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Synthesis of Protected Precursors of Chitin Oligosaccharides by Electrochemical Polyglycosylation of Thioglycosides

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Abstract

Synthesis of protected precursors of chitin oligosaccharides by electrochemical polyglycosylation of thioglycosides as a monomer is described. Oligosaccharides up to hexasaccharide were synthesized under the optimized reaction conditions. The modified method by repeating electrolysis with additional monomers enabled synthesis of oligosaccharides up to octasaccharide. Mechanism of electrochemical polyglycosylation was also discussed based on the oxidation potentials of the monomer and oligosaccharides.

Keywords

electrochemical glycosylation; glucosamine; oligosaccharide; oxidation potential; polyglycosylation

Introduction

Chitin oligosaccharides are partial structures of chitin which is an abundant β-1,4-linked polysaccharide composed of *N*-acetylglucosamine as repeating units (Figure 1) [1]. Biological activities of longer oligosaccharides such as octasaccharide have been paid much attention for many years; however, it is difficult to obtain pure oligosaccharides by isolation from natural sources and synthesis via chemical glycosylation [2]. Total synthesis of chitin and chitosan oligosaccharides have already been reported based on conventional chemical glycosylation of protected monosaccharides as building blocks. Convergent synthesis using oligosaccharide building blocks can reduce steps for the total synthesis; however, it requires manipulation of the anomeric leaving groups and deprotection of the protecting groups of the hydroxyl group at 4-position (4-OH) prior to the glycosylation. Although automated electrochemical assembly, which is a one-pot iterative synthesis of oligosaccharides based on electrochemical pre-activation of building blocks, is an alternative method for the synthesis of chitin oligosaccharides [3,4], it is also time consuming and too sophisticated to prepare oligosaccharides composed of a single repeating structure. Thus, we assume that the electrochemical polyglycosylation via the electrochemical activation of thioglycosides is a practical approach for the preparation of chitin oligosaccharides. Hashimoto and co-workers have already reported the synthesis of protected precursors of chitin oligosaccharides by polyglycosylation of thioglycosides [5]; however, this is one of a few examples of

chemical synthesis of chitin oligosaccharides through polyglycosylation of a glucosamine monosaccharide [6]. Recently, we have reported electrochemical polyglycosylation using a glucosamine derivative as a monomer [7]. This is another example of polyglycosylation of a glucosamine monosaccharide; however, *N*-acetylglucosamines are linked by α-1,4-glycosidic bonds. Here, we report electrochemical polyglycosylation of thioglycosides to produce protected precursors of chitin oligosaccharides.

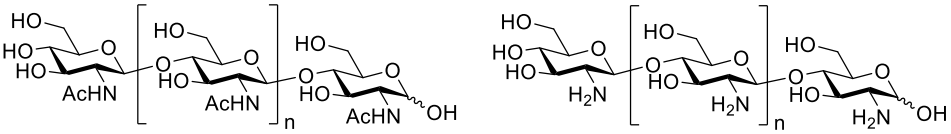


Figure 1. Structures of chitin and chitosan oligosaccharides.

Results and Discussion

Optimization of Electrochemical Polyglycosylation

We initiated our study from the optimization of the arylthio (ArS) group of thioglycoside **1** with the protecting group free 4-OH, acetyl (Ac) group at 3-OH, benzyl (Bn) group at 6-OH, and phthaloyl (Phth) group at 2-NH_2 (Figure 2) [3]. Electrochemical polyglycosylation was performed by the sequential two-step process which involved anodic oxidation at -80 °C and glycosylation at -50 °C. The crude product of the reaction was purified by gel permeation chromatography (GPC) and the monosaccharide **1a**-**d** and oligosaccharides **2a**-**d** (n = 2) ~ **7a**-**d** (n = 7) were isolated. Only thioglycoside **1a** (Ar = 4-FC_6H_4, E_{ox} = 1.70 V vs. SCE) gave oligosaccharides up to hexasaccharide **6a**, although yields of pentasaccharide **5a** (3%) and hexasaccharide

