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6-*O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-D-galactopyranose 및 유도체의 합성

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The Efficient Synthesis of 6-*O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-D-galactopyranose and Its Derivatives

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요 약. Silver triflate와 *syn*-collidine 존재하에서 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide (**2**)와 1,2;3,4-di-*O*-isopropylidene-α-D-galactopyranose (**3**)를 반응시켜 1,2;3,4-di-*O*-isopropylidene-6-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-α-D-galactopyranose (**4**)를 86 %의 수득률로 얻었다. 화합물 **4**를 hydrazine과 작용시켜 phthalimido기와 acetyl기를 동시에 제거한후, 다시 acetyl화하고 isopropylidene기와 *O*-acetyl기를 가수분해하면 6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-D-galactopyranose (**1**)가 총수득율 65.8%로 얻어졌다. 또한 화합물 **4**를 변형시켜 특정위치에 hydroxyl기를 가진 몇 가지 유도체도 합성하였다.

ABSTRACT. Condensation of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide (**2**) with 1,2 ; 3,4-di-*O*-isopropylidene-α-D-galactopyranose (**3**) in the presence of silver triflate and *syn*-collidine gave 1,2;3,4-di-*O*-isopropylidene-6-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-α-D-galactopyranose (**4**) in 86 % yield. Cleavage of phthalimido group and de-*O*-acetylation with hydrazine, acetylation, and hydrolysis of isopropylidene and *O*-acetyl groups furnished 6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-D-galactopyranose (**1**) with overall yield of 65.8 % starting from **3**. Some other derivatives of **1** which have free hydroxyl groups at the specific position have also been prepared from **4**. These compounds could be used as precursors for further glycosidation reactions.

INTRODUCTION

Synthesis of oligosaccharide containing 2-amino-2-deoxy-β-D-glycopyranoside residue has been of interest for many years. The most frequently used method has been either reaction of alcohol in the presence of silver carbonate or mercuric cyanide with 2-aminoglycosyl halides in which amino function is protected with acetyl, dichloroacetyl, 2,4-dinitrophenyl, benz-

ylsulfonyl or diphenoxyphosphinyl groups[1~6], or a strong-acid promoted reaction of 1, 2-oxazoline derivatives of aminosugar with alcohol[7]. These reactions, however, have always been resulted in a mixture of both α-and β-glycosides, from which the β-anomer has to be afterwards separated in less than 50 % yield by the laborious column chromatography.

The immunochemically important disaccharide[8~10], 6-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-D-galactopyranose (**1**), has been synthesized several times by the methods mentioned above. For instance, Kuhn *et al.*[11] first reported the condensation of 2-acetamido-3, 4, 6-tri-O-acetyl-2-deoxy-D-glucopyranosyl bromide with 1, 2, 3, 4-tetra-O-acetyl-D-galactopyranose in the presence of mercuric cyanide, but the protected β-glycoside was obtained in only 10. 9 % yield. Lloyd *et al.*[12] also synthesized the disaccharide by reacting tri-O-acetyl-2-deoxy-2-(2, 4-dinitroanilino)-α-D-glucopyranosyl bromide with 1, 2;3, 4-di-O-isopropylidene-α-D-galactopyranose (**3**) using silver carbonate as a promoter, from which the desired condensation product was obtained in 29. 4 % yield. In 1970 Khorlin *et al.*[13] improved the yield by condensing 1, 2-oxazoline derivative of 2-amino-2-deoxy-α-D-glucopyranose with **3** in the presence of *p*-toluenesulfonic acid. However, the yield was still only 48 %.

Lemieux *et al.*[14], in 1976, investigated the glycosylating capability of 2-deoxy-2-phthalimido-D-glucopyranosyl halides, and reported that the glycosidation of 3, 4, 6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide (**2**) with unreactive 2, 2, 2-trichloroethanol in the presence of silver triflate furnished only β-glycoside in very high yield.

We now report the simple and efficient synthesis of 6-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-D-galactopyranose (**1**) by applying above-mentioned method, and its derivatives which can be used as precursors for further glycosidation reactions.

