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SYNTHESIS OF D-GALACTOSAMINE DERIVATIVES AND BINDING STUDIES USING ISOLATED RAT HEPATOCYTES*

TING CHI WONG†, R. REID TOWNSEND‡, AND YUAN CHUAN LEE
Department of Biology and McCollum-Pratt Institute, The Johns Hopkins University, Baltimore, Maryland 21218 (U.S.A.)

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ABSTRACT

Derivatives of glycosides of D-galactosamine were prepared in order to study further the binding requirement of the Gal/GalNAc receptor in mammalian hepatocytes. These structures included *N*-propanoyl, *N*-benzoyl, and *N,N*-phthaloyl derivatives of 2-hydroxyethyl 2-amino-2-deoxy-β-D-galactopyranoside, 6-amino-hex-1-yl 2-deoxy-2-(trifluoroacetamido)-β-D-galactopyranoside, the mono- and di-*O*-methyl derivatives of allyl 2-acetamido-2-deoxy-β-D-galactopyranoside, and allyl 2-acetamido-2,4-dideoxy-4-fluoro-α-D-galactopyranoside. The inhibition results confirmed some of our previous findings on the involvement of the hydroxyl groups, and provided new information on the involvement of the *N*-substituent, as well as on the requirement of hydrogen bonding of the 4-hydroxyl group in binding.

INTRODUCTION

Mammalian hepatocytes possess a receptor system specific for D-galactose and *N*-acetyl-D-galactosamine, with the former being 20–30-fold less potent than the latter in inhibiting binding of ^{125}I-asialo-orosomucoid to rabbit hepatocytes[1,2].

In our earlier studies[3], neoglycoproteins prepared with 1-thioglycoside derivatives of D-galactose and D-glucose were used as inhibitors in order to probe the binding specificity. This was necessitated by the fact that D-galactose is a poor inhibitor, whereas the neoglycoproteins containing multiple D-galactose residues in each molecule are more effective inhibitors by 10^3- to 10^6-fold. Some of the conclusion on the structural specificities were deduced from the results obtained with D-glucose–neoglycoprotein of the amidino type[3], because they exhibited as high a potency as the D-galactose counterparts in inhibition. These studies led to the general conclusion that the hydroxyl groups on C-2, 3, and 4, but not on C-6, are

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†Present address: Biomira Inc., Research Center One, Edmonton Research & Development Park, 9411A-20 Ave. Edmonton, Alberta T6N1E5, Canada.
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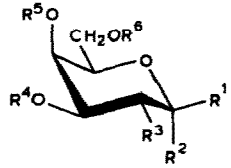
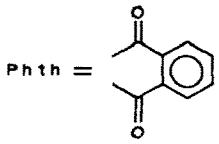
intimately involved in binding to the receptor. In the present study, derivatives of D-galactosamine were prepared and used as inhibitors (without coupling to macromolecules) in order to furnish more-direct and conclusive results on the binding requirement of the receptor, as well as to provide a way of incorporating probes into the structure by *N*-substitution.

These compounds were prepared in the form of 2-hydroxyethyl, allyl, and 6-aminohexyl glycosides. The last two can be readily coupled to multifunctional molecules[4,5] that can provide multivalent ligands or be modified in such a way that they can be radiolabelled[6,7].



1 $R^1 = H, R^2 = OAc, R^3 = NPhth, R^4 = R^5 = R^6 = Ac$
2 $R^1 = OAc, R^2 = H\ R^3 = NPhth, R^4 = R^5 = R^6 = Ac$
3 $R^1 = O(CH_2)_2OH, R^2 = H, R^3 = NPhth, R^4 = R^5 = R^6 = Ac$
4 $R^1 = O(CH_2)_2OH, R^2 = H, R^3 = NPhth, R^4 = R^5 = R^6 = H$
5 $R^1 = O(CH_2)_2OH, R^2 = H, R^3 = NH_2, R^4 = R^5 = R^6 = H$
6 $R^1 = O(CH_2)_2OH, R^2 = H, R^3 = NHCOEt, R^4 = R^5 = R^6 = H$
7 $R^1 = O(CH_2)_2OH, R^2 = H, R^3 = NHBz, R^4 = R^5 = R^6 = H$
8 $R^1 = O(CH_2)_6NHCOCMe_3, R^2 = H, R^3 = N_3, R^4 = R^5 = R^6 = Ac$
9 $R^1 = O(CH_2)_6NH_2, R^2 = H, R^3 = NHCOCF_3, R^4 = R^5 = R^6 = H$



RESULTS AND DISCUSSIONS

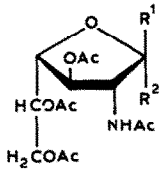
The preparation of 1,3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranose (**2**) had been reported by Ogawa and Beppu[8] and Paulsen and Bünsch[9], starting from 2-amino-2-deoxy-D-galactose hydrochloride. On following such procedures, it was found that the α-acetate **1** is also formed and may be isolated from the crude product by crystallization. The formation and isolation of the α-acetate has not previously been described. Treatment of the crude acetates (α + β) with 35% HBr in acetic acid afforded a mixture of the α- and β-bromide in the ratio of 2:3, as was evidence by ^{1}H-n.m.r. data. The pure α-bromide had been reported by Paulsen and Bünsch[9] and the pure β-bromide was obtained by Sabesan and Lemieux[10] by a different reaction-sequence. Under the conditions for

glycosylation described by Lemieux *et al.*[11], it was observed that the β-glycoside could be obtained from anomeric mixtures as well as from each of the pure anomers of the halides. The bromide mixture reacted with an excess of ethylene glycol in the presence of silver trifluoromethanesulfonate and *sym*-collidine to give the β-glycoside **3** in 66% yield. The ^{1}H-n.m.r. spectrum of this compound in Me_2SO-d_6 showed a triplet signal which disappeared on addition of D_2O, indicating a proton to be on the free hydroxyl group of the aglycon.

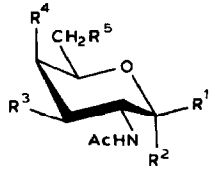
O-Deacetylation of the phthalimido compound by sodium methoxide is often accompanied by the formation of the *o*-carboxybenzamido derivative, owing to the presence of a trace of water. The *O*-deacetylation was followed by heating the crude product with pyridine, which resulted in the complete conversion of the side product into the phthalimido derivative[12] **4**. The phthalimido compound was treated with 1.2 equiv. of hydrazine in 95% ethanol to give the amino compound **5**, which was separately treated with propanoic anhydride and benzoyl chloride in the presence of triethylamine to give, respectively, the *N*-acyl derivatives **6** and **7**.

6-Aminohexyl 2-acetamido-2-deoxy-β-D-galactopyranoside had been prepared by treating 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-galactopyranosyl chloride with 6-(trifluoroacetamido)hexanol[13], and it is a useful precursor in the synthesis of multi-branch structures. The 2-deoxy-2-trifluoroacetamido analog was prepared by treating 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl bromide[14] with 6-(*tert*-butoxycarbonylamino)hexanol, using silver carbonate as a promoter. The desired glycoside was obtained in low yield (24%), apparently due to the release of the *tert*-butoxycarbonyl group during the reaction, to afford two undesired products (see experimental section). After *O*-deacetylation, the azido group was hydrogenated, giving the amine which was then *N*-(trifluoroacetyl)ated with ethyl trifluoroacetate, followed by treatment with trifluoroacetic acid to remove the *tert*-butoxycarbonyl group, giving compound **9** in 47% overall yield.

A crude mixture containing 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-galactopyranosyl chloride was obtained by treatment of *N*-acetyl-D-galactosamine with acetyl chloride, following the procedure described by Horton[15] for the efficient preparation of the *gluco*-analogue. Such material, on reaction with allyl alcohol with mercuric cyanide as promotor, afforded a low yield of the β-glycoside **12** (26%). From the reaction mixture, however, were isolated the β-furanoside **10**, the pentaacetate **13** and **11**. Similar products were also obtained in the case of the



10 $R^1 = OCH_2CH{=}CH_2$, $R^2 = H$
11 $R^1 = H$, $R^2 = OAc$

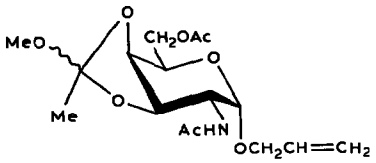


12 $R^1 = OCH_2CH{=}CH_2, R^2 = H, R^3 = R^4 = R^5 = OAc$
13 $R^1 = H, R^2 = R^3 = R^4 = R^5 = OAc$
14 $R^1 = OCH_2CH_2OH, R^2 = H, R^3 = R^4 = R^5 = OAc$
15 $R^1 = OCH_2CH{=}CH_2, R^2 = H, R^3 = R^4 = R^5 = OH$
16 $R^1 = OCH_2CH_2OH, R^2 = H, R^3 = R^4 = R^5 = OH$
17 $R^1 = H, R^2 = OCH_2CH{=}CH_2, R^2 = H, R^3 = R^4 = R^5 = OH$
18 $R^1 = OCH_2CH{=}CH_2, R^2 = H, R^3 = OCH_3, R^4 = R^5 = OH$
19 $R^1 = OCH_2CH{=}CH_2, R^2 = H, R^3 = R^4 = OH, R^5 = OCH_3$
20 $R^1 = OCH_2CH{=}CH_2, R^2 = H, R^3 = OH, R^4 = R^5 = OCH_3$
21 $R^1 = OCH_2CH{=}CH_2, R^2 = H, R^3 = R^5 = OCH_3, R^4 = OH$
22 $R^1 = H, R^2 = OCH_2CH{=}CH_2, R^3 = R^5 = OAc, R^4 = F$
23 $R^1 = H, R^2 = OCH_2CH{=}CH_2, R^3 = R^5 = OH, R^4 = F$

glycosylation using 6-trifluoroacetamido 1-hexanol[13]. The 2-hydroxyethyl glycoside **14** was obtained similarly by this procedure. The deblocking of **12** and **14** resulted in the β-glycoside, **15** and **16**. The allyl α-glycoside **16** was obtained by treating *N*-acetyl-D-galactosamine with boron trifluoride etherate in allyl alcohol.