6a (1%) were very low. In the case of thioglycoside **1b** (Ar = 4-ClC$_6$H$_4$, E_{ox} = 1.68 V vs. SCE) the highest conversion (79%) and the highest yield of tetrasaccharide **4b** (14%) were observed. Contrary, thioglycoside **1c** (Ar = 4-MeC$_6$H$_4$, E_{ox} = 1.47 V vs. SCE) which has the lowest oxidation potential showed the lowest conversion (51%) and the lowest yield of tetrasaccharide **4c** (2%) [8]. Thioglycoside **1d** (Ar = 2,4-F$_2$C$_6$H$_3$, E_{ox} = 1.73 V vs. SCE) which has the highest oxidation potential also showed low conversion (63%); however, it gave pentasaccharide **5d** in the highest yield (6%) among these four thioglycosides. Based on these results we optimized the reaction using thioglycoside **1a**, which afforded oligosaccharides **2a**-**6a** and recovered monosaccharide **1a** in the highest total yield (88%).

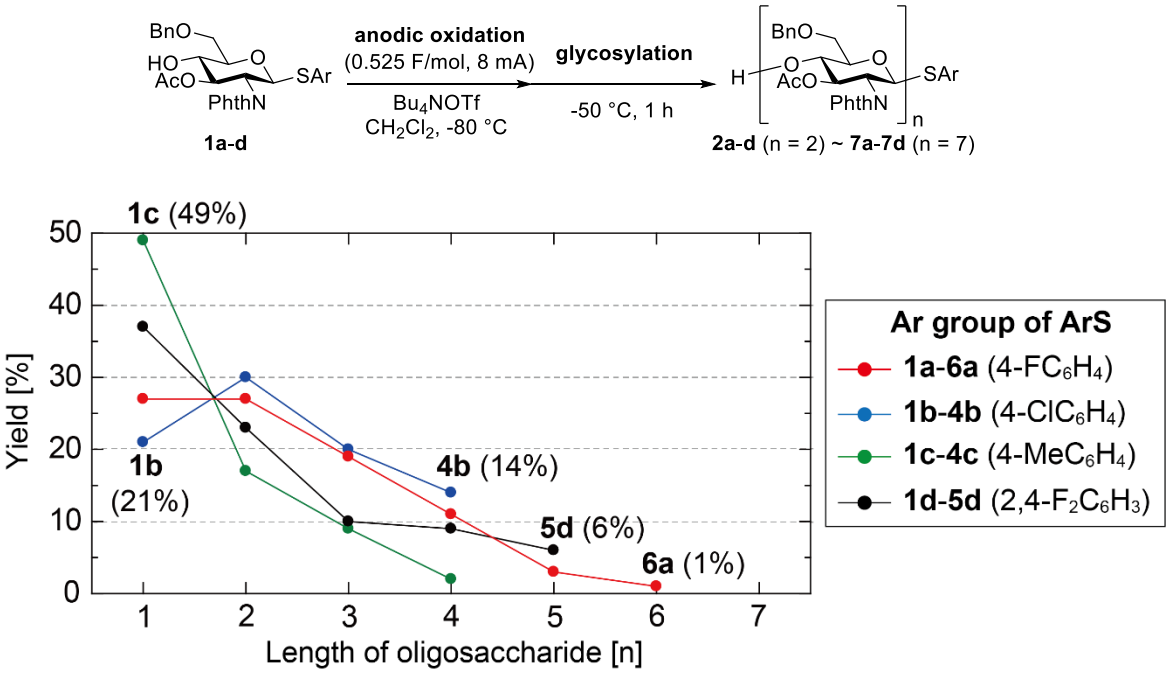
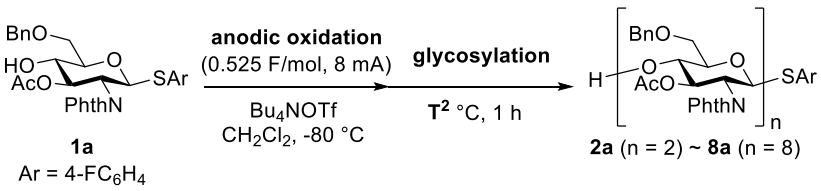


Figure 2. Effect of the anomeric leaving group on yields of oligosaccharides.

