NOTES

Synthesis and Ring-Opening Reaction of 1,6-Anhydro-6-deoxy-6-thio-2, 3, 4-tri-*O*-benzyl- β -D-glucopyranose

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Numerous glycoconjugates are biologically important. Recently, many neo-glycoconjugates have been investigated to clarify the functions of glycoconjugates. The neo-glycoconjugate is a synthetic carbohydrate compound conjugated to a variety of materials. Pseudo sugar often used for the synthesis of neo-glycoconjugate is a term given to carbohydrate of which one oxygen atom is replaced by another atom. For example, one of the oxygen atoms is replaced by sulfur atom in "thio-sugar". S-glycoside is more difficult to be hydrolyzed in acidic solution than O-glycoside. In enzyme-catalyzed hydrolysis, S-glycoside is stable (not hydrolyzed) and may work as an competitive inhibitor. Since sulfur and oxygen have the same valence, several enzymes may recognize both of them. The difference in affinity for proton and/or the difference in stability of protonated ion may cause different reactivity in hydrolysis. The stable enzyme-inhibitor complex may give information on the active conformation of enzyme.

Monad *et al.* reported¹ that phenyl 1-thio- β -D-galactopyranoside has the same affinity with β -galactosidase from *E. coli* (EC 3.2.1.23) as phenyl β -D-galactopyranoside and phenyl 1-thio- β -D-galactopyranoside is a competitive inhibitor of the β -galactosidase. Wallenfels *et al.* reported² that against β -galactosidase, the inhibitory constant of *o*-nitrophenyl 1-thio- β -D-galactopyranoside is 1.2×10^{-4} M and the Michaelis constant of *o*-nitrophenyl- β -D-galactopyranoside is 9.5×10^{-3} M, indicating the effect of the substitution of anomeric oxygen by sulfur atom on the binding of enzyme with substrate.

In this study, 1, 6-anhydro-6-deoxy-6-thio-2, 3, 4-tri-*O*-benzyl- β -D-glucopyranose was synthesized and reacted with a Lewis acid to give a thio-polysaccharide which may be an inhibitor of dextran synthetase of *Streptococcus mutans*.

EXPERIMENTAL

General Methods

¹H spectra were obtained with Varian Gemini-100 and UNITY plus 400 spectrometers for solutions in chloroform-*d* with tetramethylsilane as internal stan-

dard. ³¹P NMR spectrum of the polymerization system was measured with the UNITY plus 400 spectrometer on solution in dichloromethane with phosphoric acid as an external reference. Optical rotations were measured for solutions in chloroform at room temperature using a JASCO LIP 1000 digital polarimeter. Gel permeation chromatography (GPC) of the polymerization products was carried out on a Shimadzu LC9A liquid chromatograph (GPC-802, 803 and 804 columns) using tetrahydrofuran (THF) as solvent and polystyrene standards. Melting points were determined with a Yanaco MP-S 3 Melting Point Apparatus. Merck Silica gel was used for column chromatography.

Pentachlorophenyl 6-O-tosyl-2,3,4-tri-O-acetyl- β -D-glucopyranoside

To the suspension of pentachlorophenyl 2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranoside⁴ (91 g, 150 mmol) in methanol (1.5 L) was added gaseous ammonia. The reaction mixture was evaporated just before the biproduct spot was detected on a TLC plate. The resulting white powder was washed with acetone and dried under vacuum to give crude pentachlorophenyl β -D-glucopyranoside. To a cooled solution (0°C) of crude pentachlorophenyl β -D-glucopyranoside (45 g, 105 mmol) in dried pyridine (300 mL), *p*-toluenesulfonyl chloride (25 g, 130 mmol) in dried pyridine (40 mL) was added dropwise over a period of 30 min. The reaction mixture was stirred at 0°C for 4 h. After adding acetic anhydride (300 mL), the system was stirred at room temperature for 12 h. The chloroform solution of the product was neutralized by aqueous NaHCO₃ and the organic layer was washed with water, dried on sodium sulfate, and evaporated. Pentachlorophenyl 6-*O*-tosyl-2, 3, 4-tri-*O*-acetyl- β -D-glucopyranoside was purified by column chromatography of silica gel followed by crystallization from benzene solution. Yield 27.0 g (25.0%). mp 184°C.