Suitably protected derivatives of the GalNAc glycoside **15** were needed to prepare the required partially *O*-methylated derivatives. The 4,6-*O*-benzylidene derivative was obtained in 41% yield in crystalline form by treating **15** with benzaldehyde and zinc chloride. The benzylidene derivative was methylated with dimethyl sulfate in the presence of sodium hydroxide as described by Stoffyn and Jeanloz[16] for the methyl α-glycoside, *O*-debenzylidenation of which gave the 3-*O*-methyl derivative **18**, the peracetate of which was purified by re-acetylation followed by chromatography. Treatment of compound **15** with 2,2-dimethoxypropane, with *p*-toluenesulfonic acid as the catalyst, overnight at room temperature afforded the 3,4-*O*-isopropylidene derivative in 59% yield. This compound was then methylated with methyl iodide and silver oxide. Removal of the isopropylidene group gave the 6-*O*-methyl derivative **19**, which was subjected to purification as described for compound **18**.

A random methylation was used to produce other methyl derivatives. The unprotected compound **15** was treated with a total of 4.5 equiv. of methyl iodide in the presence of silver oxide during 3 days at room temperature until the starting material disappeared. Two major fractions were obtained by chromatography on silica gel. By acetylating the faster-moving fraction, two essentially pure compounds, were isolated by chromatography on silica gel. By acetylating the slower-moving fraction, two compounds were isolated, as well as a mixture containing a



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further two. Only the *O*-deacetylated compounds **20** and **21** were isolated in useful yields (11 and 17%, respectively).

Allyl 2-acetamido-3-*O*-acetyl-2-deoxy-α-D-glucopyranoside was obtained very efficiently from allyl 2-acetamido-2-deoxy-α-D-glucopyranoside (**3**) *via* its 4,6-*O*-benzylidene derivative. The 6-hydroxyl group of the acetylated glucopyranoside was then selectively blocked by acetylation using 1-acetylimidazole[17]. When the resultant alcohol was allowed to react with DAST (diethylaminosulfur trifluoride), in the presence[18] of 4-dimethylaminopyridine (DMAP), two major products were obtained in crystalline form, namely, **22** and **24**. For the fluorinated compound **22**, the ^{19}F-n.m.r. signal was detected at δ 218.97 as a multiplet, in accord with results of similar compounds reported by Card and Reddy[19,20]. In the ^{1}H-n.m.r. spectrum, the H-4 signal at δ 4.81 exhibits a coupling constant of 2.4 Hz (to H-3), as well as 50.7 Hz (to the fluorine atom); the small or nonexistent coupling between H-4 and H-5 is typical of galactopyranosides. The H-3 signal exhibited a vicinal coupling of 28 Hz to F-4, as well as of 2.4 Hz to H-4, and 11.3 Hz to H-2. This indicated that the compound is of the *galacto* configuration as a result of inversion of configuration at C-4 by DAST[20]. The orthoester **24** was formed, apparently due to participation of the 3-*O*-acetyl group.

The specificity of the binding of sugar to protein is most frequently determined by use of derivatives of naturally occurring substances. However, many synthetic compounds of finite structure with various structural modifications can be accepted to different extents by the combining site of a protein. Such a phenomenon can be taken advantage of for introducing probes into a sugar molecule for the study of such interactions. With respect to monosaccharides, sugar specificity is determined by the ring structure and conformation, as well as by the orientation of the hydroxyl and other groups on the ring. In the case of the Gal/GalNAc lectin of mammalian hepatocytes, the binding is mediated by the galactopyranose structure. It was found that C-6 of the sugar is not involved in the binding, and that a 2-deoxy-2-acetamido group enhances the binding. Because the amino group is much more readily modified than the hydroxyl groups, it was therefore of interest to vary the *N*-substituents and, for possible further exploitation, to examine their effect on binding.

2-Deoxy-2-phthalimidoglycoside **4** was used to prepare the β-glycoside of 2-amino-2-deoxy-D-galactopyranose, from which the propanamido (**6**) and benzamido (**7**) derivatives were also prepared. The steric requirement of the combining

TABLE I

INHIBITION RESULTS SHOWING EFFECT OF CHANGES IN *N*-SUBSTITUTION

Compound	R^1	R^3	I_{50} *(mM)*	*Relative potency*
16	OCH_2CH_2OH	$(NHCOCH_3)$	0.2	1
6	OCH_2CH_2OH	$NHCOCH_2CH_3$	0.2	1
7	OCH_2CH_2OH	$NHCOC_6H_5$	0.8	0.25
4	OCH_2CH_2OH	NPhth	8.0	0.025
9	$O(CH_2)_6NH_2$	$NHCOCF_3$	0.2	1
	$O(CH_2)_6NH_2$	$NHCOCH_3$	0.06[a]	3.333

[a]This result was personal communication from Reiko T. Lee, and has been adjusted according to the potency of GalNAc in our experiment and her experiment.

site in this position was tested by using these compounds as inhibitors. The 2-hydroxyethyl glycosides render the phthalimido and the benzamido derivatives more soluble in the aqueous medium for inhibition assay. The trifluoroacetamido group of derivative **9**, which represents a polar *N*-acyl group, and can be used in binding studies by ^{19}F-n.m.r. spectroscopy, was also prepared.

As shown in Table I and Fig. 1, the propanamido group of **6**, which is somewhat larger than the acetamido group, has no effect on the binding. Interestingly, the rather bulky benzamido group of compound **7** renders it only 4-fold less inhibitory than the acetamido compound. The phthalimido compound is about 10-fold less potent than the benzamido compound. It seems that, whereas the

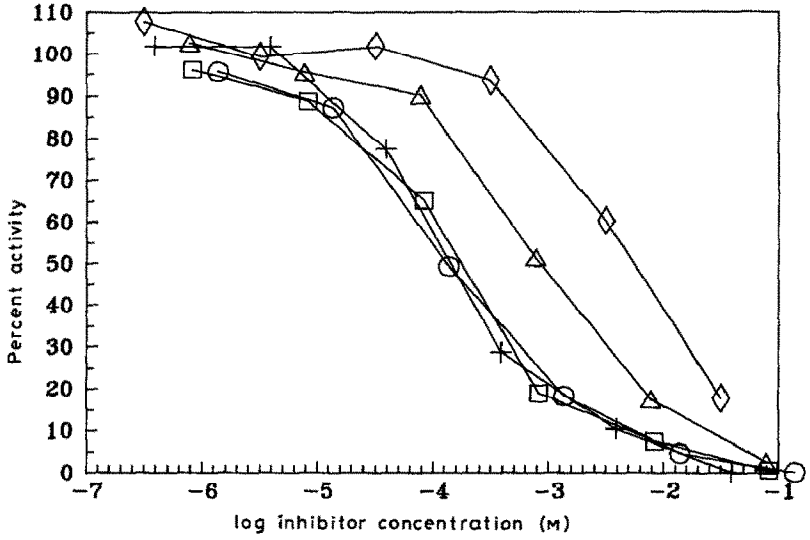


Fig. 1. Inhibition of binding of ^{125}I-asialo-orosomucoid to rat hepatocytes by compounds **16** (○), **6** (□), **7** (△), **4** (◇) and **9** (+). [Each tube contained 2.5 million cells, 125pM ^{125}I-asialo-orosomucoid, and inhibitor in a total volume of 1 mL.]