Reaction parameters of the electrochemical polyglycosylation such as amount of electricity and electrolyte were also optimized using thioglycoside **1a** (See supporting information for details). The complete conversion of monosaccharide **1a** was observed

with 0.6 F/mol; however, 0.525 F/mol was chosen as the optimized amount of electricity to prevent formation of by-products such as hydroxy sugars which have the anomeric hydroxyl group instead of the ArS group. Although we tested other ammonium triflates such as tetraethylammonium triflate (Et_4NOTf) and 1-butyl-1-methylpyrrolidinium triflate ($[Py_{41}][OTf]$) as electrolytes, both electrolytes gave oligosaccharides in lower yields.

Next, we investigated influence of glycosylation temperature (**T²**) (Figure 3). It was revealed that glycosylation proceeded even at -80 °C (pink), higher conversions of thioglycoside **1a** were observed at elevated temperatures. It is important to note that heptasaccharide **7a**, which was never obtained at -50 °C, was produced at -40 °C (blue) and -30 °C (black), although the yield of **7a** was very low (1%). These results indicate that the glycosylation temperature is an important parameter to obtain longer oligosaccharides and glycosylation might proceed during the anodic oxidation at -80 °C.



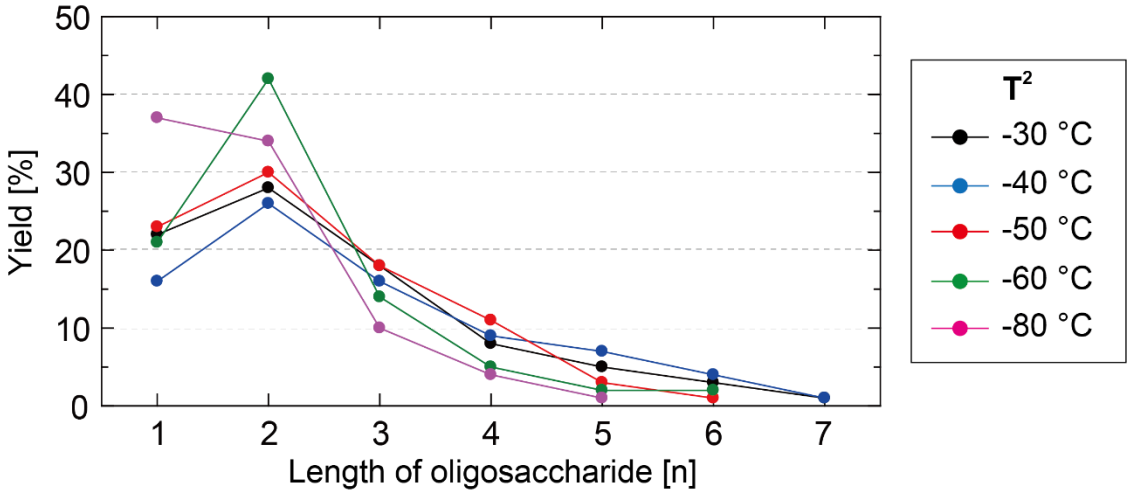


Figure 3. Influence of the glycosylation temperature on yields of oligosaccharides.

The temperature of anodic oxidation ($\mathbf{T^1}$) was also investigated together with glycosylation temperature ($\mathbf{T^2}$) because glycosylation must occur during the anodic oxidation at elevated temperature (Figure 4). Indeed, formation of oligosaccharides longer than tetrasaccharide **4a** was increased at elevated temperatures. The highest total yield of oligosaccharides **2a**-**7a** was obtained in the case of $\mathbf{T^1/T^2}$ = -60 °C /-30 °C, although heptasaccharide **7a** was not produced. Spectra of MALDI-TOF MS indicated the formation of by-products derived from longer oligosaccharides in the case of $\mathbf{T^1/T^2}$ = -30 °C /-30 °C (Figure 5). Relative intensity of molecular ion peaks of hydroxy sugars of oligosaccharides and/or trehalose-type pseudo-oligosaccharides, which were major by-products at the elevated temperature, became stronger in the corresponding peaks of longer oligosaccharides such as hexasaccharide **6a** and heptasaccharide **7a**. Proposed structures of by-products of trisaccharide **3a**, which are hydroxy sugar **9** and trehalose-type product **10**, are shown in Figure 6. There were obtained as inseparable mixture because of the same molecular weights with similar polarity. Moreover, the trehalose-type product of longer oligosaccharides has more than two possible structures. For example, there are two pseudo-tetrasaccharide

structures **11a** and **11b** which must be hard to separate by preparative-scale purification techniques.

