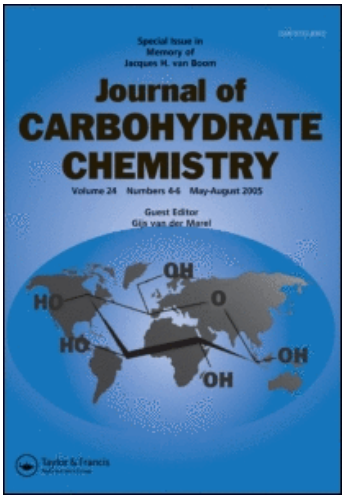

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SYNTHESIS AND BINDING ACTIVITY OF 3- AND 4-DEOXY-N-ACETYL-GALACTOSAMINE DERIVATIVES

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ABSTRACT

Allyl 3- and 4-deoxy-N-acetyl-β-D-galactosaminides were synthesized from galactosamine and glucosamine, respectively. Using inhibition assay, we found that neither the 3-deoxy nor the 4-deoxy derivative had any binding affinity to the Gal/GalNAc lectins of rabbit and rat liver at the highest concentration tested (40 mM), indicating that the absence of either 3- or 4-hydroxyl group of N-acetyl-galactosamine (GalNAc) caused a decreace in afffinity of at least 100-fold. Therefore, both 3- and 4-hydroxyl groups of GalNAc are required for the binding to the mammalian hepatic lectins.

INTRODUCTION

The Gal/GalNAc-specific lectins of mammalian hepatocytes and rat peritoneal macrophages have been studied extensively with respect to their mode of carbohydrate-recognition.[1,2] For both lectins, 3- and 4-hydroxy groups of Gal or GalNAc appeared to be important, since methylation of these positions weakens the binding affinity considerably.[1d,2b] In the case of the rat hepatic lectin, methylation of 3-OH of GalNAc decreaseo the affinity by more than a hundredfold.[1d] Although 6-O-methyl-GalNAc bound to the lectin as strongly as GalNAc, 4,6-di-O-

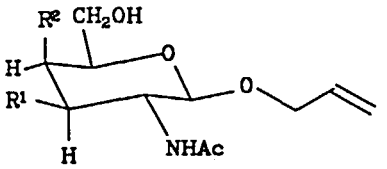
methyl-GalNAc showed a hundredfold weaker affinity than GalNAc,[1d] indicating that the 4-O-methyl group also interfered with binding. In order to differentiate whether such a decrease in affinity is due to the loss of hydrogen atom on the 3-or 4-OH group or to the steric hindrance by the methyl group, we synthesized both 3- and 4-deoxy derivatives of GalNAc (and Gal). In this report, we describe a simple and efficient synthesis of 3- and 4-deoxy GalNAc derivatives (2 and 3, respectively) having an allyl aglycon that can be used for further derivatization,[3] or can be removed to generate the reducing group.

RESULTS AND DISCUSSION

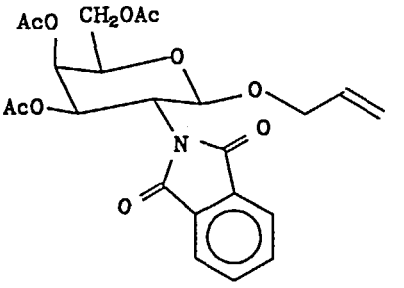
Galactosamine hydrochloride was converted by the method of Ogawa and Beppu,[4] to an N-phthaloyl derivative which was condensed with allyl alcohol in the presence of tin (IV) chloride[5] to give an 86% yield of allyl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranoside (4) as a sole product. After removal of acetyl groups of 4, the resulting triol was subjected to benzylidenation with α,α-dimethoxytoluene[6] to afford a 4,6-O-benzylidene derivative (5) in 86% yield.

For deoxygenation we first attempted the tributyltin hydride reduction[7] of the phenoxythiocarbonyl derivative 6. When the benzylidene derivative 5 was treated with phenoxythiocarbonyl chloride in pyridine, 6 was obtained in 50% yield concomitant with a by-product which might have resulted from β-elimination of 6 during the reaction. Reduction of 6 with tributyltin hydride in the presence of azobisisobutyronitrile (AIBN) in toluene successfully afforded a 59% yield of the 3-deoxy derivative 8. However, the chemical yield of 6 from 5 was unsatisfactorily low.