TABLE II

INHIBITION RESULTS SHOWING EFFECT OF CHANGES OF HYDROXYL GROUPS

Compound	*R¹*	*R²*	*R³*	*R⁴*	*R⁵*	I_{50} *(mM)*	*Relative potency*
15	$OCH_2CH{=}CH_2$	H	OH	OH	OH	0.2	1
19	$OCH_2CH{=}CH_2$	H	OH	OH	OCH_3	0.08	2.5
18	$OCH_2CH{=}CH_2$	H	OCH_3	OH	OH	70	0.0029
20	$OCH_2CH{=}CH_2$	H	OH	OCH_3	OCH_3	31	0.0065
17	H	$OCH_2CH{=}CH_2$	OH	OH	OH	0.12	1.7
23	H	$OCH_2CH{=}CH_2$	OH	F	OH	>1.5	Inactive at 1.5mM

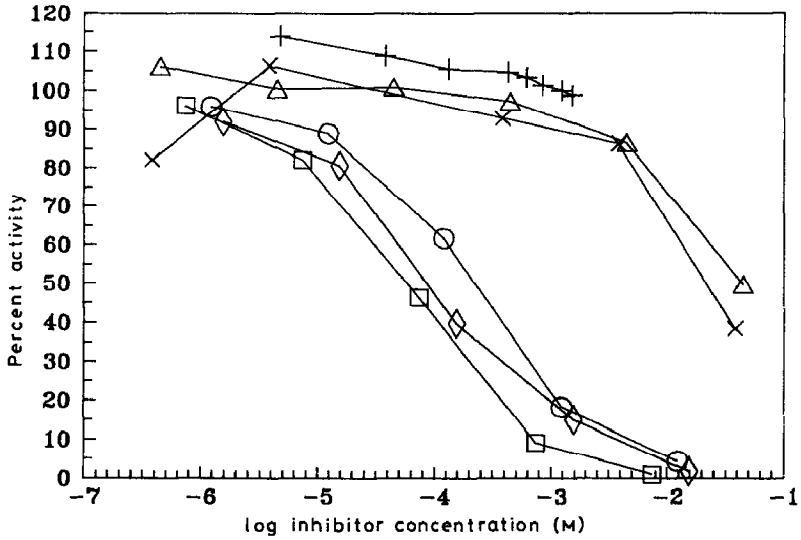
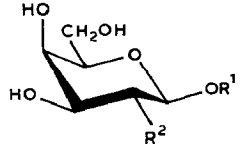
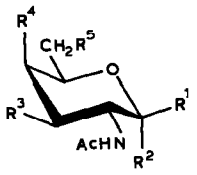


Fig. 2. Inhibition of binding of ^{125}I-asialo-orosomucoid to rat hepatocytes by compounds **15** (○), **19** (□), **18** (△), **20** (×), **17** (◇) and **23** (+). [Each tube contained 2.5 million cells, 125pM ^{125}I-asialo-orosomucoid, and inhibitor in a total volume of 1 mL.]

benzamido group is sufficiently flexible to avoid serious interaction, this is not so for the conformationally more rigid phthalimido group. Because the phthalimido ring lies orthogonal to the pyranose ring[21], it is conceivable that one of the carbonyl groups intrudes into the relatively hydrophobic α face of the galactopyranose ring, thus making the surface more hydrophilic and less compatible for binding. The binding is also shown to be little affected by the polar trifluoroacetamido group. Such a binding mode towards the 2-substituent is very similar to that of the *G. simplicifolia* I-A_4 isolectin studied by Kaifu *et al.*[22]. On the other hand, a marked adverse effect was observed by changing the *N*-acyl group in the binding of *N*-acetyl-lactosamine to Anti-I Ma antibody studied by Lemieux *et al.*[23], where the acetamido group was envisaged to be situated in a hydrophobic pocket.

As shown in Table II and Fig. 2, the fact that the 6-*O*-methyl derivative is a good inhibitor, whereas the 3-methyl ether, and the 4,6-dimethyl ether, are, respectively, 350- and 155-fold less inhibitory than the parent GalNAc glycoside, confirms the previous findings obtained with the neoglycoproteins. Compound **23** bearing a fluorine atom in place of the OH-4 group was found to be a poor inhibitor. Inasmuch as the similarity of both bond-length and polarity between the C–F and C–OH, binding of the OH-4 group seems to involve a hydrogen bond,





with OH-4 as a proton donor. The binding may involve a more-hydrophobic surface arising from the intramolecular hydrogen-bond between OH-3 and OH-4 similar to that proposed by Lemieux *et al.*[24] for the binding of D-galactose to an antibody. It may be noted that changes in the OH-3 and OH-4 groups produce a more adverse effect on binding than those in the *N*-acyl group. This suggests that OH-3 and OH-4 are critical elements for binding.

EXPERIMENTAL

General methods. — All solvents and other chemical compounds were reagent grade, and in the cases where further purification was required, standard procedures[25] were followed. All evaporations were conducted in a rotary evaporator under diminished pressure at 20–40°.

Melting points (uncorrected) were determined with a Fisher–Johns melting-point apparatus. Optical rotations were measured at room temperature (usually 23°) with a Perkin–Elmer 141 spectropolarimeter. Elemental analyses were performed by Galbraith Lab., Inc. (Knoxville, TN). Thin-layer chromatography (t.l.c.) was performed on sheets precoated with silica gel 60 F_{254} (E. Merck, Cat. No. 5534) and visibilized by quenching of fluorescence, or charring after spraying with 15% sulfuric acid in 50% aqueous ethanol, or both. Column chromatography was performed on silica gel 60 (15–40 μm), using the stated solvent mixture for which solvent components and volume ratios are indicated. Unless otherwise indicated, gel filtration was performed on a column (3.0 × 25 cm) of Sephadex LH-20 (Pharmacia) (samples usually <0.5 g) eluted with the solvent mixture stated. Nuclear magnetic resonance spectra were recorded with a Varian XL-400 spectrometer (400 MHz for protons), at 20° for samples in $CDCl_3$, and 27° for samples in D_2O. Proton chemical shifts are in p.p.m. relative to an internal reference of tetramethylsilane (δ 0.00) in $CDCl_3$ and Me_2SO-d_6 or HOD signal (δ 4.75) in D_2O. ^{13}C-Chemical shifts are referenced to the internal 1,4-dioxane signal set at δ 67.86 in D_2O. ^{19}F-Chemical shifts are referenced to internal trichlorofluoromethane.

Hepatocyte preparation. — Rat hepatocytes were prepared by a modifiation[6] of the two-step collagenase-perfusion method[26]. Prior to the inhibition assay, hepatocytes were incubated for 30 min at 37°, recovered by centrifugation at 50*g*, and resuspended to 10 million cells/mL in medium[6] at 4°.

Inhibition assay. — Freshly isolated rat hepatocytes (0.25 mL) were added to 0.75 mL of medium[6] containing the desired concentrations of inhibitors and 125pM

^{125}I-asialo-orosomucoid in 1.5-mL polypropylene tubes. After incubation for 2 h at 0–2° with end-over-end rotation, the total cell-associated radioactivity was determined by centrifuging 200 μL of the cell suspension over 4:1 silicone–mineral oil at 10,000*g* for 30 s. The snipped pellets were counted for radioactivity in a Packard Prias Auto-Gamma counter. Total cell-associated radioactivity in the absence of inhibitor was also determined. Nonspecific binding was measured by placing 200 μL of cell suspension from tubes not containing inhibitor in a tube containing 4 μL of 0.5M EDTA [(ethylenedinitrilo)tetraacetate], pH 7.8, solution and determining after 10 min the cell-associated radioactivity as already described.

SYNTHETIC METHODS

1,3,4,6-Tetra-O-acetyl-2-deoxy-2-phthalimido-α,β-D-galactopyranose (**1** *and* **2**). — The title compounds were prepared by a method similar to that used for the *gluco* analog by Lemieux *et al.*[21]. After *O*-acetylation and decolorization with charcoal, the α anomer crystallized from dichloromethane. Concentration of the mother liquor and treatment with ether yielded the crystalline β anomer.

Compound **1** had m.p. 184–185.5°; ^{1}H-n.m.r. ($CDCl_3$): δ 7.86–7.75 (m, 4 H, phthalimido protons), 6.52 (dd, 1 H, $J_{2,3}$ 12, $J_{3,4}$ 3.6 Hz, H-3), 6.34 (d, 1 H, $J_{1,2}$ 3.2 Hz, H-1), 5.67 (bd, 1 H, $J_{4,5}$ 2 Hz, H-4), 4.91 (dd, 1 H, H-2), 4.50 (m, 1 H, H-5), 4.15 (m, 2 H, H-6a and H-6b), 2.19, 2.06, 2.06, and 1.89 (4 s, 12 H, $COCH_3$).

Anal. Calc. for $C_{22}H_{23}NO_{11}$: C, 55.35; H, 4.86; N, 2.93. Found: C, 54.89; H, 4.86; N, 2.87.

Compound **2** had m.p. 127–131°, $[\alpha]_D$ +34.94° (*c* 1.3, chloroform) lit.[8] m.p. 99–101° (from iPr_2O), $[\alpha]_D$ +31.1°; lit.[9] $[\alpha]_D$ +68.7°; ^{1}H-n.m.r. data are similar to the literature values[8,9].

Anal. Calc. for $C_{22}H_{23}NO_{11}$: C, 55.35; H, 4.86; N, 2.93. Found: C, 54.57; H, 4.73; N, 2.84.

Crude product containing **1** and **2** was obtained in 40% yield from D-galactosamine hydrochloride, and was used for preparation of bromide.

