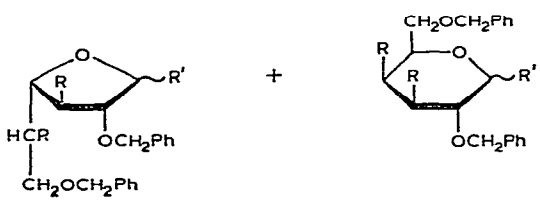
TABLE I

SYNTHESIS OF 6-AMINOHEXYL GLYCOSIDES

Intermediate	*Yields*		
	Overall[a] (%)	*of furanoside*[b] (%)	*of pyranoside*[b] (%)
2-*O*-Benzyl-D-galactose	42	81	19
2,3-Di-*O*-benzyl-D-galactose	51	83	17
2,6-Di-*O*-benzyl-D-galactose	64	22	78
2-*O*-Benzyl-D-glucose	49	0	100

[a]Based on the respective intermediate. [b]Percentage of total glycosides formed.

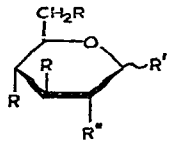
benzyl-D-galactose, the major portion of the glycoside originating from 2,6-di-*O*-benzyl-D-galactose was in the pyranose form. The high percentage of furanosides in two of these samples was not expected, because, in general, pyranoses are more stable than furanoses. Although the axial OH-4 in D-galactose tends to increase the proportion of furanosides as compared to D-glucose, it is not clear why the 2-benzyl and 2,3-dibenzyl ethers yielded such high proportions of furanosides.



	R	R'
14	OH	OH
15	$OCOC_6H_4NO_2$-*p*	$OCOC_6H_4NO_2$-*p*
16	$OCOC_6H_4NO_2$-*p*	Br
17	$OCOC_6H_4NO_2$-*p*	$O(CH_2)_6NHCOCF_3$
18	OH	$O(CH_2)_6NHCOCF_3$
19	OH	$O(CH_2)_6NH_2$

The ring isomers were, perhaps, formed prior to, or during, *p*-nitrobenzoylation, because, after *p*-nitrobenzoylation of the available hydroxyl groups, ring isomerization would be unlikely to occur. In 2,6-di-*O*-benzyl-D-galactose, repulsive interactions between the 2-*O*-benzyl and 6-*O*-benzyl groups are expected to be greater in the furanose form than in the pyranose form. The furanose form is probably more favored for 2,3-di-*O*-benzyl-D-galactose, as it allows for further separation of the adjacent benzyl ether groups. The reasons for the large proportion of furanosidic product obtained with 2-*O*-benzyl-D-galactose are, however, not clear.

These results indicate that, although the 2-benzyl and 2,3-dibenzyl ethers of D-galactose may be useful for synthesis of α- and β-D-galactofuranosides, only the 2,6-dibenzyl ether appears to have potential in the synthesis of α-D-galactopyranosides.



	R	R'	R''
20	OH	OH	OCH_2Ph
21	$OCOC_6H_4NO_2$-*p*	$O(CH_2)_6NHCOCF_3$	OCH_2Ph
22	OH	$O(CH_2)_6NH_2$	OCH_2Ph
23	OH	$O(CH_2)_6NH_2$	OH

Methanolysis of 2-*O*-benzyl-3,4,6-tri-*O*-*p*-nitrobenzoyl-D-glucopyranosyl bromide has been reported[4] to give the α-glycoside stereoselectively (α:β = 24:1). In the current study, estimations of the anomeric ratio of the 6-aminohexyl D-glucopyranosides by integration of p.m.r. signals indicated α:β = 7:3. Thus, either the nature of the nucleophile [6-(trifluoroacetamido)-1-hexanol], or its ratio to the 1-bromide, appears to have some effect on the anomeric ratio, although the preponderance of the α anomer still remains.