An efficient deoxygenation method employing an imidazoylthiocarbonyl derivative of sugars was reported by Rasmussen et al.[8] Using this method, the alcohol 5 smoothly reacted with 1,1'-thiocarbonyldiimidazole in boiling 1,2-dichloroethane to give 7 in 96% yield. Reduction of 7 with tributyltin hydride in toluene at 100 °C gave 8 in 78% yield. In the ^{1}H NMR spectrum of 8, signal for the equatorial H-3 appeared at δ 2.19 ppm as a doublet of doublet of doublets with $J_{2,3eq}$=4.64, $J_{3eq,3ax}$= 13.87, and $J_{3eq,4}$=2.39 Hz, and signal for the axial H-3 at δ 2.78 ppm as a doublet of triplets with $J_{2,3ax} = J_{3ax,3eq}$=13.86 and $J_{3ax,4}$=3.61 Hz. In general,



	R^1	R^2
1	OH	OH
2	H	OH
3	OH	H



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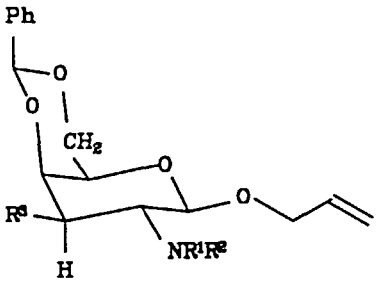
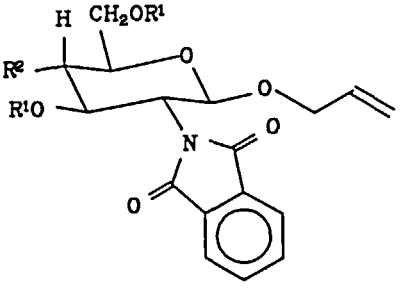


	R^1, R^2	R^3
5	phthaloyl	OH
6	phthaloyl	-OC(=S)-OPh
7	phthaloyl	-OC(=S)-imidazoyl
8	phthaloyl	H
9	Ac, H	H



	R^1	R^2
10	Ac	OAc
11	H	OH
12	Bz	OH
13	Bz	-OC(=S)-imidazoyl
14	Bz	H

the ^{1}H NMR spectrum of a 3-deoxygenated pyranose such as methyl 2-O-acetyl-4,6-O-benzylidene-3-deoxy-β-D-galactopyranoside[9] shows that the signal for the 3-axial proton (δ 1.75 ppm, ddd, J=13.7, 11.5, and 2.6 Hz) appears at a higher chemical shift than the 3-equatorial proton (δ 2.46 ppm, ddd, J=13.7, 5.3, and 2.6 Hz). In the case of 8, however, the 2-phthalimido group probably has a strong deshielding effect on the 3-axial proton so that the resonance of H-3_{ax} appears at a lower field than H-3_{eq}.

Removal of the N-phthaloyl group of 8 by treatment with hydrazine hydrate[14] followed by N-acetylation gave 9 in 87% yield. The benzylidene group of 9 was removed with 80% acetic acid to afford 2 in 28% yield after direct crystallization. Alternatively, successive treatment of 8 with 80% acetic acid, butylamine[10] in boiling methanol, and acetic anhydride gave 2 in 56% overall yield. Similarly, allyl β-GalNAc 1 was obtained from 4 in 41% overall yield by removal of the N-phthaloyl group followed by N-acetylation as described for the preparation of 2.

For the preparation of 4-deoxy-GalNAc, glucosamine hydrochloride was converted to its β-allyl glycoside[5,11] (10) *via* 1,3,4,6-tetra-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose.[12] After removal of the acetyl groups of 10, the resulting triol (11) was treated with two equivalent amounts of benzoyl chloride[13] to give a 42% yield of the 3,6-di-O-benzoyl derivative (12). As described for the preparation of 3-deoxy-GalNAc derivative (2), compound 12 was successively treated with thiocarbonyldiimidazole and tributyltin hydride to give a 4-deoxy derivative (14) in 91% overall yield. Deprotection and subsequent N-acetylation of 14 afforded a 4-deoxy GalNAc derivative (3) in 67% yield.