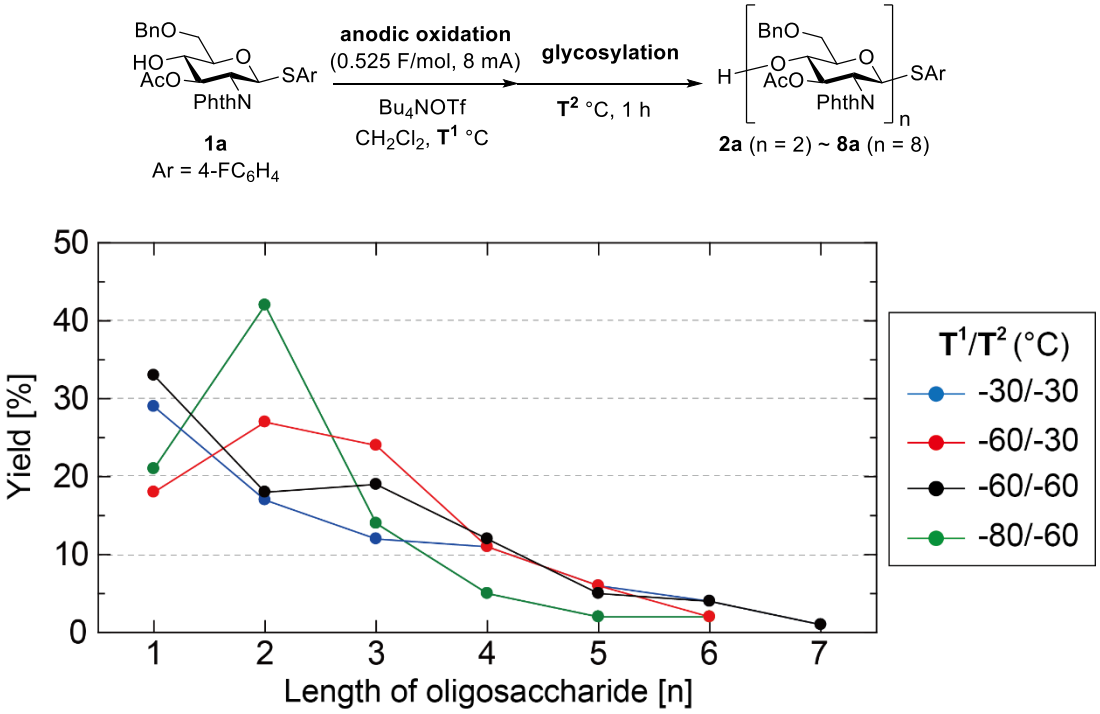


Figure 4. Influence of temperatures of anodic oxidation and glycosylation.