EXPERIMENTAL

General. — Pyridine and dimethyl sulfoxide (Me_2SO) were dried by refluxing over calcium hydride, followed by distillation, and were stored over Linde Type 4A Molecular Sieve (Union Carbide) or calcium hydride. All other solvents used were of reagent grade and were dried by storage over Molecular Sieve. Saturated solutions of hydrogen bromide (Matheson Gas Products, East Rutherford, N.J.) in dichloromethane were prepared fresh each time by introducing the gas (led through a solution of phenol in benzene, followed by a column of calcium chloride) into chilled dichloromethane until saturation was achieved.

Silver oxide, methyl iodide, mercuric cyanide, and *p*-nitrobenzoyl chloride were purchased from J. T. Baker Chemical Co.; the last was recrystallized from benzene prior to use. Palladium (10%) on charcoal was obtained from Matheson, Coleman and Bell (Norwood, Ohio).

Methyl α-D-galactopyranoside monohydrate and methyl β-D-galactopyranoside were obtained from Pfanstiehl Laboratories, Inc. (Waukegan, Illinois), and methyl β-D-galactofuranoside was obtained by chromatography of a crude mixture of methyl D-galactosides on Dowex AG-1 X-2 (OH^-) (200–400 mesh), (Bio-Rad Laboratories, Richmond, California) as described by Austin *et al.*[6]. 6-(Trifluoroacetamido)-1-hexanol was prepared as already described[7].

G.l.c. of 1-*O*-acetyl-tetra-*O*-methyl sugars was conducted in a Perkin–Elmer model 990 gas chromatograph with flame-ionization detectors, on a column (1.8 m × 2 mm. i.d.) of 3% of OV-225 on Gas Chrom Q (100–120 mesh, Applied Sciences Laboratories, Inc., State College, Pennsylvania), with helium (60 ml/min) as the carrier gas at 140°. Mass spectrometry of the g.l.c. effluent was performed with a DuPont model 21-491 mass spectrometer operated at 70 eV and a source temperature of 160°.

All other general experimental procedures were the same as previously described[1,7,8].

*2-O-Benzyl-1,3,4(5),6-tetra-*O-(p-*nitrobenzoyl*)-D-*galactose* (**2**). — A solution of 2-*O*-benzyl-D-galactose* (2.7 g, 10 mmoles) in dry pyridine (14 ml) was heated for 3 h at 55°. A solution of *p*-nitrobenzoyl chloride (9.94 g, 53.5 mmoles) in pyridine (41 ml) was then added, and the mixture was stirred overnight at room temperature under anhydrous conditions. Cold, saturated sodium hydrogen carbonate (100 ml) was added, the mixture was extracted with dichloromethane (250 ml), and the extract was successively washed with saturated sodium hydrogen carbonate solution (250 ml) and water (2 × 200 ml), dried (sodium sulfate), and evaporated to a glassy solid (8.06 g, 93% yield).

T.l.c. in 7:1 (v/v) benzene–ether revealed the presence of two closely migrating components in approximately equal amounts; these were purified by fractional recrystallization from ethyl acetate.

Faster-moving component (**2-F**): m.p. 224–225°, $[\alpha]_D^{20}$ +123.3° (*c* 0.50, dichloromethane). The p.m.r. spectrum (Me_2SO-d_6) was consistent with the assigned structure: the signals from the *p*-nitrobenzoyl groups, complex multiplets (δ 7.8–8.6, m, 16 H), the benzyl group (δ 7.12, m, 5 H), and the anomeric proton (δ 6.94, d, 1 H, $J_{1,2}$ 3 Hz) were in the expected ratios.

Anal. Calc. for $C_{41}H_{30}N_4O_{18}$: C, 56.81; H, 3.49; N, 6.46. Found: C, 56.79; H, 3.60; N, 6.39.

Slower-moving component (**2-S**): m.p. 205–206°, $[\alpha]_D^{20}$ +75.4° (*c* 0.80, dichloromethane). The p.m.r. spectrum again showed the expected ratios of *p*-nitrobenzoyl group to benzyl group, and the overall characteristics were similar to those of **2-F**. P.m.r. data (Me_2SO-d_6): δ 6.36 (d, 1 H, anomeric proton, $J_{1,2}$ 8 Hz), 7.08 (m, 5 H, benzyl protons), and 7.90–8.48 (m, 16 H, *p*-nitrobenzoyl protons).