Compound 1, its corresponding 3- and 4-deoxy derivatives (2 and 3) were tested by an inhibition assay using the detergent-solubilized, purified rat and rabbit hepatic lectins and isolated rat hepatocytes[14] to assess their binding affinity. As expected, 1 showed a strong inhibitory activity, causing >99% inhibition of ^{125}I-ASOR (asialoorosomucoid) binding to the soluble hepatic lectins of rat and rabbit at 2 mM, while the 3- and 4-deoxy derivatives, 2 and 3, did not show measurable inhibitory activity at 40 mM. Half-maximal inhibition concentration (I_{50}) of 1 was 100 μM and 15 μM for the rabbit and rat hepatic lectins, respectively. In the case of the isolated rat hepatocytes,

allyl GalNAc (1) has an I_{50} value of 24 μM, whereas 3- and 4-deoxy-GalNAc derivatives (2 and 3) were totally inactive at 40 mM. At this concentration, 3-O-methyl and 4,6-di-O-methyl GalNAc derivatives showed some inhibition (40 and 55% inhibition, respectively) in the isolated rat hepatocyte system.[14] Therefore, it appears that both oxygen and hydrogen atoms of 3-and 4-OH may be needed for a strong affinity.

EXPERIMENTAL

General methods.--- Melting points were determined with a Fisher-Johns melting point apparatus and are uncorrected. Elemental analyses were performed by Galbraith Lab. Inc. (Knoxville, TN). Solutions were concentrated under diminished pressure, and solvent extracts were dried over anhydrous sodium sulfate. Chromatography was conducted on a column of Silica Gel 60 (15--40 μm; E. Merck, Darmstadt, Germany). Thin-layer chromatography was conducted with Silica Gel 60 F_{254} precoated on glass plates (layer thickness, 0.25 mm; E. Merck, Darmstadt, Germany). Gel filtration was conducted on a column of Sephadex LH-20 or G-25 (Pharmacia). Nuclear magnetic resonance spectra were recorded with a Varian XL-400 spectrometer in $CDCl_3$ solutions unless otherwise specified. Proton chemical shift are in ppm relative to an internal reference of tetramethylsilane (δ 0.00) or HOD in D_2O at 24 °C (δ 4.778). Fast-atom-bombardment (f.a.b.) mass spectra were recorded with a JEOL HX110 spectrometer in the negative-ion mode: the sample was applied in a 3-nitrobenzylalcohol matrix and bombarded with Zenon atom having a kinetic energy equivalent to 10 KeV. Inhibition assay for assessment of binding affinity of GalNAc derivatives to the detergent-solubilized, purified rabbit and rat hepatic lectins and to the lectin on the surface of isolated rat hepatocytes was carried out as described.[14] Half-maximal inhibition concentration (I_{50}) is obtained graphically from a plot of percent inhibition *vs* inhibitor concentration.

Allyl 3,4,6-Tri-O-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranoside (4).--- Tin (IV) chloride[5] (2.23 g, 8.55 mmol; 1.0 mL) was added dropwise to a solution of 1,3,4,6-tetra-O-acetyl-2-deoxy-2-phthalimido-D-galactopyranose[4] (1.55 g, 3.25 mmol) and allyl alcohol (754 mg, 13.0 mmol; 0.88 mL) in dichloromethane (100 mL) at room temperature and the mixture was stirred for 10 h at room temperature. The mixture was