RESULTS AND DISCUSSION

Condensation of 3, 4, 6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide (**2**) with 1, 2;3, 4-di-O-isopropylidene-α-D-galactopyranose (**3**) in the presence of silver triflate and *syn*-collidine, and simple crystallization of the crude product from diethyl ether furnished a 86 % yield of the desired 1, 2, ;3, 4-di-O-isopropylidene-6-O-(3, 4, 6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-α-D-galactopyranose (**4**). The ^{1}H-NMR spectrum of the compound **4** indicated the presence of β-glycosidic linkage. The large coupling constant (8 Hz) observed for H-1′ at 5. 44 ppm is consistent with the reported value for the β-glycosides,[14] compared to the relatively small one (4~5 Hz) for the α-anomers.

Deacetylation and cleavage of phthalimido group with hydrazine in refluxing ethanol, followed by selective *N*-acetylation with acetic anhydride-methanol gave an impure 1, 2;3, 4-di-O-isopropylidene-6-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-galactopyranose (**6**), contaminated with phthalhydrazide derivatives which could not be easily removed. This complication could be solved by complete *N*- and *O*-acetylation of the hydrazine-treated impure product with acetic anhydride and pyridine. The insolubility of the acetylated phthalhydrazides in chloroform made it possible to separate in 90 % yield the fully acetylated 1, 2;3, 4-di-O-isopropylidene-6-O-(2-acetamido-3, 4, 6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-α-D-galactopyranose (**5**). Hydrolysis of the O-acetyl groups of the compound **5** with sodium methoxide in methanol yielded the pure **6** in 85 %.

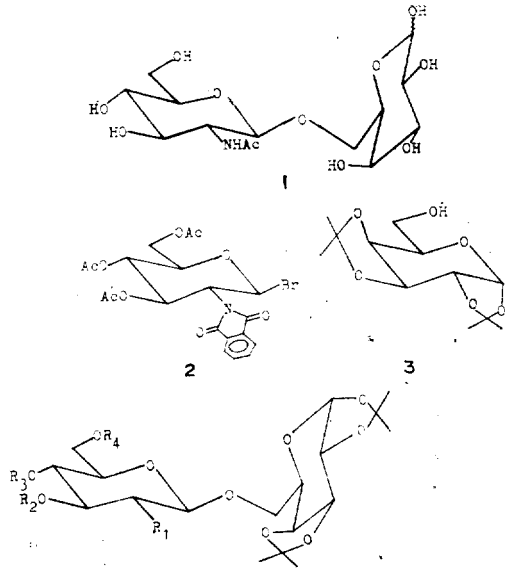
Hydrolysis of the two isopropylidene functi-

ons of **6** with 90 % aqueous trifluoroacetic acid gave syrupy disaccharide **1** contaminated with partially hydrolyzed byproduct; which confirms the rates of hyydrolsis of 1, 2-*O*-and 3, 4-*O*-isopropylidene functions are quite different[15]. Crystallization of the syrupy product from ethanol gave the disaccharide **1** (yield; 73%) as a chromatographically pure compound. The disaccharide **1** was also prepared in 85 % yield by direct treatment of **5** with acetone-water-conc. hydrochloric acid (10:5:1).

As a result, the disaccharide **1** was obtained in the overall yield of 65.8 % starting from **3** *via* the above-described three steps, **3**→**4**→**5**→**1**.

Suitable protection of the free hydroxyl groups of the compound **6** was also carried out. Protection of the 4′- and 6′-hydroxyl functions of **6** with benzylidene group by the reaction of **6** with benzaldehyde dimethyl acetal gave a 82 % yield of 1, 2;3, 4-di-*O*-isopropylidene-6-*O*-(2-acetamido-4, 6-*O*-benzylidene-2-deoxy-*β*-**D**-glucopyranosyl)-*α*-**D**-galactopyranose (**7**). Acetylation of **7** and removal of benzylidene function of the resulting 1, 2;3, 4-di-*O*-isopropylidene-6-*O*-(2-acetamido-3-*O*-acetyl-4, 6-*O*-benzylidene-2-deoxy-*β*-**D**-glucopyranosyl)-*α*-**D**-galactopyranose (**8**) by hydrogenolysis furnished 1, 2; 3, 4-di-*O*-isopropylidene-6-*O*-(2-acetamido-3-*O*-acetyl-2-deoxy-*α*-**D**-glucopyranosyl)-*α*-**D**-galactopyranose (**9**).