Anal. Calc. for $C_{41}H_{30}N_4O_{18}$: C, 56.81; H, 3.49; N, 6.46. Found: C, 56.81; H, 3.55; N, 6.29.

*2-O-Benzyl-3,4(5),6-tri-*O-(p-*nitrobenzoyl*)-D-*galactosyl bromide* (**3**). — Cold dichloromethane (200 ml) saturated with hydrogen bromide (about 9%) was added to **2** (a mixture of **2-F** and **2-S** was used) (4.16 g, 4.8 mmoles), and the resultant yellow solution was kept at 4°. After 1.5 h, t.l.c. in 7:1 (v/v) benzene–ether indicated that **2-F** had reacted completely, but that **2-S** had remained unchanged. The mixture was then

*Of the two forms of 2-*O*-benzyl-D-galactose[1], form B, having m.p. 143–144°, was used exclusively in this study.

diluted with dichloromethane (75 ml), filtered to remove precipitated *p*-nitrobenzoic acid, and the filtrate successively washed with cold, saturated sodium hydrogen carbonate (2 × 150 ml), water (2 × 150 ml), dried (sodium sulfate), and evaporated to a yellow syrup that became a glass on desiccation *in vacuo*. This material was used (without removal of unreacted **2-S**) in the Koenigs–Knorr reactions described.

6-(Trifluoroacetamido)hexyl 2-O-benzyl-3,4(5),6-tri-O-(p-nitrobenzoyl)-D-galactosides (**4**). — A 1:1 (v/v) mixture (140 ml) of nitromethane and benzene was concentrated to approximately half its volume and allowed to cool to 40°. In the resulting mixture were dissolved **3** (3.75 g, 4.8 mmoles) and 6-(trifluoroacetamido)-1-hexanol (1.02 g, 4.8 mmoles), and to this solution was added mercuric cyanide (1.22 g, 4.8 mmoles). The heterogeneous mixture was stirred for 18 h at 40° under anhydrous conditions, the mercuric salts were filtered off, and the precipitate was washed with benzene (75 ml). The filtrate and washing were combined, washed successively with cold, saturated sodium hydrogen carbonate (2 × 75 ml), and water (2 × 75 ml), dried (sodium sulfate), and evaporated to a syrup which afforded a glass on further drying *in vacuo*. This was purified by gel filtration on a column (5 × 150 cm) of Sephadex LH-20 with 90% acetic acid as the eluant, the effluent being analyzed by colorimetry (for sugar) and by t.l.c. (for sugar and trifluoroacetamido group). Repeated gel-filtration successfully removed most of the 6-(trifluoroacetamido)-1-hexanol and the unreacted *p*-nitrobenzoate, to give **4** as a syrup (1.92 g; 44%, based on **2**). The p.m.r. spectrum ($CDCl_3$) was consistent with the structure assigned. Although no anomeric proton signal(s) was clearly discernible at this stage, there was a broad peak at δ 1.27–1.87, indicating the presence of the internal methylene protons (8 H) of an aminohexanol derivative. The terminal methylene protons (4 H) appeared as a broad multiplet at δ 3.24–3.52. Aromatic proton signals of the *p*-nitrobenzoyl groups, at δ 7.8–8.3 (m, 12 H), were in the expected ratio to those of the benzyl group, which were at δ 7.18 (m, 5 H).

Anal. Calc. for $C_{42}H_{39}F_3N_4O_{16}$: C, 55.26; H, 4.30; N, 6.13. Found: C,55.19; H, 4.33; N, 6.02.

The p.m.r. spectrum of the unreacted *p*-nitrobenzoate corresponded to that of **2-S**, indicating that this *p*-nitrobenzoate had not reacted in the sequence of reactions described.