3,4,6-Tri-O-acetyl-2-deoxy-2-phthalimido-α,β-D-galactopyranosyl bromide. — The crude product (3.35 g) from the previous experiment was treated for 4 h at room temperature with a mixture of 35% HBr in acetic acid (18 mL) and acetic anhydride (2 mL). The usual work-up gave a white foam (2.31 g). T.l.c. in 1:1 ethyl acetate–hexane showed a single spot (R_F 0.57); ^{1}H-n.m.r. ($CDCl_3$): δ 7.86–7.74 (m, 4 H, phthalimido protons), 6.67 (d, 0.38 H, $J_{1\alpha,2\alpha}$ 3.7 Hz, H-1α), 6.52 (dd, $J_{2\alpha,3\alpha}$ 12.1, $J_{3\alpha,4\alpha}$ 3.1 Hz, H-3α), 6.38 (d, 0.62 H, $J_{1\beta,2\beta}$ 9.8 Hz, H-1β), 5.76 (dd, $J_{2\beta,3\beta}$ 11.3, $J_{3\beta,4\beta}$ 3.4 Hz, H-3β), 5.71 (bd, H-4α), 5.53 (bd, H-4β), 4.85–4.80 (m, H-2α and H-2β overlapping), 4.58 (m, H-5α), 4.10 (m, H-5β, H-6 overlapping), 2.22, 2.16, 2.06, 1.90, and 1.84 (5 s, 9 H, 3 $COCH_3$).

2-Hydroxyethyl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranoside (**3**). — To a stirred mixture of silver trifluoromethanesulfonate (triflate; 0.58 g,

2.26 mmol), *sym*-collidine (0.91 mL, 2.01 mmol), ethylene glycol (5 mL), and nitromethane (5 mL), kept at −20°, was added dropwise to the bromide (1 g, 2.01 mmol) in nitromethane (2 mL) during 0.5 h. The mixture was transferred to a cold room, and kept overnight at 4°, with stirring. Dichloromethane (25 mL) was added and the solid was filtered off. The filtrate was successively washed with M HCl, sodium thiosulfate solution, water, saturated sodium hydrogencarbonate solution, and water, dried (sodium sulfate), and evaporated to give syrup that was applied to a column of silica gel and eluted with 3:2 ethyl acetate–hexane; the main fractions were combined, and evaporated to a syrup (0.63 g, 1.32 mmol, 65.7%); ^{1}H-n.m.r. ($CDCl_3$): δ 7.95–7.77 (m, 4 H, aromatic protons), 5.79 (dd, 1 H, $J_{2,3}$ 11.3, $J_{3,4}$ 3.44 Hz, H-3), 5.49 (bd, 1 H, H-4), 5.38 (d, 1 H, $J_{1,2}$ 8.5 Hz), 4.58 (dd, 1 H, H-2), 4.22–3.61 (m, 7 H, other protons), 2.22, 2.09, and 1.86 (3 s, 9 H, 3 $COCH_3$); ^{1}H-n.m.r. (Me_2SO-d_6): δ 4.41 (t, 1 H, OH).

*2-Hydroxyethyl 2-deoxy-2-phthalimido-β-*D*-galactopyranoside* (**5**). — The triacetate **3** (0.524 g, 1.09 mmol) was treated with 0.1M sodium methoxide solution for 15 min at room temperature the base neutralized with Dowex-50 (H^+) resin for 1 min, and the suspension filtered. The filtrate evaporated and the resultant syrup was dissolved in pyridine, heated for 11 h at 100°, and evaporated to give a syrup (0.328 g, 0.93 mmol, 85%) that was used directly for the preparation of the *N*-acylated compounds to be described. For inhibition study, the material was purified by applying it to a column of Dowex 1 (OH^-, 200–400 mesh) resin, and eluting with water; ^{1}H-n.m.r. (D_2O): δ 7.93–7.84 (m, 4 H, phthalimido protons), 5.26 (d, 1 H, $J_{1,2}$ 8.9 Hz, H-1), 4.57 (dd, 1 H, $J_{2,3}$ 11, $J_{3,4}$ 3.1 Hz, H-3), 4.28 (dd, 1 H, H-2), 4.06 (bd, 1 H, H-4), and 3.82–3.53 (m, 7 H, other protons).

*2-Hydroxyethyl 2-amino-2-deoxy-*D*-galactopyranoside* (**5**). — A mixture of compound **4** and hydrazine (1.2 equiv.) in 95% ethanol was refluxed for 2 h, the solid was filtered off, and the filtrate evaporated to a syrup which was used directly for *N*-acylation.

*2-Hydroxyethyl 2-deoxy-2-propanamido-β-*D*-galactopyranoside* (**6**). — The amine **5** (0.15 mmol, calculated from compound **4**) was treated overnight at room temperature with propanoic anhydride (4 equiv.) in methanol, and evaporated. Chromatography on a column of LH-20 eluted with 1:1 ethanol–water gave fractions that were pure by t.l.c. (9:4:2, ethyl acetate–2-propanol–water, R_F 0.20); they were pooled and lyophilized to a solid (25 mg, 0.09 mmol, 60%); ^{1}H-n.m.r. (D_2O): δ 4.50 (d, 1 H, $J_{1,2}$ 8.1 Hz), 4.00–3.62 (m, 10 H, other protons), 2.30 (q, 2 H, CH_2 of $COCH_2CH_3$), and 1.13 (t, 3 H, CH_3 of $COCH_2CH_3$).

*2-Hydroxyethyl 2-benzamido-2-deoxy-β-*D*-galactopyranoside* (**7**). — The amine **5** (0.14 mmol) was treated with benzoyl chloride (4 equiv.) in the presence of triethylamine in methanol. Evaporation resulted in an oil that was applied to a column (1.5 × 10 cm) of AG501-X 8(D) mixed-bed resin (Bio-Rad) and eluted, first with water and then with methanol, to give fractions containing pure compound **7** by t.l.c. (9:4:2 ethyl acetate–2-propanol–water, R_F 0.54) which were pooled, evaporated to remove methanol, and then lyophilized to a solid (23 mg,

0.07 mmol, 50%); ^{1}H-n.m.r. (D_2O): δ 7.85–7.55 (m, 5 H, COC_6H_5), 4.70 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 4.21 (dd, 1 H, $J_{2,3}$ 11.2 Hz, H-2), and 4.04–3.70 (m, 9 H, other protons).

*6-(*tert*-Butoxycarbonylamino)hex-1-yl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-*D*-galactopyranoside* (**8**). — To a stirred mixture of silver carbonate (6.3 g, 22.8 mmol), finely ground calcium sulfate (5 g), 6-(*tert*-butoxycarbonylamino)hexanol (2.5 g, 11.5 mmol) in dichloromethane (40 mL) was added dropwise 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl bromide (2.73 g, 6.93 mmol), obtained as a syrup having ^{1}H-n.m.r. comparable to that reported by Sabesan and Lemieux[10], in dichloromethane (10 mL) during 0.5 h at room temperature. The mixture was stirred overnight, and the solid was filtered off through a bed of diatomaceous earth. Evaporation of the filtrate gave a syrup that was applied to a column of silica gel and eluted first with 3:7 ethyl acetate–hexane and then with 3:2 ethyl acetate–hexane. Three major fractions were isolated from the column (R_F 0.44, 0.14, and 0.06 in 3:7 ethyl acetate–hexane).

The first fraction (0.87 g, 1.64 mmol, 24% yield based on the bromide) was identified as compound **8**; ^{1}H-n.m.r. ($CDCl_3$): δ 5.32 (bd, 1 H, $J_{3,4}$ 3.2 Hz, H-4), 4.76 (dd, 1 H, $J_{2,3}$ 11.2 Hz, H-3), 4.35 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.14 (m, 2 H, H-6a and H-6b), 3.94 (m, 1 H, aminohexyl CH_2), 3.84 (m, 1 H, H-5), 3.67 (dd, 1 H, H-2), 3.57 (m, 1 H, aminohexyl CH_2), 2.15, 2.05, 2.05 (3 s, 9 H, 3 $COCH_3$), 1.80–1.20 (m, 17 H, aminohexyl CH_2 and *tert*-butoxy protons).

The second fraction (0.38 g) was identified as 6′-(3′,4′,6′-tri-*O*-acetyl-2′-azido-2′-deoxy-β-D-galactopyranosylamino)hex-1-yl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-D-galactopyranoside; ^{1}H-n.m.r. ($CDCl_3$): δ 5.52 (d, 1 H, $J_{1',2'}$ 9.2 Hz, H-1′), 5.37 (bd, 1 H, $J_{3',4'}$ 3.2 Hz, H-4′), 5.32 (bd, 1 H, $J_{3,4}$ 3.2 Hz, H-4), 4.89 (dd, 1 H, $J_{2',3'}$ 10.4, $J_{3',4'}$ 2.4 Hz, H-3′), 4.76 (dd, 1 H, $J_{2,3}$ 10.4, $J_{3,4}$ 3.2 Hz, H-3), 4.35 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.19–4.08 (m, 4 H, H-6a, H-6b, H-6a′ and H-6b′), 4.00 (m, 1 H, H-5″), 3.95 (m, 1 H, aminohexyl CH_2), 3.84 (m, 1 H, H-5), 3.79 (dd, 1 H, H-2″), 3.66 (dd, 1 H, H-2), 3.56, 3.23 (m, 3 H, aminohexyl CH_2), 2.15, 2.15, 2.05, 2.04, 2.03 (5 s, 18 H, 6 $COCH_3$), and 1.76–1.30 (m, 8 H, aminohexyl CH_2).