It is well known[6] that the hydroxyl function at 4-position of D-galactopyranosides or **D**-glucopyranosides is quite unreactive compared with 3-hydroxyl or primary 6-hydroxyl groups. Consequently, compounds **7** and **9**, in which each has free hydroxyl group at 3- and 6-position, could be used as precursors for further glycosidation reactions.



4 R_1=phthalimido, R_2=R_3=R_4=Ac
5 R_1=NHAc, R_2=R_3=R_4=Ac
6 R_1=NHAc, R_2=R_3=R_4=H
7 R_1=NHAc, R_2=H, R_3=R_4=benzylidene
8 R_1=NHAc, R_2=Ac, R_3=R_4=benzylidene
9 R_1=NHAc, R_2=Ac, R_3=R_4=H

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EXPERIMENTAL

All the chemicals used were of reagent grade and purified prior to use, if necessary, by the methods reported in the literature. Melting points are uncorrected. The ^{1}H-NMR spectra were recorded on a Varian HA-100 or T-60A spectrometer. Unless otherwise stated, deuteriochloroform was used as a solvent and internal TMS as a standard. For deuterium oxide, 50 % TMS in carbon tetrachloride was used as a standard. Doublets, triplets, and quartets in the spectra were recorded as the center of the peaks and the multiplets as their range of absor-

ption. All NMR spectra are described in ppm.

1,2;3,4-Di-*O*-isopropylidene-6-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-α-D-galactopyranose (4). A solution of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide (2) (5.98 g, 12 mmole) in dry nitromethane (10 m*l*) was added to the cooled (−30°) solution of 1,2;3,4-di-*O*-isopropylidene-α-D-galactopyranose (3) (2.70 g, 10 mmole), silver trifluoromethanesulfonate (3.09 g, 12 mmole) and *syn*-collidine (1.46 g, 12 mmole) in dry nitromethane (30 m*l*) under nitrogen and the resulting mixture was stirred at the same temperature for 2 hr. Dilution of the mixture with chloroform (100 m*l*), filtration and evaporation gave a foam which was dissolved in chloroform (100 m*l*) and washed with cold water, dilute hydrochloric acid and aqueous sodium bicarbonate. Solvent removal after drying over sodium sulfate left a foam which was passed through a short alumina (Activity I, neutral) column using ethyl acetate as an eluant. Evaporation of the solvent gave a foam which was crystallized from diethyl ether (5.75 g, 86 % yield based on 3). Recrystallization from ethanol gave a colorless solid (5.42 g, 80 % yield), m.p 214~215 °C, $[\alpha]_D^{28}$ −24.4° (*c*, 0.5, $CHCl_3$).

^{1}H-NMR: δ 7.88~7.64 (*m*, 4, phthalimido), 5.83 (*q*, *J*=11 Hz and 9 Hz, 1, H-3'), 5.44 (*d*, *J*=8 Hz, 1, H-1'), 5.14 (*t*, *J*=9 Hz, 1, H-4'), 5.10 (*d*, *J*=5 Hz, 1, H-1), 4.47~3.56 (*m*, 10) 2.10, 2.05, 1.84 (each *s*, 9, OAc), 1.38, 1.22 (each *s*, 6, isopropylidene), 1.04 (*s*, 6, isopropylidene).

Anal. Calcd. for $C_{32}H_{39}NO_{15}$: C, 56.7; H, 5.80; N, 2.07. Found: C, 56.6; H, 5.73; N, 2.05.

1,2;3,4-Di-*O*-isopropylidene-6-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-α-D-galactopyranose (5). A solution of the disaccharide **4** (9.56 g, 14 mmole) and 85 % hydrazine (6.0 g) in ethanol (100 m*l*) was refluxed for 2 hr. Thorough solvent removal left a solid which was suspended in cold ethanol and filtered. The impure solid material (10.2 g) was stirred overnight with acetic anhydride (20 m*l*) and pyridine (20 m*l*) at room temperature, poured into ice-water and extracted with chloroform (100 m*l*). The chloroform solution was washed with water, dilute hydrochloric acid and aqueous sodium bicarbonate. Drying over sodium sulfate and solvent removal gave a foamy solid which was crystallized from diethyl ether. Recrystallization from ethanol gave a colorless solid (7.51 g, 90% yield), m.p 107~109 °C, $[\alpha]_D^{28}$ −66.6° (*c*, 0.5, $CHCl_3$).