6-Aminohexyl 2-O-benzyl-D-galactoside (**6**). — A solution of **4** (0.5 g, 0.54 mmole) in dry chloroform (1.9 ml) was mixed with dry methanol (1.9 ml) and 0.34M sodium methoxide (0.2 ml), and the mixture was kept overnight at room temperature. Dowex-50 X-8 (H^+) (0.5 ml) was then added to remove cations, and the resin was filtered off, and washed extensively with 1:1 (v/v) chloroform–methanol. The filtrate and washings were combined, and evaporated to a white solid which was stirred with 2 ml of 50% ethanol. Insoluble material (methyl *p*-nitrobenzoate) was filtered off, and washed with 50% ethanol, and the combined filtrate and washings were evaporated to give 6-(trifluoroacetamido)hexyl 2-*O*-benzyl-D-galactoside (**5**) as a syrup in almost quantitative yield.

Compound **5** (253 mg) was dissolved in 2:1 (v/v) ethanol–water (3.8 ml) and

stirred with Amberlite IRA-400 (OH^-) ion-exchange resin (4 ml) for 3 h at room temperature to achieve *N*-deacylation. After filtration and extensive washing of the resin with 67% ethanol and 50% ethanol successively, the filtrate was evaporated to a syrup (190 mg; 95% yield, based on **4**). P.m.r. spectra in two solvents (acetone-d_6 and D_2O) indicated the presence of benzyl and 6-aminohexyl groups. In acetone-d_6: δ 1.2–1.8 (broad peak, 8 H, –C–CH_2–C–) and 7.20–7.48 (m, 5 H, aromatic protons). A downfield signal appearing as a doublet at δ 4.90 (1 H, $J_{1,2}$ 4 Hz) was assigned as an anomeric proton. In D_2O: δ 1.5–2.2 (broad peak, 8 H, –C–CH_2–C–) and 7.72 (m, 5 H, aromatic protons). In this solvent, an anomeric proton signal could not be unambiguously assigned.

6-Aminohexyl D-*galactosides* (**7**). — Compound **6** (190 mg, 0.51 mmole) was hydrogenolyzed in 90% ethanol (1.5 ml) with hydrogen in the presence of 10% palladium-on-charcoal (270 mg) in a Brown hydrogenator[9]. Debenzylation was complete after 40 h, and the catalyst was then filtered off and washed extensively with methanol; the filtrate and washings were combined, and evaporated to give an almost quantitative yield (based on **6**) of syrupy **7**. T.l.c. of this material in 3:2:1 (v/v) ethyl acetate–acetic acid–water revealed traces of 6-amino-1-hexanol; gel-filtration on Sephadex G-15 (2 × 140 cm) with 0.1M acetic acid as the eluant removed this impurity.

The p.m.r. spectrum (D_2O) was in agreement with the structure assigned: δ 1.7–2.3 (m, 8 H, –C–CH_2–C–), 2.97–3.37 (m, 2 H, N–CH_2–), 3.96–4.77 (m, 8 H, O–CH_2–, and sugar-ring protons), two doublets totaling 1 H; δ 5.44 ($J_{1,2}$ 2 Hz, H-1α, for pyranoside), and δ 5.50 [$J_{1,2}$ 3 Hz, furanosidic anomeric proton(s)]. No signal was seen at the position corresponding to the anomeric proton of 6-aminohexyl β-D-galactopyranoside* (δ 4.89, $J_{1,2}$ 7.0 Hz) (D_2O), thus indicating that the β-pyranoside was absent from the glycosides derived from 2-*O*-benzyl-D-galactose.

*2,3-Di-*O*-benzyl-1,4(5),6-tri-*O*-(*p*-nitrobenzoyl)-*D*-galactose* (**9**). — To a solution of syrupy **8** (3.78 g, 10.5 mmoles) in pyridine (75 ml) was added *p*-nitrobenzoyl chloride (6.9 g, 37.1 mmoles) at room temperature under anhydrous conditions. After 24 h, the mixture was processed as for **2**, giving a syrup which, on further drying, gave a glass (8.33 g, 98% yield). T.l.c. in 7:1 or 10:1 (v/v) benzene–ether showed three components in approximately equal amounts. P.m.r. data ($CDCl_3$): the ratio of *p*-nitrobenzoyl protons at δ 8.15–8.55 (m, 12 H) to benzyl protons at δ 7.31–7.51 (m, 10 H) was as expected. Two broad signals (δ 6.03–6.17 and 6.73–6.91), corresponding to about one proton each, were tentatively assigned to the anomeric protons of at least two of the three *p*-nitrobenzoates seen in t.l.c.

