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NOTES

Convenient Synthetic Method for 1,2,3,6-Tetra-*O*-Acetyl-β-D-Glucopyranose: A Starting Material for β(1→4)-D-Glucan

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As a tool for the chemical synthesis of a saccharide chain, preparation of the partially protected monosaccharide is important. For example, the chemical synthesis of cellulose would require activation of the anomeric position and protection of hydroxyl groups other than 4-OH. To introduce protective groups into the hydroxyl groups other than 4-OH, the method of changing the benzylidene group of methyl α-D-glucopyranoside into a benzyl group using a reducing agent is reported.[1] By using this method, either 4-*O*-benzyl derivative or 6-*O*-benzyl derivative can be obtained under the appropriate reaction condition.[1] However, it is often difficult to remove benzyl groups after the saccharide chain was constructed. This research was carried out to synthesize conveniently 1,2,3,6-tetra-*O*-acetyl-α-D-glucopyranose which can be a simple starting material for the synthesis of (1→4)-D-glucan. Moreover, the condensation reaction of 1,2,3,6-tetra-*O*-acetyl-β-D-glucopyranose by Lewis acid as catalyst was investigated to give (1→4)-D-glucan.

Cellulose and amylose which are natural (1→4)-D-glucan are synthesized by glycosyltransferase by using UDP-glucose or GDP-glucose as starting materials in the living body.[2,3] Glycogen which is a (1→4)-D-glucan in the animal cell is also synthesized by the saccharide transfer reaction by using UDP-glucose. Cellulose and amylose are hydrolyzed by cellulase and amylase, respectively, to give glucose and its oligomer (cellobiose, maltose). On the other hand, glycogen which has the same skeleton as amylose is phosphorolyzed by phospholylase to give a glucose-1-phosphate. It is possible to make artificially these cleavage reactions for reverse (for a balance to be turned to the starting materials side), and to prepare polysaccharides. Cellobiosyl fluoride is polymerized by cellulase to afford a high-molecular-weight cellulose,[4] and glucose-1-phosphate is polymerized by phosphorylase to provide amylose.[5] It is also possible to synthesize (1→4)-D-glucan chemically. Nakatsubo *et al.* synthesized cellulose by cationic ring-opening polymerization of 3,6-di-*O*-benzyl-α-D-glucose 1,2,4-orthopivalate.[6] They concluded that the selection of protective groups for hydroxyl groups is very important for the synthesis of stereoregular polysaccharide. Moreover, (1→4)-β-D-xylan which is hemicellulose was synthesized by ring-opening polymerization of intramolecular orthoester derivative of xylose.[7] In this investigation, polycondensation reaction of partially acetylated glucose was carried out by Lewis acid-catalyzed glycosylation in order to construct the (1→4)-linkage of glucose.

EXPERIMENTAL

^{1}H and ^{13}C NMR spectra were obtained with JEOL JNM-ECP 600 spectrometers in chloroform-*d* with tetramethylsilane as internal standard. Gel permeation chromatography (GPC) of the polymerization products was carried out on a Shimadzu LC 10 AD liquid chromatograph (TOSOH Multipore HXL-Mx3 columns or G 2000 HXL + G 1000 HXL columns) using chloroform as solvent and polystyrene standards. Merck silica gel was used for column chromatography. The MALDI-TOF mass spectrum was recorded on a Bruker MALDI-TOF mass spectrometer with a 2,5-dihydroxybenzoic acid (DHB) matrix.

*1,2,3,4-Tetra-O-acetyl-6-trityl-β-D-glucopyranose (**1**)*

D-Glucose (20.2 g, 112 mmol) and triphenylmethyl chloride (trityl chloride) (34.6 g, 122 mmol) in

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200 mL pyridine was stirred overnight at room temperature under a stream of nitrogen. To this solution was gradually added 100 mL acetic anhydride and the solution stirred for another 6.5 h at room temperature. The reaction was monitored by thin layer chromatography with hexane:ethyl acetate (1:1, v/v) as eluent. Pyridine was evaporated by azeotropic removal with toluene and the residue was dissolved in chloroform, neutralized with sodium hydrogen carbonate, washed with water and aqueous sodium chloride, dried over anhydrous sodium sulfate, evaporated *in vacuo* and the resulting residue was subjected to column chromatography (hexane:ethyl acetate, 2:1, v/v). The ^{1}H NMR spectrum of the obtained product showed that the mixture contained 11.6% of α-form and 88.4% of β-form. Recrystallization with ethanol gave the desired β-glucose derivative in 40.0% yield. ^{1}H NMR ($CDCl_3$, 600 MHz): 1.97–2.12 (12H, CH_3), 7.26–7.44 (15H, Ph_3), 3.07 (1H, dd, H6′), 3.35 (1H, dd, H6), 3.72 (1H, d, H5), 5.30 (1H, t, H4), 5.15–5.25 (2H, H2 and H3), 5.75 (1H, d, H1); ^{13}C NMR ($CDCl_3$, 150 MHz): 20.9 (CH_3), 170.3, 169.4 and 169.1 (C=O), 127.1, 127.9, 128.8 and 143.6 (Ph_3), 86.7 (C–Ph_3), 61.6 (C6), 74.1 (C5), 68.3 (C4), 73.2 (C3), 70.5 (C2), 92.0 (C1).