poured into ice-cold aqueous sodium hydrogen carbonate and the precipitate was filtered through a Celite bed. The filtrate was extracted with chloroform and the extracts, without washing, were dried, and concentrated. The residue was chromatographed on silica gel with 16:1 toluene--ethyl acetate, to give 4 as a syrup (1.25 g, 81%); δ_H: 1.854, 2.075, and 2.203 (s, 3 H each, 3 x OAc), 4.03--4.08 (m, 1 H, allylic proton of allyl group), 4.08 (br t, 1 H, J=6.58 Hz, H-5), 4.13--4.27 (m, 2 H, H-6a,6b), 4.28--4.30 (m, 1 H, allylic proton of allyl group), 4.58 (dd, 1 H, J=8.48 and 11.42 Hz, H-2), 5.04--5.16 (2 H, olefinic protons of allyl group), 5.35 (d, 1 H, J=8.48 Hz, H-1), 5.48 (br d, 1 H, J=3.36 Hz, H-4), 5.65--5.74 (m, 1 H, olefinic proton of allyl group), and 5.81 (dd, 1 H, J=3.39 and 11.42 Hz, H-3). *m/z*: 476.5 [$(M^+ + 1)$, calc. for $C_{23}H_{25}NO_{10}$: 475.5].

Benzylidenation of 4.--- To a solution of 4 (1.11 g, 2.33 mmol) in methanol (30 mL) was added 0.1 M methanolic sodium methoxide (0.7 mL) and the mixture was stirred for one h at room temperature. After neutralizing with Dowex 50W-X8 [H^+] resin, the resin was filtered off, and the filtrate was concentrated. A solution of the residue, α,α-dimethoxytoluene (1.49 g, 9.81 mmol; 1.5 mL), p-toluenesulfonic acid monohydrate (30 mg, 0.16 mmol) in N,N-dimethylformamide (DMF) (10 mL) was heated for one h at 50 °C. After cooling, triethylamine (0.2 mL) was added and the resulting mixture was concentrated. The residue was chromatographed on silica gel, with 5:1 toluene--ethyl acetate, to give allyl 4,6-O-benzylidene-2-deoxy-2-phthalimido-β-D-galactopyranoside (5) as a syrup (878 mg, 86%); δ_H: 3.651 (br s, 1 H, H-5), 4.06--4.13 (m, 1 H, allylic proton of allyl group), 4.16 (dd, 1 H, J=1.57 and 12.45 Hz, H-6a), 4.29--4.38 (m, 2 H, H-4, allylic proton of allyl group), 4.40 (d, 1 H, J=12.45 Hz, H-6b), 4.47 (dd, 1 H, J=8.06 and 10.93 Hz, H-2), 4.53 (dd, 1 H, J=3.66 and 10.93 Hz, H-3), 5.04--5.17 (m, 2 H, olefinic proton of allyl group), 5.31 (d, 1 H, J=8.06 Hz, H-1), 5.619 (s, 1 H, benzylic proton), and 5.71--5.78 (m, 1 H, olefinic proton of allyl group).

Phenoxythiocarbonylation of 5.--Phenoxythiocarbonyl chloride (158 mg, 0.91 mmol) was added dropwise to a cooled solution of 5 (180 mg, 0.41 mmol) in pyridine (5 mL) at 0--5 °C, and the mixture was stirred for one h with cooling. The mixture was poured into ice-water and extracted with ethyl acetate. The combined extracts were successively

washed with ice-cold dilute hydrochloric acid, aqueous sodium hydrogencarbonate, and water, and then dried, and concentrated. The residual syrup was chromatographed on silica gel with 5:1 hexane--ethyl acetate to remove excess reagent, and yielded the product concomitant with an unidentified UV-positive compound. The impure material was rechromatographed on silica gel, with 20:1 toluene--ethyl acetate to give allyl 3-O-phenoxythiocarbonyl-4,6-O-benzylidene-2-deoxy-2-phthalimido-β-D-galactopyranoside (6) as an amorphous powder (117 mg, 50%). δ_H: 3.70 (d, 1 H, J=1.10 Hz, H-5), 4.06 (m, 1 H, allylic proton of allyl group), 4.16 (dd, 1 H, J=1.76 and 12.45 Hz, H-6a), 4.30--4.36 (m, 1 H, allylic proton of allyl group), 4.41 (dd, 1 H, J=0.57 and 3.73 Hz, H-4), 4.89 (dd, 1 H, J=8.46 and 11.34 Hz, H-2), 5.02--5.16 (m, 2 H, olefinic protons of allyl group), 5.36 (d, 1 H, J=8.46 Hz, H-1), 5.634 (s, 1 H, benzylic proton), and 5.71 (dd, 1 H, J=3.73 and 11.34 Hz, H-3). This compound was unstable and was used for the deoxygenation reaction immediately for the preparation of 8.