This mixture was used, without further purification, for preparation of the 1-bromide.

*2,3-Di-*O*-benzyl-4(5),6-di-*O*-(*p*-nitrobenzoyl)-*D*-galactosyl bromide* (**10**). — A solution of **9** (4.0 g, 4.95 mmoles) in dry dichloromethane (58 ml) was cooled to 4° and mixed with saturated hydrogen bromide in dichloromethane (58 ml). A crystalline precipitate of *p*-nitrobenzoic acid was formed immediately, but the mixture was kept

*M. Naoi, S. Roseman, and Y. C. Lee, unpublished data.

for 30 min at 4°. The products were processed as described for **3**, to yield a glass. T.l.c. in 10:1 (v/v) benzene–ether showed that the two faster-migrating components had reacted completely, whereas the slowest-migrating component had not reacted. As with **3**, this mixture (containing unreacted *p*-nitrobenzoate) was used in the following Koenigs–Knorr reaction.

*6-(Trifluoroacetamido)hexyl 2,3-di-*O*-benzyl-4(5),6-di-*O*-(p-nitrobenzoyl)-*D*-galactoside* (**11**). — A mixture of **10** (3.57 g, 4.95 mmoles), 6-(trifluoroacetamido)-1-hexanol (1.06 g, 4.95 mmoles), and mercuric cyanide (1.25 g, 4.95 mmoles) in nitromethane–benzene was stirred for 18 h at 40°. The products were processed as for **4**, to yield a syrup (2.28 g; 54% yield, based on **9**).

The p.m.r. spectrum ($CDCl_3$) showed the presence of various groups in the correct ratios: δ 1.21–1.82 (m, 8 H, –C–CH_2–C–), 7.16–7.37 (m, 10 H, aromatic protons of benzyl groups), 7.94–8.30 (m, 8 H, aromatic protons of *p*-nitrobenzoyl groups). As with the glycoside **4**, the anomeric signal(s) could not be unambiguously assigned. The p.m.r. spectrum ($CDCl_3$) of the unreacted *p*-nitrobenzoate (recovered as for **4**) resembled the spectrum of the mixture of *p*-nitrobenzoates used in the bromination, except that the complex peak at δ 6.73–6.91 was not present, indicating that this signal was attributable to the *p*-nitrobenzoate components that reacted with hydrogen bromide.

*6-Aminohexyl 2,3-di-*O*-benzyl-*D*-galactoside* (**13**). — A solution of **11** (1.72 g, 2.82 mmoles) in dry chloroform (9 ml) was treated with 2 mmoles of sodium methoxide in dry methanol (10 ml) overnight. Decationization with Dowex-50 X-8 (H^+) ion-exchange resin, and removal of methyl *p*-nitrobenzoate as described for **5**, yielded syrupy 6-(trifluoroacetamido)hexyl 2,3-di-*O*-benzyl-D-galactoside (**12**).

A solution of **12** (1.12 g, 2.02 mmoles) in 2:1 (v/v) ethanol–water (14 ml) was stirred with 16 ml of Amberlite IRA-400 (OH^-) ion-exchange resin for 6 h to remove the *N*-trifluoroacetyl group, and the suspension was then filtered. The filtrate and washings were combined, and evaporated to give syrupy **13** (0.91 g, 98.5% yield).

The p.m.r. spectrum ($CDCl_3$) of this material was in agreement with the structure assigned: δ 1.15–1.80 (m, 8 H, –C–CH_2–C–) and 7.13–7.42 (m, 10 H, aromatic protons). Unambiguous assignment of anomeric proton signal(s) was not possible, owing to the proximity of benzyl methylene resonances.