Pentachlorophenyl 6-deoxy-6-S-acetyl-2, 3, 4-tri-O-acetyl- β -D-glucopyranoside

To a solution of pentachlorophenyl 6-*O*-tosyl-2, 3, 4-tri-*O*-acetyl- β -D-glucopyranoside (17.5 g, 24.7 mmol) in dried *N,N*-dimethylformamide (46 mL), potassium thioacetate (20.6 g, 180 mmol) was added at 90°C under an atmosphere of argon. After 2 min, the reaction mixture

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Table I. Ring-Opening Reaction of 1,6-anhydro-6-deoxy-6-thio-2, 3, 4-tri-*O*-benzyl- β -D-glucopyranose (**1**)^a

No.	Monomer mg	Solvent mL	Initiator mol%	Temp. °C	Time h	Yield mg	Main product ^b ($\times 10^3$)	$[\alpha]_D$ deg
1	250	1.0	10	-40	24	Trace		
2	125	0.5	200	-40	24	120	1.1	+2.9
3	250	1.0	200	-40	24	212	1.1	+3.0
4	250	1.0	100	0	24	Trace	0.7	
5	125	0.5	200	0	24	110	1.1	+1.4
6	125	0.5	200	0	24	114	1.1	+1.9

^a Solvent, dichloromethane; initiator PF₅. ^b Determined by GPC (polystyrene standard).

was cooled in an ice bath and poured into chloroform. The chloroform solution was washed with water, dried on sodium sulfate, and evaporated. Crystallization from ethanol solution gave crystals of pentachlorophenyl 6-deoxy-6-*S*-acetyl-2, 3, 4-tri-*O*-acetyl- β -D-glucopyranoside. Yield 8.43 g (55.7%). M.p.: 172°C.

1, 6-Anhydro-6-deoxy-6-thio- β -D-glucopyranose

To 230 mL of 3 *N* potassium hydroxide solution was added pentachlorophenyl 6-deoxy-6-*S*-acetyl-2, 3, 4-tri-*O*-acetyl- β -D-glucopyranoside (30.7 g, 50 mmol). Gentle reflux was maintained for 20 h. At the end of the reflux period, the solution was cooled to the room temperature and neutralized with 3 *N* sulfuric acid. After filtration and evaporation, the product was dried under reduced pressure at 40°C. The residue was thoroughly extracted with diethyl ether. The solution was concentrated to dryness. 1, 6-Anhydro-6-deoxy-6-thio- β -D-glucopyranose was obtained as colorless crystal. Yield 2.70 g (30.7%). mp 179°C.

1, 6-Anhydro-6-deoxy-6-thio-2, 3, 4-tri-*O*-benzyl- β -D-glucopyranose (**1**)

To a solution of 1,6-anhydro-6-deoxy-6-thio- β -D-glucopyranose (1.85 g, 10.4 mmol) in dried *N,N*-dimethylformamide (60 mL) was added sodium hydride (3.2 g, 73 mmol) activated by washing with *n*-hexane. After the reaction mixture was stirred at room temperature for 30 min, 8 mL of benzyl bromide were added dropwise. The reaction mixture was vigorously stirred for 4 h, and methanol was added to stop the reaction. The product was extracted with chloroform and the chloroform solution was washed with water, dried on anhydrous sodium sulfate, and concentrated to a syrup. The obtained compound was purified by chromatography on a silica gel column (benzene : ethyl acetate = 4 : 1 as eluent) and was crystallized from *n*-hexane and *n*-butyl chloride. Yield 3.10 g (66.5%). mp 83°C, $[\alpha]_D$: -7.9° (c1, chloroform), ¹H NMR: 2.79 (d, 1H, H-6en, $J_{6en,6ex}$ = 9.6 Hz), 2.95 (dd, 1H, H-6ex, $J_{5,6ex}$ = 6.1 Hz), 3.36 (dd, 1H, H-4, $J_{4,5}$ = 2.0 Hz, $J_{3,4}$ = 5.6 Hz), 3.54 (d, 1H, H-2, $J_{2,3}$ = 4.0 Hz), 3.67 (dd, 1H, H-3), 4.54–4.73 (m, 6H, -CH₂Ph), 4.81 (dd, 1H, H-5, $J_{5,6en}$ = 1.7 Hz), 5.48 (s, 1H, H-1), 7.25–7.45 (m, 15H, aromatic protons), elemental analysis (Calcd: C, 72.29%, H, 6.29%, S, 7.15%. Found: C, 71.78%, H, 6.17%, S, 7.10%).