Allyl 3-O-Imidazoylthiocarbonyl-4,6-O-benzylidene-2-deoxy-2-phthalimido-β-D-galactopyranoside (7).--- A solution of 5 (295 mg, 0.67 mmol) and thiocarbonyldiimidazole (240 mg, 1.35 mmol) in 1,2-dichloroethane (30 mL) was heated under gentle reflux for 20 h. After cooling the mixture, it was concentrated and the residual syrup was chromatographed on silica gel, with 6:1 toluene--ethyl acetate, to give 7 as an amorphous powder (353 mg, 96%); δ_H: 3.77 (br s, 1 H, H-5), 4.08--4.14 (m, 1 H, allylic proton of allyl group), 4.19 (dd, 1 H, J=1.53 and 12.48 Hz, H-6a), 4.33--4.38 (m, 1 H, allylic proton of allyl group), 4.45 (dd, 1 H, J=1.32 and 12.48 Hz, H-6b), 4.79 (br d, 1 H, J=3.63 Hz, H-4), 5.04 (dd, 1 H, J=8.39 and 11.17 Hz, H-2), 5.03--5.18 (m, 2 H, olefinic protons of allyl group), 5.51 (d, 1 H, J=8.39 Hz, H-1), 5.586 (s, 1 H, benzylic proton), 5.62--5.74 (m, 1 H, olefinic proton of allyl group), 6.35 (dd, 1 H, J=3.66 and 11.17 Hz, H-3), 7.00 (br s, 1 H, imidazole group), and 8.36 (br s, 1 H, imidazole group). *m/z*: 548 [(M^++1), calc. for $C_{28}H_{25}N_3O_7S$: 547]

Allyl 4,6-O-Benzylidene-2,3-dideoxy-2-phthalimido-β-D-xylo-hexopyranoside (8).--- From 6: A mixture of 6 (496 mg, 0.86 mmol), tributyltin hydride (755 mg, 2.59 mmol; 0.7 mL), and azobisisobutylnitrile (AIBN) (241 mg, 1.47 mmol) in toluene (25 mL) was heated for 1.5 h at 75 °C.

After cooling, the mixture was concentrated and the residual syrup was chromatographed on silica gel, with 5:1 hexane--ethyl acetate, to give 8 (215 mg, 59.3%); mp 189--192 °C (from ether--hexane); δ_H: 2.19 (ddd, 1 H, J=2.39, 4.64, and 13.87 Hz, H-3_{eq}), 2.78 (ddd, 1 H, J=3.61, 13.86, and 13.86 Hz, H-3_{ax}), 3.68 (s, 1 H, H-5), 4.05--4.09 (m, 1 H, allylic proton of allyl group), 4.12 (dd, 1 H, J=1.71 and 12.40 Hz, H-6a), 4.14 (br s, 1 H, H-4), 4.30--4.34 (m, 1 H, allylic proton of allyl group), 4.35 (d, 1 H, J=11.91 Hz, H-6b), 4.68 (ddd, 1 J=4.64, 8.54, and 13.68 Hz, H-2), 5.00--5.14 (m, 2 H, olefinic protons of allyl group), 5.30 (d, 1 H, J=8.54 Hz, H-1), 5.58 (s, 1 H, benzylic proton), and 5.68--5.77 (m, 1 H, olefinic proton of allyl group). *m/z*: 420 [(M^+-1), calc. for $C_{24}H_{23}NO_6$: 421].

From 7: A solution of 7 (1.05 g, 1.92 mmol) in toluene (20 mL) was added dropwise over 30 min to a refluxing mixture of tributyltin hydride (1.12 g, 3.83 mmol) in toluene (40 mL), and the reaction mixture was heated under reflux for 5 h. After cooling, the mixture was concentrated. The residual syrup was chromatographed on silica gel, with 25:1 toluene--ethyl acetate, to give 8 (500 mg, 78% yield based on the unrecovered 7) and 7 (210 mg, 0.38 mmol).