6-Aminohexyl D-*galactoside* (**7**) *from* **13**. — Compound **13** (0.87 g, 1.89 mmoles) was hydrogenolyzed for 48 h in 90% ethanol (15 ml) containing 1.3 g of 10% palladium-on-charcoal, and then purified on Sephadex G-15, according to the procedures already described, to give syrupy **7** (0.5 g, 95% yield). P.m.r. data (D_2O): δ 1.60–2.32 (m, 8 H, –C–CH_2–C–), 4.90 (d, 0.1 H, $J_{1,2}$ 7.0 Hz, β-anomeric proton). In addition, two doublets, totaling 0.9 H, were observed: δ 5.45 ($J_{1,2}$ 2 Hz, H-1α of the pyranoside), and 5.51 [$J_{1,2}$ 3 Hz, furanosidic anomeric proton(s)].

Anal. Calc. for $C_{12}H_{25}NO_6$: C, 51.59; H, 9.02; N, 5.01. Found: C, 51.62; H, 9.10; N, 4.88.

*2,6-Di-*O*-benzyl-1,3,4(5)-tri-*O*-(p-nitrobenzoyl)-*D*-galactose* (**15**). — When 2,6-di-*O*-benzyl-D-galactose (**14**) was treated with *p*-nitrobenzoyl chloride in pyridine at

4° as described for **9**, two major products could be seen in t.l.c. in 29:1 (v/v) benzene–ether. These components (designated **15-F** and **15-S** for the faster- and slower-migrating components) were partially purified by chromatography on silica gel with 29:1 (v/v) benzene–ether as the eluant. The p.m.r. spectra of the two fractions were consistent with the structure proposed: *p*-nitrobenzoyl protons (**15-F**, δ 7.99–8.55; **15-S**, 7.96–8.56) were present in the correct ratios to benzyl protons (**15-F**, δ 7.13–7.55; **15-S**, 7.09–7.61). Compound **15-F** had a doublet ($J_{1,2}$ 3.5 Hz) at δ 7.03 and was more dextrorotatory, $[\alpha]_D^{20}$ +83.1° (*c* 1.57, chloroform), than component **15-S**, $[\alpha]_D^{20}$ +69.0° (*c* 1.87, chloroform), the anomeric doublet signal ($J_{1,2}$ 8 Hz) of which appeared at δ 6.26.

Under the conditions employed here, **15-F** was reactive towards hydrogen bromide in dichloromethane, whereas **15-S** was not. The relative proportion of **15-S** was increased by the following treatment (as used for **2**). A solution of compound **14** (2.46 g, 6.8 mmoles) in pyridine (34 ml) was heated for 4 h at 55°; then additional pyridine (34 ml) was added, followed by *p*-nitrobenzoyl chloride (4.5 g, 24.1 mmoles). The mixture was stirred overnight at room temperature, and was processed in the usual way, to give a glass in almost quantitative yield. T.l.c. in 29:1 or 10:1 (v/v) benzene–ether showed a greater proportion of the **15-S** component in the mixture.

*2,6-Di-*O*-benzyl-3,4(5)-di-*O*-(*p*-nitrobenzoyl)-*D*-galactosyl bromide* (**16**). — A solution of **15** (3.23 g, 4.0 mmoles) in dichloromethane (86 ml) at 4° was mixed with cold dichloromethane (86 ml) saturated with hydrogen bromide, and the mixture was kept for 12 h at 4°, and then processed in the usual way, to give a glass that still contained unreacted *p*-nitrobenzoate; this was used directly in the following reaction.

*6-(Trifluoroacetamido)hexyl 2,6-di-*O*-benzyl-3,4(5)-di-*O*-(p-nitrobenzoyl)-D-galactoside* (**17**). — To a solution of **16** (2.9 g, 4.0 mmoles) in 1:1 (v/v) nitromethane–benzene (58 ml) were added 6-(trifluoroacetamido)-1-hexanol (0.85 g, 4.0 mmoles) and mercuric cyanide (1.01 g, 4.0 mmoles), and the mixture was stirred for 18 h at 40°; the reaction was then complete, as judged by t.l.c. The products were processed as already described, to give a syrup. After purification by gel filtration on Sephadex LH-20 (see procedure for **4**), syrupy **17** was obtained (2.32 g; 68% yield, based on **15**).