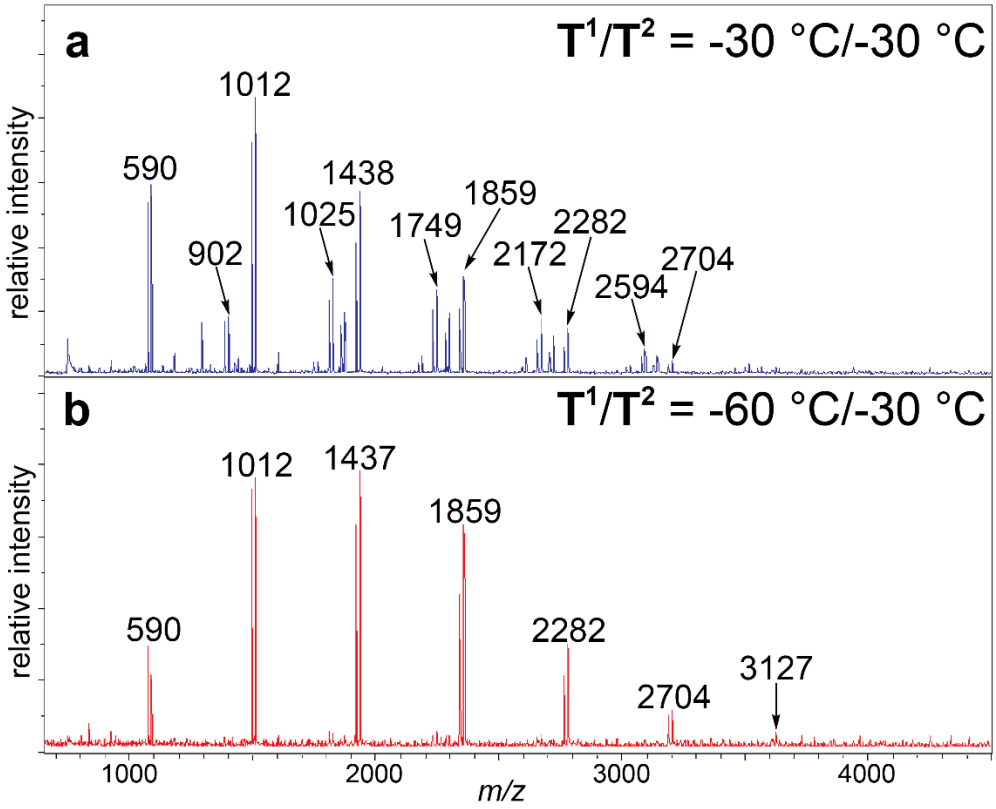


Figure 5. Spectra of MALDI-TOF MS of oligosaccharides.

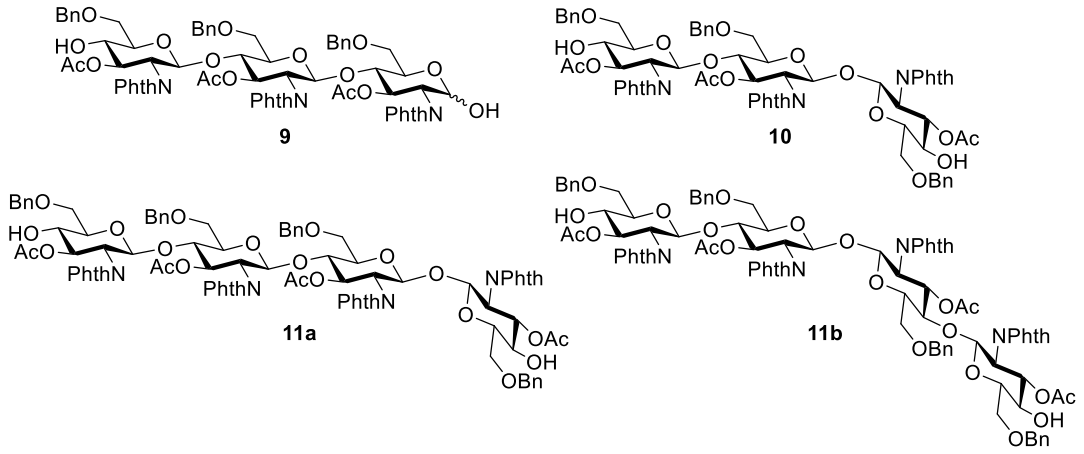


Figure 6. Proposed structures of by-products of electrochemical polyglycosylation.

Reaction Mechanism

There are two possible pathways for chain elongation in electrochemical polyglycosylation (Figure 7). In the path a, monosaccharide **1a** is converted to the

corresponding glycosyl triflate **12** and 4-OH of oligosaccharide **2a**-**6a** reacts with **12**. In the path b, oligosaccharide **2a**-**6a** are converted to the corresponding glycosyl triflate **13**-**17** and 4-OH of monosaccharide **1a** reacts with **13**-**17**. It is hard to exclude the possibility of reactions between oligosaccharides; however, polyglycosylation has been carried with slight excess amount of electricity (0.525 F/mol) and monosaccharide **1a** has always been recovered more than 15%. Moreover, longer oligosaccharides might be less reactive from viewpoints of mass transfer because electrochemical activation occurs at the surface of the anode and the substrates must move to the surface of the electrode.

To confirm reactivity of oligosaccharides we measured oxidation potentials of monosaccharide **1a**, disaccharide **2a**, and trisaccharide **3a** using rotating disk electrode (RDE) made of glassy carbon (Figure 8). Oxidation potentials of oligosaccharides **2a** and **3a** (E_{ox} = 1.76 and 1.74 V vs. SCE) were higher than that of monosaccharide **1a** (E_{ox} = 1.70 V vs. SCE). We also examined electrochemical activation of tetrasaccharide **4a** to obtain octasaccharide **8a** through the dimerization of **4a**; however, a trace amount of **8a** was formed together with by-products and recovered yield of tetrasaccharide **4a** was 64% (Scheme 1). These results strongly suggested that path a of Figure 7 is the most probable mechanism of the reaction.