The third fraction (0.69 g) was identified as 6-(3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-D-galactopyranosylamino)hexanol; ^{1}H-n.m.r. ($CDCl_3$): δ 5.49 (d, 1 H, $J_{1,2}$ 8.8 Hz, H-1), 5.32 (bd, 1 H, $J_{3,4}$ 3.2 Hz, H-4), 5.09 (bs, OH), 4.86 (dd, 1 H, $J_{2,3}$ 10.4, $J_{3,4}$ 3.2 Hz, H-3), 4.09 (m, 1 H, H-6a and H-6b), 3.98 (m, 1 H, H-5), 3.75 (dd, 1 H, H-2), 3.58, 3.19 (m, 4 H, aminohexyl CH_2), 2.11, 2.01, 1.99 (3 s, 9 H, 3 $COCH_3$), and 1.07–1.30 (m, 8 H, aminohexyl CH_2).

6-Aminohex-1-yl 2-deoxy-2-(trifluoroacetamido)-β-D-galactopyranoside (**9**). — Compound **8** (1.067 g, 2.01 mmol) was treated with 0.02M sodium methoxide (10 mL) for 1 h at room temperature, the base neutralized with Dowex-50W (H^+) resin, and the solution evaporated to give a syrup (0.8617 g, 100%) that was pure by t.l.c. in 9:1 ethyl acetate–methanol (R_F 0.67). This was hydrogenated in methanol at room temperature with 10% palladium-on-charcoal as the catalyst. The catalyst was filtered off, the filtrate was treated directly with trifluoroacetate

(0.71 mL) and triethylamine (0.28 mL), evaporated to an oil which was applied to a column of silica gel and eluted with 84:11:5 ethyl acetate–2-propanol–water, to yield a fraction that was pure by t.l.c. in 84:11:5 ethyl acetate–2-propanol–water (R_F 0.54). The eluate was evaporated, the residue was mixed with 60% trifluoroacetic acid, kept for 45 min at room temperature, the acid evaporated, and the residue applied to a column of LH-20 which was eluted with 1:1 ethanol–water. The fractions pure by t.l.c. in 3:2:1 ethyl acetate–acetic acid–water (R_F 0.59) were combined, concentrated by evaporation, and then lyophilized, to afford a solid (0.352 g, 0.94 mmol, 47%); ^{1}H-n.m.r. (D_2O): δ 4.53 (d, 1 H, $J_{1,2}$ 8.9 Hz, H-1), 4.00–3.55 (m, 8 H, H-2 and other protons), 2.97 (t, 2 H, aminohexyl CH_2), and 1.70–1.25 (aminohexyl CH_2).

*Crude N-acetyl-tri-*O*-acetyl-2-amino-2-deoxy-*D*-galactopyranosyl chloride.* — Acetyl chloride (12 mL) was added to *N*-acetyl-D-galactosamine (4.9261 g, 22.29 mmol), and the suspension was stirred for 3 h at room temperature and then kept for 4 days at 4°. Dichloromethane (100 mL) was added to the resultant pink syrup and the solution was washed successively with ice–water (100 mL) and cold saturated sodium hydrogencarbonate solution (100 mL), dried (sodium sulfate), and evaporated to a foam (4.852 g).

*Allyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-galactopyranoside* (**12**). — Freshly distilled allyl alcohol (12 mL), mercuric cyanide (3.5 g), nitromethane (10 mL), toluene (10 mL), and ground 4A molecular sieve (5 g) were stirred for 15 min at room temperature before a solution of crude chloride in 1:1 nitromethane–toluene (15 mL) was added dropwise during 1 h, and the mixture was stirred overnight. The solid was filtered off, and evaporation of the filtrate left a solid residue which was extracted with 9:1 chloroform–methanol (15 mL); the extract was applied to a column of silica gel and eluted first with 4:1 ethyl acetate–toluene and then with ethyl acetate. Four major fractions were isolated from the column (R_F 0.56, 0.50, 0.44, and 0.40; ethyl acetate).

Evaporation of the second fraction gave a solid (2.59 g, 5.79 mmol, 26% yield based on *N*-acetylgalactosamine) which was identified as compound **13**, m.p. 172°; ^{1}H-n.m.r. ($CDCl_3$): δ 5.88 (m, 1 H, allyl proton), 5.40 (d, 1 H, $J_{NH,2}$ 8.5 Hz, NH), 5.37 (bd, 1 H, $J_{3,4}$ 3.4 Hz, H-4), 5.32 (dd, 1 H, $J_{2,3}$ 11 Hz, H-3), 5.31–5.20 (m, 2 H, allyl protons), 4.76 (d, 1 H, $J_{1,2}$ 8.9 Hz, H-1), 4.36 (m, 1 H, allyl proton), 4.20–4.08 (m, 4 H, H-6a, H-6b and allyl protons), 3.99 (m, 1 H, H-2), 3.92 (m, 1 H, H-5), 2.10, 2.00, 1.89, and 1.78 (4 s, 12 H, 4 $COCH_3$).

Anal. Calc. for $C_{17}H_{25}NO_9$: C, 52.71; H, 6.50; N, 3.62. Found: C, 52.77; H, 6.65; N, 3.57.

The first fraction (0.84 g, 8.4%) was identified as allyl 2-acetamido-3,5,6-tri-*O*-acetyl-2-deoxy-β-D-galactofuranoside, m.p. 115–116°; ^{1}H-n.m.r. ($CDCl_3$): δ 5.97 (d, 1 H, $J_{NH,2}$ 7.9 Hz, NH), 5.90 (m, 1 H, allyl proton), 5.37 (m, 1 H, $J_{4,5}$ 4.3, $J_{5,6a}$ 4.6, $J_{5,6b}$ 7 Hz), 5.34–5.18 (m, 2 H, allyl protons), 4.99 (d, 1 H, $J_{1,2}$ 0.9 Hz, H-1), 4.76 (dd, 1 H, $J_{2,3}$ 2.4, $J_{3,4}$ 5.2 Hz, H-3), 4.41 (m, 1 H, H-2), 4.36 (dd, 1 H, $J_{H\text{-}6a,H\text{-}6b}$ 11.3 Hz, H-6a), 4.24 (dd, 1 H, H-6b), 4.22 (dd, 1 H, H-4), 4.20–3.99 (m, 2 H, allyl protons), 2.15, 2.09, 2.07, and 2.01 (4 s, 12 H, 4 $COCH_3$).

Anal. Calc. for $C_{17}H_{25}NO_9$: C, 52.71; H, 6.50; N, 3.62. Found: C, 52.86; H, 6.68; N, 3.29.

The third fraction (0.38 g) was identified as 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-galactopyranose (**13**), ^{1}H-n.m.r. ($CDCl_3$): δ 6.22 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 5.46 (d, 1 H, $J_{NH,2}$ 10 Hz, NH), 5.42 (dd, 1 H, $J_{3,4}$ 3.2, $J_{4,5}$ 1.2 Hz, H-4), 5.22 (dd, 1 H, $J_{2,3}$ 11.6 Hz, H-3), 4.73 (m, 1 H, H-2), 4.25 (m, 1 H, H-5), 4.09 (m, 2 H, H-6a and H-6b), 2.18, 2.04, 2.03, and 1.96 (4 s, 15 H, 5 $COCH_3$).

The fourth fraction (0.3 g) was identified as 2-acetamido-1,3,5,6-tetra-*O*-acetyl-2-deoxy-α-D-galactofuranose; ^{1}H-n.m.r. ($CDCl_3$): δ 6.20 (d, 1 H, $J_{1,2}$ 4.3 Hz, H-1), 5.93 (d, 1 H, $J_{NH,2}$ 7.9 Hz, NH), 5.34 (dd, 1 H, $J_{2,3}$ 9.6, $J_{3,4}$ 8 Hz, H-3), 5.20 (m, 1 H, $J_{4,5}$ 6.4, $J_{5,6a}$ 4.2, $J_{5,6b}$ 6 Hz, H-5), 4.75 (m, 1 H, H-2), 4.24 (dd, 1 H, $J_{5a,6b}$ 12.8 Hz, H-6a), 4.23 (bd, 1 H, H-4), 4.12 (dd, 1 H, H-6b), 2.14, 2.11, 2.10, 2.06, and 1.99 (5 s, 15 H, 5 $COCH_3$).