Polymerization of **1**

Ring-opening polymerization of **1** was carried out with phosphorus pentafluoride as initiator as described previously.³

RESULTS AND DISCUSSION

1, 6-Anhydro-6-deoxy-6-thio-2, 3, 4-tri-*O*-benzyl- β -D-glucopyranose (**1**)

Generally, sodium methoxide is used for de-*O*-acetylation. However, pentachlorophenyl glucoside was decomposed by sodium methoxide. Deacetylation was carried out with gaseous ammonia. Tosylation is relatively selective on primary hydroxyl group. In this study, 6-OH was selectively tosylated. Since the resulting 6-*O*-tosyl derivative was unstable and decomposed during purification by column chromatography, it was purified after acetylation. Substitution reaction with potassium thioacetate readily occurred because the site was the primary carbon atom. Substitution reaction was performed in oxygen-free atmosphere, because, potassium thioacetate may react with oxygen to form a disulfide compound.

Pentachlorophenyl 6-deoxy-6-*S*-acetyl-2, 3, 4-tri-*O*-acetyl- β -D-glucopyranoside was synthesized *via* different reaction route in which 6-*S*-acetyl group was introduced before glycosylation with pentachlorophenol.

Ring closure reaction was performed according to Kuzuhara *et al.*⁴ The yield of ring closure reaction was 31%, while the ring closure reaction of pentachlorophenyl 2, 3, 4, 6-tetra-*O*-acetyl- β -D-glucopyranoside gave levoglucosan in 56% yield.

1, 6-Anhydro-6-deoxy-6-thio- β -D-glucopyranose was finally benzylated in order to be polymerized.

Ring-Opening Reaction

Cationic ring-opening polymerization of **1** with phosphorus pentafluoride as an initiator was attempted. The results are summarized in Table I. Since the specific rotations of the products were in the range of +1.4° to +3.0°, ring-opening reaction occurred. However, the gel permeation chromatography of the product suggested that polymerization did not occur and the reaction product was a disaccharide derivative.

The mechanism of initiation and propagation reactions in the polymerization of levoglucosan tribenzylether (**2**) is well-investigated.⁵ PF₅ reacts with oxygen of the anhydro-ring of the monomer and then, after the attack of the next monomer, another PF₅ is added to form PF₄O at the initiating end and PF₆⁻ as counter ion. In this study, ³¹P NMR of the reaction mixture containing **1** and PF₅ (5 : 1 in molar ratio) in methylene chloride under high vacuum was measured at -40°C, as shown in Figure 1. Quartet, quintet, and septet peaks appeared at -35, -20, and -144 ppm, respectively. Quartet, quintet, and septet peaks can be assigned to PF₃O which was produced from PF₅ reacted with Pyrex, ⁵PF₄SCH₂⁻,

and PF_6^- , respectively. Since in the case of the reaction of **2** and PF_5 , quintet peak assigned to $\text{PF}_4\text{OCH}_2^-$ appeared at around -75 ppm, the quintet peak at -20 ppm in Figure 1 was assignable to $\text{PF}_4\text{SCH}_2^-$, indicating that PF_5 reacted with ring sulfur atom instead of ring oxygen. In the ring-opening polymerization of **1**, 3-oxathiolane with $\text{BF}_3 \cdot \text{etherate}$,^{6,7} cleavage of C–O bond instead of C–S bond occurred to give a polymer. Since the cleavage of C–O bond of **1** may provide a compound containing unstable seven-membered ring, C–S bond cleavage which results in the normal pyranose ring may be preferable.

Ring-opening reaction of **1** with PF_5 is shown in Scheme 1. After the addition of second PF_5 , trialkyl sulfonium ion was formed. It is speculated that the third monomer addition, *i.e.*, propagation, did not occur because the sulfonium ion was not adequately active.

To elucidate the reason of low polymerizability of **1**, copolymerization of **1** with **2** was attempted. The main products were low-molecular-weight poly(**2**), indicating that cation chain end derived from **1** was not elongated. In other words, **1** and **2** monomers attack **2**-derived oxonium ion, while neither **1** nor **2** attack the **1**-derived sulfonium ion. The low reactivity of **1**-derived sulfonium ion may be caused by lower electrophilicity of sulfonium ion, compared with oxonium ion, and/or the conformational stability of the anhydrosugar.

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Figure 1. ^{31}P NMR spectrum of the reaction mixture containing **1** and PF_5 in CH_2Cl_2 at -40°C .

Scheme 1.