^{1}H-NMR: δ 5.87 (*d*, *J*=9 Hz, 1, NH), 5.50 (*d*, *J*=5 Hz, 1, H-1), 5.20 (*t*, *J*=9 Hz, 1, H-3') 5.04 (*t*, *J*=9 Hz, 1, H-4'), 4.70 (*d*, *J*=8 Hz, 1, H-1'), 4.56 (*q*, *J*=2 Hz and 8 Hz, 1, H-3), 4.28 (*q*, *J*=2 Hz and 5 Hz, 1, H-4), 4.23~3.58 (*m*, 8), 2.06 (*s*, 3, NAc), 2.00 (*s*, 6, two OAc), 1.94 (*s*, 3, OAc), 1.50, 1.42 (each *s*, 6, isopropylidene), 1.40 (*s*, 6, isopropylidene).

Anal. Calcd. for $C_{26}H_{39}NO_{14}$: C, 52.9; H, 6.67; N, 2.28. Found: C, 52.6; H, 6.65; N, 2.32.

1,2;3,4-Di-*O*-isopropylidene-6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-galactopyranose (6). The acetylated compound **5** (5.90 g, 10 mmole) was stirred with sodium methoxide in methanol at room temperature for 6 hr and passed through a short column of acidic resin [Amberlite, IR 120 (H)]. Evaporation of methanol left a solid which was recrystallized from 2-propanol (4.72 g, 85 % yield), m.p 197~198 °C, $[\alpha]_D^{28}$ −61.2° (*c*, 0.5, CH_3OH).

^{1}H-NMR (D_2O): δ 5.86 (*d*, *J*=5 Hz, 1, H-1), 4.74 (*d*, *J*=8 Hz, 1, H-1') 2.30 (*s*, 3,

NAc), 5.06~3.58 (*m*, 12), 1.82, 1.72 (each *s*, 6, isopropylidene), 1.65 (*s*, 6, isopropylidene).

Anal. Calcd. for $C_{20}H_{33}NO_{11}$: C, 51.8; H, 7.18; N, 3.02. Found: C, 51.4; H, 7.16; N, 2.94.

1,2:3,4-Di-*O*-isopropylidene-6-*O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-α-D-galactopyranose (7). A solution of the hydroxy compound **6** (3.50 g, 7.55 mmole), benzaldehyde dimethyl acetal (2.29 g, 15 mmole) and *p*-toluenesulfonic acid (100 mg) in a 1 : 4 mixture of dimethylformamide and acetonitrile (100 m*l*) was stirred at room temperature for 16 hr. Neutralization with triethylamine (2 m*l*) and evaporation left a solid which was dissolved in chloroform (100 m*l*). Washing with water, drying over sodiun sulfate and evaporation gave a solid which was recrystallized from ethanol (3.42 g, 82 % yield), m.p 235~237 °C (dec.), $[\alpha]_D^{29}$ −133.6° (*c*, 1.0, $CHCl_3$).

^{1}H-NMR: δ 7.58~7.24 (*m*, 5, phenyl), 6.38 (*d*, *J*=5 Hz, 1, NH), 5.74 (broad *s*, 1, OH, D_2O exchangeable), 5.56 (*d*, *J*=5 Hz, 1, H-1), 5.52 (*s*, 1, CH of benzylidene), 4.57 (*d*, *J*=8 Hz, 1, H-1′), 4.56 (*q*, *J*=2 Hz and 8 Hz, 1, H-3), 4.64~3.30 (*m*, 11), 2.06 (*s*, 3, NAc), 1.62, 1.45 (each *s*, 6, isopropylidene), 1.30 (*s*, 6, isopropylidene).

Anal. Calcd. for $C_{27}H_{37}NO_{11}$: C, 58.8; H, 6.76; N, 2.54. Found: C, 58.5; H, 6.75; N, 2.27.

