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NOTES

Facile Synthesis of β(1→6)-Linked Gluco-Oligosaccharide Derivative

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Synthetic polysaccharides are indispensable models for the physicochemical and biological studies that are directed toward elucidation of the role played by carbohydrates in biological processes and to understanding the structure-function relationship. The diversity of structures of natural polysaccharides gave rise to the rapid development of methods for the synthesis of their analogues. However, strategies aimed at the preparation of polysaccharides with a strictly regular structure have been of primary concern. A slight variation in the structural and stereochemical regularity of the monomeric linkage within the polymeric chain changes sharply the macromolecular conformation, altering essentially its physicochemical properties, and, therefore, its biological specificity.[1]

The synthesis of polysaccharides with a regular structure can be accomplished by (1) step-by-step attachment of monosaccharide or oligosaccharide units;[2] (2) enzymatic polymerization of the monosaccharide or oligosaccharide precursors;[3] (3) ring-opening polymerization of orthoester and anhydro sugar derivatives;[4] and, (4) polycondensation of corresponding monosaccharide or oligosaccharide derivative by chemical methods.[5]

Recently, a branched β(1→6)-glucan fragment has been isolated from the mycelial walls of the fungal pathogen Pmg and was found to be one of the most potent biotic elicitors.[6] Although the general nature of the β-glucan elicitor has already been determined, the exactness of its structure and the mechanism underlying the elicitation of phytoalexins has not yet been elucidated. To better understand the structure-function relationship involving the β-glucan elicitor, the chemical synthesis of structurally related β-glucans is necessary as an alternative to isolation from natural sources.

Gagnaire and his associates have achieved the synthesis of (1→6)-β-D-glucans by self-condensation of 2,3,4-tri-*O*-acetyl-6-*O*-(2′,3′,4′-tri-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranosyl bromide using $Hg(CN)_2$ and $HgBr_2$ in acetonitrile at 0°C.[7] In our previous work, we have reported the synthesis of an alternate heteropolysaccharide with controlled sequence by polycondensation of an acetylated monomer (1,2,3,6-tetra-*O*-acetyl-4-*O*-(2′,4′,6′-tri-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranose) using stannic tetrachloride as catalyst.[8,9]

This paper reports the facile synthesis of (1→6)-β-D-glucan by polycondensation of 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose under high vacuum using Lewis acid as catalyst.

EXPERIMENTAL

^1H and ^{13}C nuclear magnetic resonance spectra were obtained with JEOL JNM LA-400 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 H_{XL}-Mx3 columns or G 2000 H_{XL} + G 1000 H_{XL} columns) using chloroform as solvent and polystyrene standards. Merck silica gel was used for column chromatography.

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

D-Glucose (32.6 g, 181.1 mmol) and triphenylmethylchloride (41.4 g, 148.4 mmol) in 200 mL pyridine was stirred overnight at room temperature under a stream of nitrogen. To this solution was gradually added 120 mL acetic anhydride and the solution stirred for another 6.5 h at room temperature. The reaction was monitored by thin layer chromatography with

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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 bicarbonate, washed with water and aqueous sodium chloride, dried with anhydrous sodium sulfate, evaporated *in vacuo* and the resulting residue was subjected to column chromatography (hexane:ethyl acetate, 2 : 1, v/v). Recrystallization with ethanol gave the desired β-glucose derivative in 4.7% yield. ^{1}H NMR ($CDCl_3$, 400 MHz): 1.97–2.12 (CH_3,12H), 7.26–7.44 (15H, Ph_3) 3.07 (H6′, dd, 1H), 3.35 (H6, dd, 1H), 3.72 (H5, d, 1H), 5.30 (H4, t, 1H), 5.15–5.25 (H2 and H3, 2H), 5.75 (H1, d, 1H); ^{13}C NMR ($CDCl_3$, 100 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).