Allyl 2-Acetamido-4,6-O-benzylidene-2,3-dideoxy-β-D-xylo-hexopyranoside (9).--- A solution of 8 (160 mg, 0.38 mmol) and hydrazine monohydrate (57 mg, 1.14 mmol; 55 μL) in 95% ethanol (10 mL) was heated under reflux for 2.5 h. After cooling, the mixture was concentrated and treated with pyridine (10 mL) and acetic anhydride (5 mL). The reaction mixture was concentrated, and the residue was chromatographed on silica gel with 1:3 toluene--ethyl acetate. Proper fractions were combined and concentrated, and the residue was crystallized from chloroform--ether to give 9 (110 mg, 87%); mp 222--223 °C, δ_H: 1.99 (s, 3 H, NHAc), 2.16--2.44 (2 H, H-3_{ax},3_{eq}), 3.54 (br s, 1 H, H-5), 4.03--4.14 (m, 4 H, H-2, 4, 6a, allylic proton of allyl group), 4.29 (br d, 1 H, J=12.35 Hz, H-6b), 4.36--4.42 (m, 1 H, allylic proton of allyl group), 4.80 (d, 1 H, J=8.30 Hz, H-1), 5.17--5.31 (2 H, olefinic protons of allyl group), 5.52 (s, 1 H, benzylic proton), and 5.84--5.98 (m, 1 H, olefinic proton of allyl group). *m/z*: 334 [(M^++1), calc. for $C_{18}H_{23}NO_5$: 333].

Anal. Calcd for $C_{18}H_{23}NO_5$: C, 64.85; H, 6.95; N, 4.20. Found: C, 64.38; H, 7.11; N, 4.13.

Allyl 2-Acetamido-2,3-dideoxy-β-D-xylo-hexopyranoside (2).--From 8: A solution of 8 (53 mg, 0.13 mmol) in acetic acid (7 mL) and water (2 mL) was heated for one h at 90 °C, cooled, and concentrated. The residue was treated with pyridine (3 mL) and acetic anhydride (2 mL), and the mixture was concentrated. The residue was chromatographed on silica gel, with 3:1 toluene--ethyl acetate, to give the per-O-acetylated compound. A solution of the acetylated compound and butylamine (3 mL) in methanol (15 mL) was heated under gentle reflux for 15 h. After cooling, the mixture was concentrated. Acetic anhydride (3 mL) was added to a cooled solution of the residue in methanol (20 mL) at 0--5 °C, and the mixture was stirred for 1.2 h with cooling, then concentrated. Ether was added to a methanolic solution of the residue to give crystalline 2 (18 mg, 56%); mp 177--180 °C; δ_H (D_2O): 1.76 (ddd, 1 H, J=3.16, 12.76, and 13.94 Hz, H-3_{ax}), 1.986 (s, 3 H, NHAc), 2.11 (ddd, 1 H, J=3.05, 4.95, and 13.94 Hz, H-3_{eq}), 3.71--3.76 (3 H, H-5, 6a, 6b), 3.945 (distorted dd, 1 H, J=2.67 and 3.19 Hz, H-4), 3.99 (ddd, 1 H, J=4.95, 8.48, and 12.76 Hz, H-2), 4.14--4.20 (m, 1 H, allylic proton of allyl group), 4.32--4.38 (m, 1 H, allylic proton of allyl group), 4.53 (d, 1 H, J=8.48 Hz, H-1), 5.23--5.35 (m, 2 H, olefinic protons of allyl group), and 5.87--5.98 (m, 1 H, olefinic proton of allyl group).

Anal. Calcd for $C_{11}H_{19}NO_5 \cdot 0.1H_2O$: C, 53.49; H, 7.83; N, 5.67. Found: C, 53.48; H, 7.82; N, 5.64.

From 9: A solution of 9 (0.30 mmol) in acetic acid (8 mL) and water (2 mL) was heated for 2.5 h at 60 °C, cooled, and concentrated. The residue was chromatographed on silica gel, with 40:30:10 chloroform--ethyl acetate--methanol. Proper fractions were combined and concentrated, and the residue was crystallized from methanol--ether to give 2 (20.8 mg, 28.3%).