2-Hydroxyethyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-galactopyranoside (**14**). — Crude chloride (1 g) was added to a mixture of mercuric cyanide (0.7 g), finely ground calcium sulfate (2 g), ethylene glycol (6 mL), nitromethane (2 mL), and toluene (2 mL). Stirring was conducted for 2 days at room temperature, the solid was filtered off, and the filtrate was evaporated to a syrup, which was dissolved in 95% ethanol and applied to an LH-20 column eluted with the same solvent. The fractions that were detected by charring in t.l.c. were pooled and evaporated. The residue was dissolved in hot ethyl acetate and kept for 2 weeks; the crystalline material deposited (0.265 g, 0.678 mmol, 15%) was essentially pure by t.l.c. in 9:1 ethyl acetate–methanol (R_F 0.32); m.p. 207–209.5°; ^{1}H-n.m.r. (Me_2SO-d_6): δ 7.80 (d, 1 H, $J_{NH,2}$ 9.1 Hz, NH), 5.21 (bd, 1 H, $J_{3,4}$ 4 Hz, H-4), 4.97 (dd, 1 H, $J_{2,3}$ 11.3 Hz, H-3), 4.55 (d, 1 H, $J_{1,2}$ 8.6 Hz, H-1), 4.54 (t, 1 H, OH), 3.87 (m, 1 H, H-2), 3.65–3.55 (other protons), 2.10, 2.00, 1.89, and 1.78 (4 s, 12 H, 4 $COCH_3$).

Anal. Calc. for $C_{16}H_{25}NO_{10}$: C, 49.10; H, 6.44; N, 3.58. Found: C, 48.83; H, 6.44; N, 3.55.

Allyl 2-acetamido-2-deoxy-β-D-galactopyranoside (**15**). — The triacetate **13** (2.59 g) was treated with 0.1M sodium methoxide solution (7 mL). Crystals (1.168 g, 4.48 mmol, 77.4%) deposited immediately after dissolution, m.p. 212–216°; ^{1}H-n.m.r. (D_2O): δ 5.88 (m, 1 H, allyl proton), 5.33–5.24 (m, 2 H, allyl protons), 4.49 (d, 1 H, $J_{1,2}$ 8.8 Hz, H-1), 4.36–4.13 (m, 2 H, allyl protons), 3.93 (dd, 1 H, $J_{4,5}$ 4 Hz, H-4), 3.90 (dd, 1 H, $J_{2,3}$ 10.4 Hz, H-2), 3.83–3.74 (m, 2 H, H-6a and H-6b), 3.71 (dd, 1 H, H-3), 3.66 (m, 1 H, H-5), and 2.03 (s, 3 H, $COCH_3$).

Anal. Calc. for $C_{11}H_{19}NO_6$: C, 50.57; H, 7.33; N, 5.36. Found: C, 50.36; H, 7.41; N, 5.14.

2-Hydroxyethyl 2-acetamido-2-deoxy-β-D-galactopyranoside (**16**). — The triacetate **14** (51 mg, 0.1278 mmol) was treated with 0.05M sodium methoxide solution and the base neutralized with Dowex-50W (H^+) resin. The resultant material was purified by passing it through a LH-20 column eluted with 1:1 ethanol–water to yield a fraction that was pure by t.l.c. in 10:7:1 chloroform–methanol–water (R_F

0.35); lyophilization gave a solid (25 mg, 94.3 μmol, 74%); ^{1}H-n.m.r. (D_2O): δ 4.49 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 4.00–3.63 (m, 10 H, other protons), and 2.04 (s, 3 H, $COCH_3$).

*Allyl 2-acetamido-2-deoxy-α-*D*-galactopyranoside* (**17**). — *N*-Acetyl-D-galactosamine (200 mg, 0.905 mmol) was suspended in allyl alcohol (4 mL) containing boron trifluoride etherate (20 μL), and refluxed for 3 h, giving a clear solution. Evaporation resulted in a grey solid which was recrystallized from 95% ethanol, to yield **21** (60 mg, 0.23 mmol, 25%), m.p. 204–205.5°; ^{1}H-n.m.r. (D_2O): δ 6.02–5.92 (m, 1 H), 5.37–5.24 (m, 2 H, allyl protons), 4.96 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 4.24–4.19 (m, 1 H, allyl proton), 4.17 (dd, 1 H, $J_{2,3}$ 11 Hz, H-2), 4.06–4.00 (m, 1 H, allyl proton), 4.00 (bd, 1 H, H-4), 3.92 (dd, 1 H, $J_{3,4}$ 2.8 Hz, H-3), 3.77–3.75 (other protons), and 2.04 (s, 3 H, $COCH_3$).

Anal. Calc. for $C_{11}H_{19}NO_6$: C, 50.57; H, 7.23; N, 5.36. Found: C, 49.87; H, 7.39; N, 5.26.

*Allyl 2-acetamido-4,6-O-benzylidene-2-deoxy-β-*D*-galactopyranoside.* — A mixture of compound **15** (200 mg, 0.766 mmol), zinc chloride (200 mg), and benzaldehyde (1 mL) was shaken for 24 h at room temperature. Water and chloroform were then added, and the mixture was shaken for 0.5 h. The chloroform layer was separated, dried (sodium sulfate), and evaporated, to give an oil which was dissolved in 1:1 methanol–ether hexane to incipient turbidity. Storage for 2 days at 4° yielded a crystalline product (105 mg, 0.313 mmol, 41%); m.p. 215°; ^{1}H-n.m.r. (Me_2SO-d_6): δ 7.65 (d, 1 H, $J_{NH,2}$ 8.6 Hz, NH), 7.50–7.36 (m, 5 H, benzylidene aromatic protons), 5.89–5.81 (m, 1 H, allyl proton), 5.59 (s, 1 H, benzylidene CH), 5.28–5.10 (m, 2 H, allyl protons), 4.86 (d, 1 H, OH), 4.39 (d, 1 H, $J_{1,2}$ 8.9 Hz, H-1), 4.26–4.21 (m, 1 H, allyl proton), 4.10 (dd, 1 H, $J_{3,4}$ 3.4 Hz, H-4), 4.07 (m, 2 H, H-6a and H-6b), 4.03–3.98 (m, 1 H, allyl proton), 3.79 (m, 1 H, H-2), 3.63 (dd, 1 H, $J_{2,3}$ 10.7 Hz, H-3), 3.50 (m, 1 H, H-5), and 1.81 (s, 3 H, $COCH_3$).

Anal. Calc. for $C_{18}H_{23}NO_6 \cdot 0.5\ H_2O$: C, 60.32; H, 6.75; N, 3.91. Found: C, 60.20; H, 6.40; N, 3.44.

*Allyl 2-acetamido-2-deoxy-3-O-methyl-β-*D*-galactopyranoside* (**18**). — To a solution of the last produced compound (82 mg, 0.244 mmol) in 1,4-dioxane (4 mL) at 60° were added 30% sodium hydroxide solution (900 μL) in ten portions and dimethyl sulfate (340 μL) during 1 h, and heating was continued for 1 h. The solution was cooled, diluted with cold water (10 mL), excess of the base neutralized with carbon dioxide, and extracted with chloroform (2 × 25 mL). The extracts were combined, and evaporated, giving an oil which was treated with 80% acetic acid for 3 h at 60°, the solution evaporated, and the residue treated with 1:1 pyridine–acetic anhydride overnight at room temperature. The solution was evaporated, and the resultant syrup was dissolved in ethyl acetate and chromatographed on a column of silica gel to afford a main fraction identified as allyl 2-acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-methyl-β-D-galactopyranoside; ^{1}H-n.m.r. ($CDCl_3$): δ 5.90 (m, 1 H, allyl proton), 5.65 (d, 1 H, $J_{NH,2}$ 7.2 Hz, NH), 5.48 (bd, 1 H, $J_{3,4}$ 3.2 Hz, H-4), 5.32–5.19 (m, 2 H, allyl protons), 5.12 (d, 1 H, $J_{1,2}$ 8 Hz,

H-1), 4.37–4.32 (m, 1 H, allyl proton), 4.24 (dd, 1 H, $J_{2,3}$ 10.8 Hz, H-3), 4.15 (d, 2 H, H-6a and H-6b), 3.88 (m, 1 H, H-5), 3.38 (s, 3 H, OCH_3), 2.12, 2.08, and 1.99 (s, 9 H, 3 $COCH_3$). This material was *O*-deacetylated to provide compound **23** (19 mg, 69 μmol, 28%); ^{1}H-n.m.r. (D_2O): δ 5.90 (m, 1 H, allyl proton), 5.33–5.24 (m, 2 H, allyl protons), 4.50 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 4.36–4.31 (m, 1 H, allyl proton), 4.21 (bd, 1 H, $J_{3,4}$ 3.1 Hz, H-4), 4.19–4.13 (m, 1 H, allyl proton), 3.91 (dd, 1 H, $J_{2,3}$ 11 Hz, H-2), 3.80 (m, 1 H, H-6a and H-6b), 3.64 (m, 1 H, H-5), 3.43 (dd, 1 H, H-3), 3.39 (s, 3 H, OCH_3), and 2.02 (s, 3 H, $COCH_3$).