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

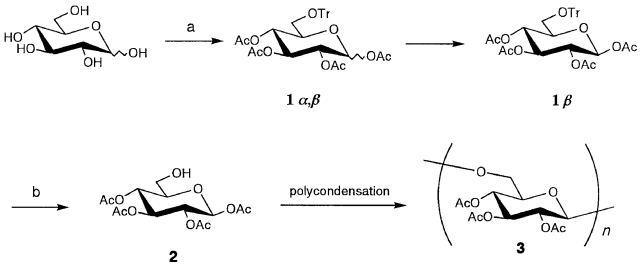
To 6.0 g (9.704 mmol) of 1,2,3,4-tetra-*O*-acetyl-6-*O*-trityl-β-D-glucopyranose was added 90 mL saturated hydrochloric acid in chloroform and stirred under a stream of nitrogen for 30 min. The solution was neutralized with sodium bicarbonate, washed with saturated sodium chloride and water, dried with anhydrous sodium sulfate and evaporated *in vacuo*. The residue was subjected to column chromatography (hexane:ethyl acetate, 1 : 1, v/v) to obtain the desired monomer. Yield 2.2 g (63.0%). ^{1}H NMR ($CDCl_3$, 400 MHz): 1.90–2.25 (CH_3, 12H), 3.48 (H6′, dd, 1H), 3.64 (H6, dd, 1H), 3.72 (H5, d, 1H), 5.30 (H3, t, 1H), 5.02–5.15 (H2 and H4, 2H), 5.75 (H1, d, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): 20.9 (CH_3), 169.4, 170.3 (C=O), 60.2 (C6), 70.6 (C5), 65.8 (C4), 75.1 (C3), 70.9 (C2), 92.0 (C1).

Polymerization

Polymerization of the monomer (0.2 g, 1.11 mmol) was carried out under high vacuum with anhydrous dichloroethane as solvent and in the presence of Lewis acid (stannic tetrachloride or boron trifluoride-diethyl ether complex) as catalyst. Polymerization was carried out at various temperatures for 6 or 24 h and the reaction was stopped by the addition of methanol. The solution was neutralized with sodium bicarbonate, washed with water, dried on anhydrous sodium sulfate, evaporated *in vacuo* and freeze dried from benzene.

RESULTS AND DISCUSSION

The monosaccharide derivative 1,2,3,4-tetra-*O*-acetyl-6-*O*-trityl-β-D-glucopyranose was prepared by one-pot synthesis in pyridine using glucose as starting material. As shown in Scheme 1, regioselective protection of the primary hydroxyl group was accomplished by the reaction of glucose with trityl chloride.



Scheme 1. Reagents and conditions for synthesis of monomer **2**: **i**. a) TrCl, pyridine, r.t., overnight; **ii**. Ac_2O, r.t., 6.5 h, 4.7%; b) HCl, $CHCl_3$, 30 min, 63.0%.

Subsequent acetylation of the remaining hydroxyl groups with acetic anhydride gave an α,β mixture of the monosaccharide derivative. Separation by column chromatography followed by recrystallization from ethanol gave only the desired β-glucose derivative. Detritylation by treatment with a strong acid (HCl) gave the desired monomer (1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose) with a free hydroxyl group at the primary position. This monomer behaves both as a glycosyl donor and acceptor that can be polymerized using a Lewis acid.

Polymerization under high vacuum was carried out at slightly elevated temperatures using Lewis acid ($SnCl_4$ or $BF_3{\cdot}OEt_2$) as catalyst. As shown in Figure 1, the ^{1}H NMR spectral result of the polycondensation product suggests that polymerization proceeded to afford the 1→6 linked glucan as evidenced by the absence of resonance at 5.75 ppm corresponding to the β-acetyl reducing end. The reaction conditions and results of polycondensation are shown in Table I.