Allyl 2-Acetamido-2-deoxy-β-D-galactopyranoside (1).-- A solution of 4 (990 mg, 2.08 mmol) and butylamine (6 mL) in methanol (30 mL) was heated under gentle reflux for 18 h, cooled, and concentrated. Acetic anhydride (2 mL) was added to a cooled solution of the residue in methanol (30 mL) at 0--5 °C, and the mixture was stirred for 2 h with cooling. The mixture was concentrated and the residue was crystallized from methanol--ethyl acetate to give 1 (224 mg, 41%); mp 208 °C (Lit.[1d] mp 212--216 °C from methanol).

Anal. Calcd for $C_{11}H_{19}NO_6$: C, 50.56; H, 7.33; N, 5.36. Found: C, 50.47; H, 7.26; N, 5.26.

Allyl 2-Deoxy-2-phthalimido-β-D-glucopyranoside (11).-- To a solution of allyl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside[5,11] (10) (1.47 g, 3.09 mmol) in methanol (50 mL) was added 0.1 M sodium methoxide in methanol (1.2 mL) and the mixture was stirred for 3 h at room temperature. After neutralizing with Dowex 50W-X8 [H^+] resin, the resin was filtered off and the filtrate was concentrated to give 11 (1.0 g, 93%), mp 188--189 °C (from methanol--ether).

Anal. Calcd for $C_{17}H_{19}NO_7$: C, 58.46; H, 5.48; N, 4.01. Found: C, 58.47; H, 5.66; N, 4.04.

Allyl 3,6-Di-O-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (12).-- Benzoyl chloride (310 mg, 2.21 mmol) was added dropwise to a cooled solution of 11 (360 mg, 1.03 mmol) in pyridine (5 mL) and dichloromethane (5 mL) at 0--5 °C, and the mixture was stirred for 10 h at room temperature. The mixture was poured into ice-cold dilute sulfuric acid and extracted with chloroform. The extracts were successively washed with aqueous sodium hydrogen carbonate and water, and then dried, and concentrated. The residue was chromatographed on silica gel, with 30:1 toluene--ethyl acetate, to give 12 (R_F 0.50 in 3:1 toluene--ethyl acetate) (245 mg, 42%) as a syrup; δ_H: 3.90 (dd, 1 H, J=8.23 and 9.80 Hz, H-4), 3.92--3.98 (m, 1 H, H-5), 4.07--4.14 (m, 1 H, allylic proton of allyl group), 4.28--4.34 (m, 1 H, allylic proton of allyl group), 4.50 (dd, 1 H, J=8.45 and 10.78 Hz, H-2), 4.70 (dd, 1 H, J=2.20 and 12.12 Hz, H-6a), 4.79 (dd, 1 H, J=4.42 and 12.12 Hz, H-6b), 5.04--5.17 (m, 2 H, vinylic protons of allyl group), 5.50 (d, 1 H, J=8.45 Hz, H-1), 5.69--5.79 (m, 1 H, vinylic proton of allyl group), and 5.92 (dd, 1 H, J=8.23 and 10.78 Hz, H-3).

Anal. Calcd for $C_{31}H_{27}NO_9$: C, 66.78; H, 4.88; N, 2.51. Found: 67.02; H, 4.95; N, 2.24.

Allyl 3,6-Di-O-benzoyl-2-deoxy-2-phthalimido-4-O-thiocarbonyl-imidazoyl-β-D-glucopyranoside (13).-- A solution of 12 (236 mg, 0.42 mmol) and thiocarbonyldiimidazole (189 mg, 1.06 mmol) in 1,2-dichloroethane (25 mL) was refluxed for 20 h, and the mixture was concentrated. The residue was chromatographed on silica gel, with 3:1 toluene--ethyl acetate, to give 13 (R_F 0.19 in 3:1 toluene--ethyl acetate) (270 mg,