*1,2,3,6-Tetra-O-acetyl-β-D-glucopyranose (**2**)*

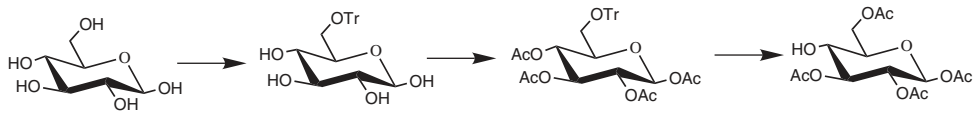
To 5.0 g (8.5 mmol) of 1,2,3,4-tetra-*O*-acetyl-6-*O*-trityl-β-D-glucopyranose in 40 mL of acetic acid was added 10.8 mL (141 mmol) trifluoroacetic acid and stirred at 65 °C under a stream of nitrogen for 1 h. 200 mL of chloroform was added to the reaction mixture, and the organic layer was washed with water and neutralized with sodium hydrogen carbonate, washed with water and saturated aqueous sodium chloride, dried over anhydrous sodium sulfate and evaporated *in vacuo*. The residue was subjected to flash column chromatography (hexane:ethyl acetate, 1:1, v/v) to obtain the 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose and 1,2,3,6-tetra-*O*-acetyl-β-D-glucopyranose. The resulting syrup was dissolved in 12 mL of pyridine and trityl chloride (2.3 g, 8.3 mmol) was added to the solution. The reaction mixture was stirred at 70 °C over night. Pyridine was evaporated by azeotropic removal with toluene and the residue was dissolved in chloroform, neutralized with sodium hydrogen carbonate, washed with water and aqueous sodium chloride, dried over anhydrous sodium sulfate, evaporated *in vacuo* and the resulting residue was subjected to column chromatography (hexane:ethyl acetate, 2:1, v/v). The yield of crude syrupy product was 1.37 g (45.9%). 1,2,3,6-Tetra-*O*-acetyl-β-D-glucopyranose was further purified by crystallization from its ethanol solution by the addition of *n*-hexane. Yield 0.70 g (24.3%). ^{1}H NMR ($CDCl_3$, 600 MHz): 2.03–2.11 (12H, CH_3), 3.59 (1H, m, H4), 3.64 (1H, m, H5), 4.28 (1H, dd, H6), 4.54 (1H, dd, H6′), 5.07 (1H, t, H2), 5.11 (1H, t, H-3), 5.70 (1H, d, H1); ^{13}C NMR ($CDCl_3$, 150 MHz): 20.9 (CH_3), 62.6 (C6), 68.5 (C4), 70.4 (C2), 75.0, 75.1 (C3, C5), 91.8 (C1), 169.1, 169.6, 171.2, 171.8 (C=O).

Polymerization

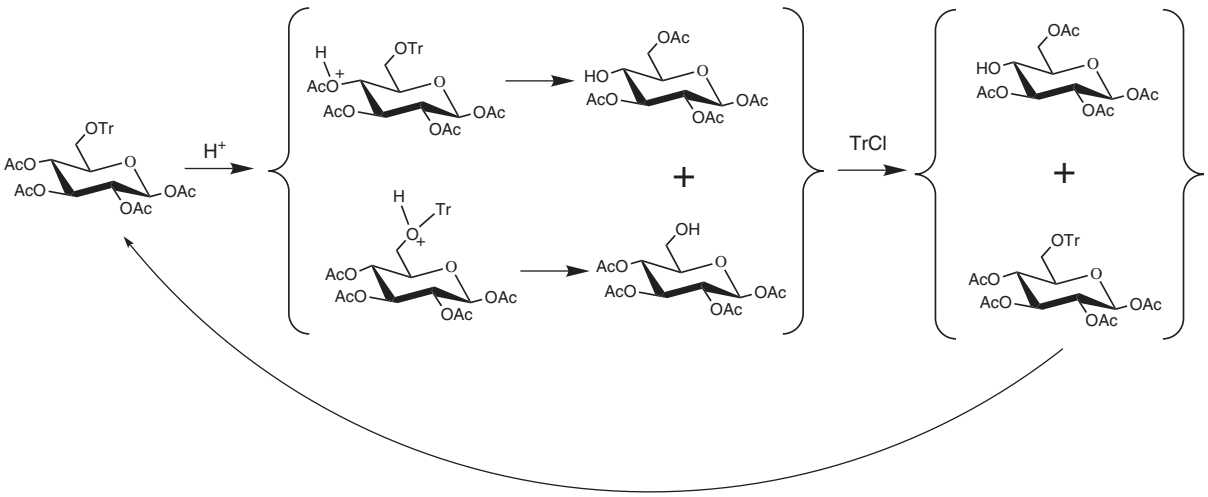
Polymerization of the monomer (0.35 g) was carried out under high vacuum (10^{-5} mmHg) with anhydrous 1,2-dichloroethane as solvent and in the presence of Lewis acid (boron trifluoride–diethyl ether complex) as catalyst. Polymerization was carried out at various temperatures for 24 h and the reaction was stopped by the addition of methanol. The solution was neutralized with sodium hydrogen carbonate, washed with water, dried on anhydrous sodium sulfate, evaporated *in vacuo* and freeze dried from benzene.