*Allyl 2-acetamido-2-deoxy-3,4-O-isopropylidene-β-*D*-galactopyranoside.* — A mixture of compound **15** (0.7363 g, 2.821 mmol), 2,2-dimethoxypropane (1.38 mL), and *p*-toluenesulfonic acid (40 mg) in *N,N*-dimethylformamide (10 mL) was stirred for 4 h at room temperature, additional 2,2-dimethoxypropane (1.2 mL) and *p*-toluenesulfonic acid monohydrate (20 mg) were added, and the solution was kept overnight. Neutralization of the acid with triethylamine followed by evaporation gave a syrup which was applied to a column of silica gel and eluted with 9:1 ethyl acetate–methanol, to give two major fractions (R_F 0.50 and R_F 0.25 in 9:1 ethyl acetate–methanol). The first fraction was identified as compound **25** (0.5 g, 1.66 mmol, 59%); m.p. 165–166.5°; ^{1}H-n.m.r. ($CDCl_3$): δ 5.95–5.85 (m, 2 H, allyl proton and NH), 5.31–5.18 (m, 2 H, allyl protons), 5.04 (d, 1 H, $J_{1,2}$ 8.6 Hz, H-1), 4.76 (dd, 1 H, $J_{2,3}$ 8.2, $J_{3,4}$ 5.3 Hz, H-3), 4.36–4.31 (m, 1 H, allyl proton), 4.18 (dd, 1 H, $J_{4,5}$ 2.1 Hz, H-4), 4.14–4.08 (m, 1 H, allyl proton), 4.02–3.93 (m, 2 H, H-6a and H-5), 3.83 (dd, 1 H, $J_{6a,6b}$ 11.3, $J_{5,6b}$ 3.1 Hz, H-6b), 3.11 (ddd, 1 H, H-2), 2.00 (s, 3 H, $COCH_3$), 1.52 and 1.34 (2 s, 6 H, isopropylidene methyl protons); ^{1}H-n.m.r. (Me_2SO-d_6): δ 4.81 (t, 1 H, *J* 5.2 Hz, OH).

The second fraction was the 4,6-*O*-isopropylidene derivative.

*Allyl 2-acetamido-2-deoxy-6-O-methyl-β-*D*-galactopyranoside* (**19**). — To a solution of the last prepared compound (150 mg, 0.50 mmol) in *N,N*-dimethylformamide (1 mL) were added methyl iodide (125 μL) and silver oxide (116 mg). After 8 h of vigorous stirring, second portions of methyl iodide (125 μL) and silver oxide (116 mg) were added, and the mixture was stirred overnight. The solid was filtered off, and the filtrate evaporated, to give a syrup which was chromatographed on a column of silica gel eluted with 9:1:0.04 chloroform–methanol–triethylamine. A fraction pure by t.l.c. (R_F 0.54 in the same solvent system) was obtained and was evaporated to an oil which was treated with 80% acetic acid for 0.5 h at 60°. Evaporation of the acid left an oil which was treated with 1:1 pyridine–acetic anhydride overnight at room temperature and the solution evaporated. The resultant material applied to a column of silica gel eluted with 39:1 chloroform–methanol, to give a pure fraction which was identified as allyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-methyl-β-D-galactopyranoside: ^{1}H-n.m.r. ($CDCl_3$): δ 5.87 (m, 1 H, allyl proton), 5.51 (d, 1 H, $J_{NH,2}$ 8.8 Hz, NH), 5.38 (bd, 1 H, $J_{3,4}$ 3.6 Hz, H-4), 5.31–5.18 (m, allyl proton), 5.25 (dd, 1 H, $J_{2,3}$ 10.4 Hz, H-3), 4.69 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 4.41–4.36 (m, 1 H, allyl proton), 4.13–4.08 (m, 1 H, allyl proton), 4.05 (m, 1 H, H-2), 3.82 (m, 1 H, H-5), 3.53–3.40 (m, 2 H, H-6a and H-6b), 3.34 (s, 3

H, OCH_3), 2.15, 2.00, and 1.96 (3 s, 9 H, 3 $COCH_3$). The material was *O*-deacetylated, to yield compound **19** (62 mg, 0.23 mmol, 46%); ^{1}H-n.m.r. (D_2O): δ 5.95–5.85 (m, 1 H, allyl proton, 5.34–5.24 (m, 2 H, allyl protons), 4.50 (d, 1 H, $J_{1,2}$ 8.6 Hz, H-1), 4.36–4.12 (m, 2 H, allyl protons), 3.90 (dd, 1 H, $J_{2,3}$ 10.4 Hz, H-2), 3.90 (dd, 1 H, $J_{3,4}$ 3.4 Hz, H-4), 3.80 (m, 1 H, H-5), 3.72 (dd, 1 H, H-3), 3.68 (m, 2 H, H-6a and H-6b), 3.41 (s, 3 H, OCH_3), and 2.03 (s, 3 H, $COCH_3$).

*Allyl 2-acetamido-2-deoxy-4,6-di-*O*-methyl-β-*D*-galactopyranoside* (**20**) *and allyl 2-acetamido-2-deoxy-3,6-di-*O*-methyl-β-*D*-galactopyranoside* (**21**). — To a vigorously stirred mixture of compound **15** (370 mg, 1.42 mmol), *N,N*-dimethylformamide (7 mL), and silver oxide (160 mg) was added methyl iodide (100 μL), and stirring was continued overnight at room temperature. Additional silver oxide and methyl iodide (a total of 4.5 equiv.) were added during the next 3 days, while vigorous stirring was maintained. Methanol was added, the solid was filtered off, and the filtrate was evaporated to a solid which was dissolved in 7:2:1 ethyl acetate–2-propanol–water and the solution applied to a column of silica gel and eluted with the same solvent system. Two major fractions (R_F 0.53 and R_F 0.37 in 7:2:1 ethyl acetate–2-propanol–water) were separated, and processed as follows.

(*1*) The first fraction (R_F 0.53) was treated with 1:1 pyridine–acetic anhydride overnight at room temperature, and evaporated; the residue was applied to a column of silica gel and eluted with ethyl acetate. Two fractions (R_F 0.37 and 0.12, ethyl acetate) were separated.

The first fraction (R_F 0.37, ethyl acetate) was identified as allyl 2-acetamido-3-*O*-acetyl-2-deoxy-4,6-di-*O*-methyl-β-D-galactopyranoside; ^{1}H-n.m.r. ($CDCl_3$): δ 5.85 (m, 1 H, allyl proton), 5.46 (d, 1 H, $J_{NH,2}$ 8.0 Hz, NH), 5.28–5.15 (m, 2 H, allyl protons), 5.21 (dd, 1 H, $J_{2,3}$ 11.6, $J_{3,4}$ 3.6 Hz, H-3), 4.67 (d, 1 H, $J_{1,2}$ 8.8 Hz, H-1), 4.36–4.02 (m, 3 H, H-2 and allyl protons overlapping), 3.51, 3.39 (2 s, 6 H, 2 OCH_3), 3.70–3.50 (m, H-5,6a,6b), 2.11 and 1.95 (2 s, 6 H, 2 $COCH_3$). The material was *O*-deacetylated, to give compound **21** (45 mg, 0.16 mmol, 11%); ^{1}H-n.m.r. (D_2O): δ 5.94–5.84 (m, 1 H, allyl proton), 5.32–5.23 (m, 2 H, allyl proton), 4.46 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.34–4.10 (m, 2 H, allyl proton), 3.85 (dd, 1 H, $J_{2,3}$ 11.2 Hz, H-2), 3.80 (m, 1 H, H-5), 3.76 (dd, 1 H, $J_{3,4}$ 3.6 Hz, H-3), 3.71 (m, 2 H, H-6a,b), 3.62 (dd, 1 H, H-4), 3.53, 3.42 (2 s, 6 H, 2 OCH_3), and 2.03 (s, 3 H, $COCH_3$).

The second fraction was identified as 2-acetamido-2-deoxy-3,4,6-tri-*O*-methyl-β-D-galactopyranoside, by ^{1}H-n.m.r. spectroscopy.

(*2*) The second fraction (R_F 0.37) was *O*-acetylated in the same way, and the product was applied to a column of silica gel eluted with ethyl acetate, to give 3 fractions (R_F 0.37, 0.21, and 0.13, ethyl acetate).

The first fraction was identified as a mixture of allyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-methyl-β-D-galactopyranoside (a) and allyl 2-acetamido-3,6-di-*O*-acetyl-2-deoxy-4-*O*-methyl-β-D-galactopyranoside (b): ^{1}H-n.m.r. ($CDCl_3$): δ 5.91–5.80 (m, 1 H, allyl proton), 5.48–5.40 (m, 1 H, NH of both compounds), 5.37 (bd, 0.5 H, H-4 of a), 5.29–5.14 (m, allyl proton overlapping with H-3 of both

compounds), 4.70 (d, 0.5 H, H-1 of a), 4.67 (d, 0.5 H, H-1 of b), 4.39–4.18 (m, allyl protons overlapping with H-6 of b), 4.11–3.97 (m, allyl protons overlapping with H-2 for both compounds), 3.82 (m, 0.5 H, H-5 of a), 3.70 (m, 0.5 H, H-5 of b), 3.61 (bd, 0.5 H, H-4 of b), 3.50 (s, 1.5 H, OCH_3), 3.47 (m, 0.5 H, H-6 of a), 3.33 (s, 1.5 H, OCH_3), 2.14, 2.10, 2.07, 2.00, 1.94, and 1.93 (6 s, 2 $COCH_3$).