Polymerization of run 4 carried out using $BF_3{\cdot}OEt_2$ at 50°C for 24 h gave the highest degree of polymerization of 4.5 in relatively good yield. The rest of the runs gave a degree of polymerization of 3.0–3.5. Neighboring group participation by the acetoxy group at C-2 position ensured the stereoselective formation of the β-linked polysaccharide. Considering the general bimolecular condensation reaction (A + B → AB), a 20% side reaction of A with no side reaction of B gives the product in 80% yield, when the molar ratio in the feed is 1 : 1. When the molar ratio in the feed is 1.25 : 1, B can completely react to give the product in 100% yield (yield based on A is 80%). Therefore, in the polycondensation of bifunctional monomers such as B-A, the number average degree of polymerization must be 5.0, because of the side reaction of 20% of A. This speculation excludes ring formation.

In the case of partially protected glucose monomer 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose, the hydroxy

Table I. Polymerization of 1,2,3,4-tetra-*O*-acetyl-*β*-D-glucopyranose

No.	Monomer[a]		Lewis acid[b]	Solvent[c]	Temp.	Time	Conversion	M_n[d,e]	DP
	(mg)	(mmol)		mL	°C	h	%		
1	200	0.556	$SnCl_4$	0.5	40	24	56	910	3.2
2	200	0.556	$SnCl_4$	0.5	60	6	71	870	3.0
3	200	0.556	$SnCl_4$	0.5	60	24	60	900	3.1
4	200	0.556	$BF_3{\cdot}OEt_2$	0.5	50	24	88	1300	4.5
5	200	0.556	$BF_3{\cdot}OEt_2$	0.5	60	6	82	1000	3.5
6	200	0.556	$BF_3{\cdot}OEt_2$	0.5	60	24	81	1020	3.5

[a]1,2,3,4-tetra-*O*-acetyl-*β*-D-glucopyranose. [b]2 equiv to monomer. [c]$ClCH_2CH_2Cl$. [d]Determined by GPC. [e]Containing oligomers.

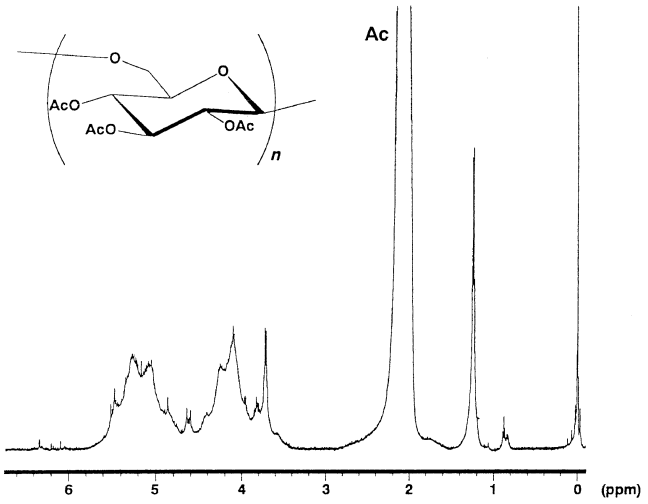


Figure 1. ^1H NMR spectrum of polymerization product in $CDCl_3$.

group at C-6 is quite stable so side reaction may not occur. On the other hand, the five-membered cyclic acyloxonium ion is obtained by the abstraction of acetate ion on C-1 by Lewis acid followed by the nucleophilic attack on the resulting oxocarbenium ion with the neighboring acetyl group, may react with a slight amount of water which is present in solvent. Considering that polymerization was carried out under high vacuum using anhydrous solvents, the presence of water molecules could not rationalize the low degree of polymerization. In glycosylation using per-*O*-acetylated sugar donor, the donor often remains intact and can be recovered because of the poor reactivity of 1-*O*-acyl group as a leaving group. However, the remaining monomers were rarely found as shown in ^1H NMR spectrum (Figure 1). In order to explain the outcome of polycondensation, the following alternative aspects can be considered. Side reactions such as the formation of an orthoester, acyl migration from O-3/O-4 to O-6, and even partial cleavage of the glycoside linkage may likely occur when strong Lewis acids like $SnCl_4$ are employed at elevated temperature.

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