P.m.r. data ($CDCl_3$): δ 1.24–1.82 (m, 8 H, –C–CH_2–C–), 7.10–7.44 (m, 10 H, aromatic protons of benzyl groups), and 7.86–8.44 (m, 8 H, *p*-nitrobenzoyl protons); the anomeric signal(s) could not be assigned with certainty. The p.m.r. spectrum of the unreacted *p*-nitrobenzoate recovered was the same as that for the purified, slowest-migrating (t.l.c.) *p*-nitrobenzoate.

*6-Aminohexyl 2,6-di-*O*-benzyl-D-galactoside* (**19**). — A solution of **17** (1.07 g, 1.25 mmoles) in a mixture of dry chloroform (6 ml) and dry methanol (6 ml) was treated with sodium methoxide as already described. The resulting syrupy 6-(trifluoroacetamido)hexyl 2,6-di-*O*-benzyl-D-galactoside (**18**) was *N*-de(trifluoroacetyl)-ated with Amberlite IRA-400 (OH^-) ion-exchange resin (10 ml) in 50% ethanol as already described, to give syrupy **19** (0.52 g; 94% yield, based on **17**).

P.m.r. data in $CDCl_3$: δ 1.15–1.79 (m, 8 H, –C–CH_2–C–) and 7.11–7.23 (m, 10 H, aromatic protons); in acetone-d_6: δ 1.19–1.81 (m, 8 H, –C–CH_2–C–) and

7.15–7.55 (m, 10 H, aromatic protons). Anomeric protons could not be definitely assigned in either solvent.

6-Aminohexyl D-*galactoside* (**7**) *from* **19**. — Debenzylation of **19** was effected by hydrogenolysis conducted as already described. Final purification on a column of Sephadex G-15 yielded syrupy **7** in almost quantitative yield. This product was indistinguishable (by t.l.c. in several solvent-systems) from **7** obtained from both **1** and **8**.

P.m.r. data (D_2O): δ 1.78–2.37 (m, 8 H, –C–CH_2–C–), 4.91 (d, 0.25 H, $J_{1,2}$ 7.0 Hz, H-1β of pyranoside). Two doublets, constituting about 0.75 H, were also observed: δ 5.46 ($J_{1,2}$ 2 Hz, H-1α of pyranoside), and 5.51 [$J_{1,2}$ 2 Hz, furanoside anomeric signal(s)].

*6-(Trifluoroacetamido)hexyl 2-*O*-benzyl-3,4,6-tri-*O*-(*p*-nitrobenzoyl)-α-*D*-glucopyranoside* (**21**). — The title glycoside was prepared from 2-*O*-benzyl-D-glucose (**20**) by the same method as that described for **4**. After gel-filtration on a column of Sephadex LH-20, syrupy **21** (55% yield) was obtained.

P.m.r. data: δ 1.20–1.90 (m, 8 H, –C–CH_2–C–), 7.08–7.44 (m, 5 H, aromatic protons of benzyl groups), and 7.92–8.44 (m, 12 H, *p*-nitrobenzoyl protons).

*6-Aminohexyl 2-*O*-benzyl-α-*D*-glucopyranoside* (**22**). — Compound **22** was obtained from **21** in 93% yield (based on **21**) by *O*-deacetylation with sodium methoxide and *N*-de(trifluoroacetyl)ation with Amberlite IRA-400 (OH^-) ion-exchange resin.

P.m.r. data in acetone-d_6: δ 1.23–1.85 (m, 8 H, –C–CH_2–C–), 7.19–7.51 (m, 5 H, aromatic protons), 4.43 (d, 0.2 H, $J_{1,2}$ 7.0 Hz, β-anomeric proton), 4.85 (d, $J_{1,2}$ 3.7 Hz, α-anomeric proton). Quantitation of the α-anomeric proton peak was hampered by the proximity of the signal of the benzyl methylene group. In D_2O: δ 1.61–2.17 (m, 8 H, –C–CH_2–C–) and 7.69–7.95 (m, 5 H, aromatic protons). Definite assignment of the β-anomeric signal could not be made in this case, and a downfield doublet (δ 5.23) was partially obscured by its proximity to the HOD signal. Thus, assignment of anomeric proton ratios was not possible for spectra obtained with either solvent.