The second fraction was identified as allyl 2-acetamido-4-*O*-acetyl-2-deoxy-3,6-di-*O*-methyl-β-D-galactopyranoside: ^{1}H-n.m.r. ($CDCl_3$): δ 5.88 (m, 1 H, allyl proton), 5.69 (d, 1 H, $J_{NH,2}$ 6.8 Hz, NH), 5.49 (bd, 1 H, $J_{3,4}$ 3.6 Hz, H-4), 5.29–5.16 (m, 2 H, allyl protons), 5.07 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1), 4.38–4.32 (m, 1 H, allyl proton), 4.18 (dd, 1 H, $J_{2,3}$ 11.2 Hz, H-3), 4.12–4.07 (m, 1 H, allyl proton), 3.77 (m, 1 H, H-5), 3.46 (m, 1 H, H-6a and H-6b), 3.35, 3.34 (2 s, 6 H, 2 OCH_3), 3.22 (m, 1 H, H-2), 2.11, and 1.97 (2 s, 6 H, 2 $COCH_3$). The material was *O*-deacetylated, to yield compound **21** (70 mg, 17%); ^{1}H-n.m.r. (D_2O): δ 5.89 (m, 1 H, allyl proton), 5.33–5.23 (m, 2 H, allyl protons), 4.49 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 4.35–4.30 (m, 1 H, allyl proton), 4.18 (bd, 1 H, $J_{3,4}$ 3.2 Hz, H-4), 4.17–4.11 (m, 1 H, allyl proton), 3.91 (dd, 1 H, $J_{2,3}$ 10.4 Hz, H-2), 3.78–3.67 (m, 3 H, H-5,6a,6b), 3.43 (dd, 1 H, H-3), 3.41, 3.39 (2 s, 6 H, 2 OCH_3), and 2.02 (s, 3 H, $COCH_3$).

The third fraction was identified as allyl 2-acetamido-6-*O*-acetyl-2-deoxy-3,4-di-*O*-methyl-β-D-galactopyranoside: ^{1}H-n.m.r. ($CDCl_3$): δ 5.89 (m, 1 H, allyl proton), 5.71 (d, 1 H, $J_{NH,2}$ 6.4 Hz, NH), 5.27–5.15 (m, 2 H, allyl protons), 5.04 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.26 (m, 2 H, H-6), 4.19 (dd, 1 H, $J_{2,3}$ 11.3, $J_{3,4}$ 3.4 Hz, H-3), 3.65 (bd, 1 H, H-4), 3.55, 3.46 (2 s, 6 H, 2 OCH_3), 3.27 (m, 1 H, H-2), 2.09, and 1.97 (2 s, 6 H, 2 $COCH_3$).

*Allyl 2-acetamido-3-*O*-acetyl-2-deoxy-α-*D*-glucopyranoside.* — A mixture of allyl 2-acetamido-2-deoxy-α-D-glucopyranoside[4] (10 g, 38.3 mmol), benzaldehyde (60 mL), and zinc chloride (10 g) was stirred overnight at room temperature, shaken with water (150 mL) and hexane (150 mL) for 15 min, the precipitate filtered off, and the filtrate washed with water and hexane. The material (10 g) was suspended in acetic anhydride (25 mL) and pyridine (50 mL), and stirred overnight, resulting in a clear solution which was evaporated. The residue crystallized from ethyl acetate, to give compound (8.42 g, 22.2 mmol, 58%); m.p. 192–195°. This was treated with 80% acetic acid for 4 h at 70°, evaporated, and washed with 1:1 acetone–diethyl ether, to give a white solid (5.12 g, 16.9 mmol, 76%), m.p. 174–177.5°.

*Allyl 2-acetamido-3,6-di-*O*-acetyl-2-deoxy-α-*D*-glucopyranoside.* — To a stirred solution of imidazole (0.9241 g, 13.57 mmol) in dry chloroform (130 mL) cooled at 0° was added a solution of acetyl chloride (0.516 g, 7.26 mmol) in dry chloroform (30 mL). The precipitate was filtered off, and to the filtrate was added the compound prepared in the previous section (2 g, 6.6 mmol). The suspension was heated for 3 days at 80° cooled and washed with water (60 mL) which was extracted with chloroform (2 × 20 mL). The solution and extract were combined, dried (sodium sulfate), and evaporated to dryness. The residue was dissolved in ethyl acetate, and the solution applied to a column of silica gel eluted with the same solvent. Evapora-

tion of the combined major fraction afforded a solid which was recrystallized from ethyl acetate–hexane, to give product (1.2498 g, 3.62 mmol, 55%), m.p. 137.5–138°; ^{1}H-n.m.r. ($CDCl_3$): δ 5.94–5.87 (m, 1 H, allyl proton), 5.81 (d, 1 H, NH), 5.33–5.23 (m, 2 H, allyl protons), 5.12 (dd, 1 H, $J_{2,3}$ 10.8, $J_{3,4}$ 9.2 Hz, H-3), 4.86 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 4.52 (dd, 1 H, $J_{6a,6b}$ 12.8, $J_{5,6a}$ 4.6 Hz, H-6a), 4.30–4.24 (m, 2 H, H-2 and H-6b overlapping), 4.21–3.98 (m, 2 H, allyl proton), 3.83 (m, 1 H, H-5), 3.62 (m, 1 H, H-4), 3.07 (d, 1 H, OH), 2.14, 2.09, and 1.96 (3 s, 9 H, 3 $COCH_3$).

*Allyl 2-acetamido-3,6-di-*O*-acetyl-2,4-dideoxy-4-fluoro-α-*D*-galactopyranoside* (**22**). — To a solution of the last prepared compound (1 g, 2.9 mmol) and 4-(dimethylamino)pyridine (1.4165 g, 11.59 mmol) in dry dichloromethane (6 mL) cooled to −10° was added diethylaminosulfur trifluoride (DAST, 1.41 mL, 11.6 mmol). After stirring overnight at room temperature, the mixture was cooled to −20° and methanol (10 mL) was added. Chloroform (100 mL) was added to the dark red solution and this was washed with saturated sodium hydrogencarbonate solution (40 mL) which was extracted with chloroform (50 mL). The solution and extracts were combined, and evaporated to dryness, and the residue was chromatographed on a column of silica gel eluted with ethyl acetate. The fraction having R_F 0.54 in t.l.c. in ethyl acetate were combined, concentrated to ~5 mL by evaporation, and hexane was added to incipient opalescence. On storage for 2 days at 4°, crystals were deposited (163 mg, 0.47 mmol, 16%), and these were identified as **22**, m.p. 98–100°; ^{1}H-n.m.r. ($CDCl_3$): δ 5.89 (m, 1 H, allyl proton), 5.64 (d, 1 H, $J_{NH,2}$ 9.6 Hz, NH), 5.33–5.23 (m, 2 H, allyl protons), 5.16 (ddd, 1 H, $J_{2,3}$ 11.29, $J_{3,4}$, $J_{3H,4F}$ 28 Hz, H-3), 4.94 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1), 4.81 (dd, 1 H, $J_{4H,4F}$ 50.7 Hz, H-4), 4.65 (ddd, 1 H, H-2), 4.33–4.00 (m, H-5, H-6a, H-6b, and allyl protons overlapping), 2.12, 2.09, and 1.98 (3 s, 9 H, 3 $COCH_3$); ^{19}F-n.m.r. ($CDCl_3$): δ −218.97.

The second fraction eluted from the column (having R_F 0.45) was processed as for compound **22**, to give crystals (148 mg, 0.47 mmol, 16%) identified as allyl 2-acetamido-6-*O*-acetyl-2-deoxy-3,4-*O*-(methyl orthoacetyl)-α-D-galactopyranoside (**24**), m.p. 122.5–125°; ^{1}H-n.m.r. ($CDCl_3$): δ 5.90 (m, 1 H, allyl proton), 5.80 (broad s, 1 H, NH), 5.30–5.22 (m, 2 H, allyl protons), 4.84 (d, 1 H, $J_{1,2}$ 2.8 Hz, H-1), 4.42–3.95 (m, 8 H, other protons), 3.30 (s, 3 H, orthoacetate OCH_3), 2.10, 2.04 (2 s, 6 H, 2 $COCH_3$), and 1.67 (orthoacetate CH_3).

*Allyl 2-acetamido-2,4-dideoxy-4-fluoro-α-*D*-galactopyranoside* (**23**). — Compound **22** (85 mg, 0.245 mmol) was treated with 0.1M sodium methoxide solution for 3 h at room temperature. The usual work-up afforded a solid residue which was applied to a column of silica gel and eluted with 12:2:1 ethyl acetate–2-propanol–water. The purified material was passed through a LH-20 column and eluted with 1:1 ethanol–water. The resultant material was recrystallized from ethyl acetate to give **23** (35 mg, 0.133 mmol, 54%), m.p. 197.5–198°; ^{1}H-n.m.r. (D_2O): δ 5.97 (m, 1 H, allyl proton), 5.31 (m, 2 H, allyl protons), 5.00 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 4.91 (bdd, 1 H, $J_{3,4}$ 2.4, $J_{4H,4F}$ 50.4 Hz, H-4), 4.25–3.79 (m, 7 H, other protons), and 2.04 (s, 3 H, $COCH_3$).

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