RESULTS AND DISCUSSION

Synthetic route of 1,2,3,6-tetra-*O*-acetyl-β-D-glucopyranose is shown in Scheme 1. 1,2,3,4-Tetra-*O*-acetyl-6-*O*-trityl-D-glucopyranose (α:β = 1:8) was synthesized by the reaction of D-glucose with 1.1 equivalent of trityl chloride followed by acetylation of the remaining hydroxyl groups. Since the obtained 1,2,3,4-tetra-*O*-acetyl-6-*O*-trityl-D-glucopyranose was a mixture of α-form and β-form, the β-form was isolated by crystallization from ethanol solution. Removal of trityl group from 1,2,3,4-tetra-*O*-acetyl-6-*O*-trityl-β-D-glucopyranose was carried out by trifluoroacetic acid in acetic acid solution. Detritylation under acidic condition gave the mixture of 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose which was prepared by the simple cleavage of the trityl ether bond and 1,2,3,6-tetra-*O*-acetyl-β-D-glucopyranose which was produced by acyl migration from C-4 position to C-6 position during detritylation reaction. The detritylation reaction was affected by reaction temperature and reaction time. When the reaction was carried out at 80 °C, the TLC data showed that a lot of by-products,



Scheme 1.



Scheme 2.

which were presumably over-acetylated derivative and decomposed compounds, were formed. The 90 min reaction at 65 °C resulted in the best production of 1,2,3,6-tetra-*O*-acetyl-*β*-D-glucopyranose even if the unreacted trityl derivative remained. Higher reaction temperature than 65 °C or longer reaction time than 90 min caused the increase of by-product. Since the separation of 1,2,3,4-tetra-*O*-acetyl-*β*-D-glucopyranose and 1,2,3,6-tetra-*O*-acetyl-*β*-D-glucopyranose by column chromatography was very difficult, the mixture which included two kinds of isomers and unreacted trityl derivative was tritylated again. The obtained product contained 1,2,3,6-tetra-*O*-acetyl-*β*-D-glucopyranose and 1,2,3,4-tetra-*O*-acetyl-6-*O*-trityl-*β*-D-glucopyranose, and they were easily separated by silica gel column chromatography. Moreover, the by-product of 1,2,3,4-tetra-*O*-acetyl-6-*O*-trityl-*β*-D-glucopyranose was reusable as a starting material of detritylation (Scheme 2). Therefore, the substantial yield was estimated to be more than 70% though the yield of pure 1,2,3,6-tetra-*O*-acetyl-*β*-D-glucopyranose crystal in single detritylation reaction was about 24%. 1,2,3,6-Tetra-*O*-acetyl-*β*-D-glucopyranose was crystallized from its ethanol solution by the addition of *n*-hexane.

Condensation reaction of 1,2,3,6-tetra-*O*-acetyl-*β*-D-glucopyranose was carried out with boron trifluoride etherate as catalyst in 1,2-dichloroethane under high vacuum. The results are shown in Table I. When the reaction was examined at 60 °C, the reaction mixture was colored after 2 h, and the insoluble precipitate appeared after 24 h. It seems that it decomposed. On the other hand, when it reacted at 0 °C, low molecular weight compounds such as dimer and trimer were obtained. The reaction at 40 °C provided the highest molecular weight of cellooligosaccharide of which the number average degree of polymerization was 5.7. The ^{13}C NMR spectrum of the oligosaccharide showed both α- and β-anomeric carbon (α:β = 1:1), indicating that the neighboring group participation by the acetyl group of the C-2 position was imperfect. The MALDI-TOF MS data of the oligosaccharide showed that the highest molecular weight of the polymer was 4447, indicating that the resulting product contained (1→4)-D-glucan with DP of 15.

Table I. Polymerization of 1,2,3,6-tetra-*O*-acetyl-*β*-D-glucopyranose[a]

No.	Temp. (°C)	Yield (mg)	Mn[b]	DP
1	60	65	n.d.	n.d.
2	50	114	1400	4.8
3[c]	50	114	1250	4.3
4	40	113	1630	5.7
5[c]	0	206	889	3.1

[a]Monomer: 350 mg (1.0 mmol); catalyst: $BF_3 \cdot OEt_2$, 2.0 equivalent to themonomer; solvent: 1,2-dichloroethane, 0.5 mL; time: 24 h. [b]Determined by GPC. [c]1.5 equivalent to the monomer.

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