6-Aminohexyl α-D-*glucopyranoside* (**23**). — Compound **22** (0.64 g, 1.73 mmoles) was hydrogenolyzed, and purified on a column of Sephadex G-15, as already described, to give **23** as a syrup (0.46 g, 96% yield).

The p.m.r. spectrum (D_2O) supported the structure expected: δ 1.55–2.41 (m, 8 H, –C–CH_2–C–); the β-anomeric signal (δ 4.96, $J_{1,2}$ 7.7 Hz) had the same chemical-shift as that of 6-aminohexyl β-D-glucopyranoside* (δ 4.95, $J_{1,2}$ 7.7 Hz); the α-anomeric proton appeared as a doublet ($J_{1,2}$ 4.7 Hz) at δ 5.43. Integration of the anomeric-proton peaks showed $\alpha:\beta = 67:33$.

Anal. Calc. for $C_{12}H_{25}NO_6$: C, 51.59; H, 9.02; N, 5.01. Found: C, 51.37; H, 8.92; N, 4.87.

Methylation analysis. — *N*-Acetylation of 6-aminohexyl glycosides prior to

*M. Naoi, S. Roseman, and Y. C. Lee, unpublished results.

methylation was accomplished by the method of Wheat[10]. The *N*-acetylated 6-aminohexyl D-galactosides (~5 mg) derived from the three benzyl ethers were permethylated, either with methyl iodide and silver oxide in *N*,*N*-dimethylformamide[11] or with methyl iodide and methylsulfinyl sodium[12]. Portions of *N*-acetylated, permethylated samples (100–200 μg) were hydrolyzed with 2M trifluoroacetic acid for 6 h at 100°. After evaporation, followed by repeated co-evaporation with water and then toluene, the hydrolyzates were acetylated for 18 h at room temperature with 1:1 (v/v) pyridine–acetic anhydride (1 ml). The mixture was then evaporated to a syrup, and this was dissolved in 20–50 μl of chloroform for g.l.c. analysis.

Methyl α- and β-D-galactopyranosides and methyl β-D-galactofuranoside were treated as described for the 6-aminohexyl D-galactosides, and were used as standards in the assignment of pyranoside and furanoside peaks.

It was possible to separate, completely, all four possible isomers on a column of OV-225 at 140°: the 1-*O*-acetylated α- and β-pyranoses had retention times of 16 and 23 min, and the 1-*O*-acetylated α- and β-furanoses had retention times of 18 and 20 min. Differentiation between the anomeric furanoses and the anomeric pyranoses was not made.

The relative ratios of furanosides and pyranosides in the reaction products were also determined by the following method. The permethylated glycosides (prepared as already described) were hydrolyzed with trifluoroacetic acid, the products reduced with sodium borohydride, and the products acetylated with acetic anhydride and pyridine; the products were then subjected to g.l.c. analysis. 1,5-Di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-galactitol (derived from the D-galactopyranosides) and 1,4-di-*O*-acetyl-2,3,5,6-tetra-*O*-methyl-D-galactitol (derived from the D-galactofuranosides) were completely separable by g.l.c., and each showed characteristic mass spectra (see Table II), in agreement with the well established pattern[12b,13].

TABLE II

PROMINENT PEAKS[a] (*m*/*e*) IN THE MASS SPECTRA OF ACETATES OF METHYLATED ALDITOLS

Parent sugar	m/e												
	43	*45*	*59*	*71*	*87*	*89*	*101*	*117*	*129*	*145*	*161*	*205*	*277*
2,3,4,6-Me_4-Gal*p*	+	+		+	+		+	+	+	+	+	+	
2,3,5,6-Me_4-Gal*f*	+	+	+		+	+	+	+				+	+[b]
2,3,4,6-Me_4-Glc*p*	+	+		+	+		+	+	+	+	+	+	

[a]Peaks of intensity greater than 10% of the base peak (*m*/*e* 43). [b]Six percent of the intensity of the base peak.

Similar methylation analysis of 6-aminohexyl D-glucoside gave only 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol, indicating that only the pyranoid form of this glycoside was produced.

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