96%) as a syrup; δ_H: 4.10--4.16 (m, 1 H, allylic proton of allyl group), 4.29--4.35 (m, 1 H, allylic proton of allyl group), 4.33--4.38 (m, 1 H, H-5), 4.55 (dd, 1 H, J=4.75 and 12.24 Hz, H-6a), 4.64 (dd, 1 H, J=3.66 and 12.24 Hz, H-6b), 4.66 (dd, 1 H, J=8.48 and 10.57 Hz, H-2), 5.07--5.18 (m, 2 H, vinylic protons of allyl group), 5.61 (d, 1 H, J=8.48 Hz, H-1), 5.69--5.79 (m, 1 H, vinylic proton of allyl group), 6.27 (br t, 1 H, J=9.33 Hz, H-4), 6.35 (dd, 1 H, J=9.09 and 10.60 Hz, H-3), 6.98 (br s, 1 H, imidazolyl), and 8.309 (br s, 1 H, imidazolyl). *m/z*: 668 [$(M^+ + 1)$, calc. for $C_{35}H_{29}N_3O_9S$: 667].

Allyl 2-Acetamido-2,4-dideoxy-β-D-xylo-hexopyranoside (3).-- A solution of tributyltin hydride (271 mg, 0.93 mmol; 0.25 mL) in toluene (15 mL) was added dropwise to a refluxing solution of 13 (270 mg, 0.40 mmol) in toluene (10 mL) over 5 min and the mixture was refluxed for another 30 min. The mixture was concentrated, and the residue was chromatographed on silica gel, with 30:1 toluene--ethyl acetate as eluant, to give allyl 3,6-di-O-benzoyl-2,4-dideoxy-2-phthalimido-β-D-xylo-hexo-pyranoside (14) (R_F 0.72 in 3:1 toluene--ethyl acetate) (206 mg, 95%) as a syrup; δ_H: 1.841 (br dt, 1 H, J=11.65 and 12.65 Hz, H-4_{ax}), 2.54 (ddd, 1 H, J= 1.96, 5.30, and 12.60 Hz, H-4_{eq}), 4.06--4.12 (m, 1 H, allylic proton of allyl group), 4.12--4.19 (m, 1 H, H-5), 4.27--4.33 (m, 1 H, allylic proton of allyl group), 4.44 (dd, 1 H, J=8.47 and 10.72 Hz, H-2), 4.46 (dd, 1 H, J=4.35 and 11.56 Hz, H-6a), 4.52 (dd, 1 H, J=5.95 and 11.56 Hz, H-6b), 5.02--5.16 (m, 2 H, vinylic protons of allyl group), 5.44 (d, 1 H, J=8.47 Hz, H-1), 5.69--5.79 (m, 1 H, vinylic proton of allyl group), and 5.90 (ddd, 1 H, J=5.30, 10.72, and 10.91 Hz, H-3).

A solution of 14 (160 mg, 0.30 mmol) and butylamine (2 mL) in methanol (10 mL) was refluxed for 10 h, and concentrated. Acetic anhydride (2 mL) was added dropwise to a cooled solution of the residue in methanol (10 mL) at 0--5 °C, and the mixture was stirred for 2 h with cooling. The mixture was concentrated, and the product was crystallized from methanol--ether to give 3 (46.7 mg, 67%); mp 192.5--194 °C, δ_H (D_2O): 1.45 (dt, 1 H, J=11.23 and 12.74 Hz, H-4_{ax}), 2.03 (ddd, 1 H, J=1.75, 5.01, and 12.74 Hz, H-4_{eq}), 2.036 (s, 3 H, NHAc), 3.55 (dd, 1 H, J=8.50 and 10.05 Hz, H-2), 3.61--3.72 (m, 3 H, H-5,6a,6b), 3.78 (ddd, 1 H, J=5.01, 10.05, and 11.23 Hz, H-3), 4.12--4.17 (m, 1 H, allylic proton

of allyl group), 4.31--4.36 (m, 1 H, allylic proton of allyl group), 4.48 (d, 1 H, J=8.50 Hz, H-1), 5.23--5.33 (m, 2 H, vinylic protons of allyl group), and 5.85--5.96 (m, 1 H, vinylic proton of allyl group).

Anal. Calcd for $C_{11}H_{19}NO_5 \cdot H_2O$: C, 53.47; H, 7.83; N, 5.67. Found: C, 53.45; H, 7.82; N, 5.56.

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