1,2;3,4-Di-*O*-isopropylidene-6-*O*-(2-acetamido-3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-α-D-galactopyranose (8). The 3′-hydroxy compound **7** (2.76 g, 5 mmole) was acetylated with acetic anhydride and pyridine. Usual work-up left a solid which was recrystallized from ethanol (2.82 g, 95 % yield), m.p 199~201°C, $[\alpha]_D^{29}$ −113.6° (*c*, 1.0, $CHCl_3$).

^{1}H-NMR: δ 7.50~7.26 (*m*, 5, phenyl), 5.83 (*d*, *J*=9 Hz, 1, NH), 5.54 (*d*, *J*=5 Hz, 1, H-1), 5.50 (*s*, 1, CH of benzylidene), 5.20 (*t*, *J*=9 Hz, H-3′), 4.65 (*d*, *J*=8 Hz, 1, H-1′), 4.58 (*q*, *J*=2 Hz and 8 Hz, 1, H-3), 4.40~3.36 (*m*, 10), 2.08 (*s*, 3, NAc), 1.98 (*s*, 3, OAc), 1.52, 1.46 (each *s*, 6, isopropylidene), 1.32 (*s*, 6, isopropylidene).

Anal. Calcd. for $C_{29}H_{39}NO_{12}$: C, 58.7; H, 6.62; N, 2.36. Found: C, 58.8; H, 6.82; N, 2.40.

1,2:3,4-Di-*O*-isopropylidene-6-*O*-(2-acetamido-3-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-α-D-galactopyranose (9). A solution of the benzylidene compound **8** (1.20 g, 2 mmole) in a 1 : 1 mixture of ethyl acetate and ethanol (20 m*l*) was hydrogenated over 5 % Pd-C at room temperature for 18 hr under the hydrogen pressure of 3 atm. Filtration and evaporation left a solid which was recrystallized from ethanol (0.91 g, 90 % yield), m.p 114~117 °C, $[\alpha]_D^{29}$ −85.1° (*c*, 1.0, $CHCl_3$).

^{1}H-NMR: δ 6.46 (*d*, *J*=9 Hz, 1, NH), 5.58 (*d*, *J*=5 Hz, 1, H-1), 5.08 (*t*, *J*=9 Hz, 1, H-3′), 4.66 (*d*, *J*=8 Hz, 1, H-1′), 4.68 (*q*, *J*=2 Hz and 8 Hz, 1, H-3), 4.40~3.40 (*m*, 12), 2.15 (*s*, 3, NAc), 2.00 (*s*, 3, OAc), 1.56, 1.48 (each *s*, 6, isopropylidene), 1.36 (*s*, 6, isopropylidene).

Anal. Calcd. for $C_{22}H_{35}NO_{12}\cdot H_2O$: C, 50.5; H, 7.07; N, 2.67. Found: C, 50.6; H, 6.65; N, 2.73.

6-*O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-D-galactopyranose (1). A solution of the di-*O*-isopropylidene compound **6** (465 mg, 1mmole) in 90 % aqueous trifluoroacetic acid (10 m*l*) was stirrred for 30 min and solvent evaporated in high *vacuo*. The resulting solid was found to be contaminated with some partially hydrolized by-product. Crystallization from ethanol gave 280 mg (73%) of the

colorless solid (Method A). (B) A solution of the compound **5** (570 mg, 1mmole) in acetone (10 m*l*), water (5 m*l*) and conc. hydrochloric acid (1 m*l*) was stirred at 70°C for 1 hr. Evaporation of the solvent gave a solid which was suspended in ethanol and filtered. Drying over P_2O_5 gave 325 mg (85 %) of the chromatographically pure solid, m. p: softening at 49~60°C, $[\alpha]_D^{24}$ +9.3° (*c*, 1.0, H_2O) [*lit*.[12] $[\alpha]_D^{20}$ +9.9° (H_2O), m. p: softening at 50~60 °C].

^{1}H-NMR (D_2O): δ 4.90~3.50 (*m*), 2.00, 2.12 (each *s*, 3, NAc, ratio=1 : 4).

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