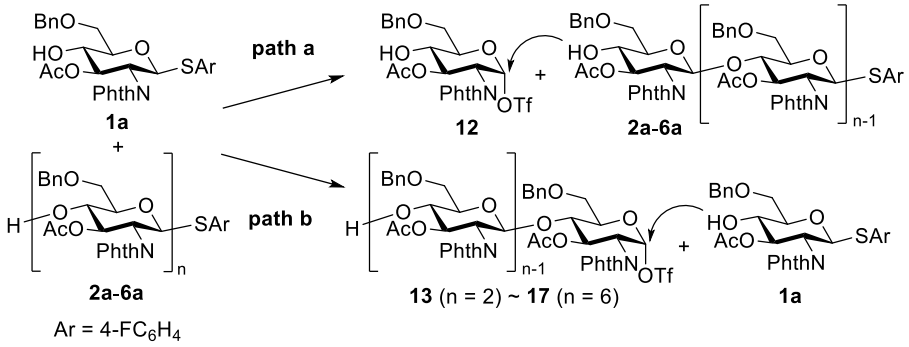


Figure 7. Proposed mechanisms of electrochemical polyglycosylation.

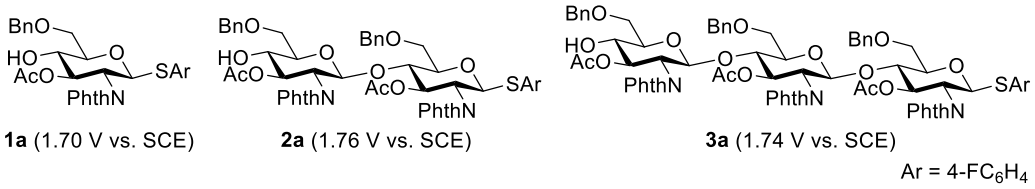
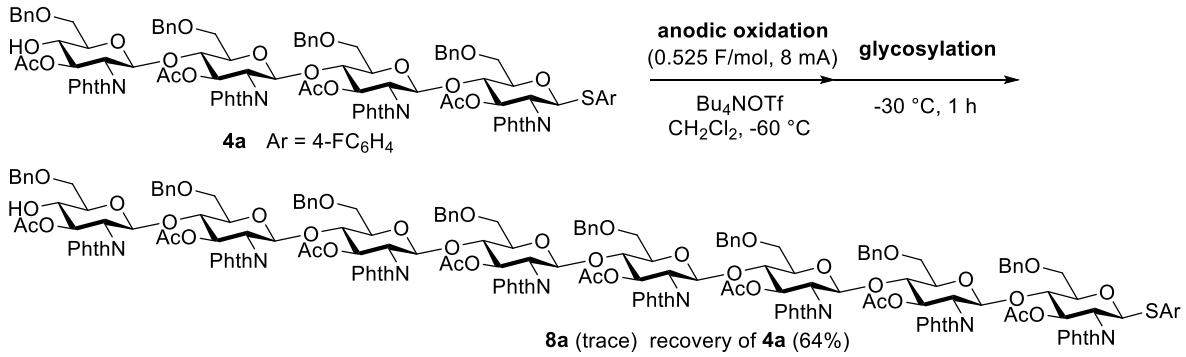


Figure 8. Oxidative potential of monosaccharide, disaccharide, and trisaccharide.



Scheme 1. Electrochemical dimerization of tetrasaccharide

Protocol Modification of Electrochemical Polyglycosylation

The optimized conditions of electrochemical polyglycosylation can afford oligosaccharides up to hexasaccharide **6a**; however, we were also interested in longer oligosaccharides such as heptasaccharide **7a** and octasaccharide **8a** because of their biological activities [9]. Higher reactivity of monosaccharide **1a** than oligosaccharides encouraged us to modify the protocol of electrochemical polyglycosylation (Figure 9). We developed the modified method of electrochemical polyglycosylation by repeating addition of monosaccharide **1a** and anodic oxidation as a single cycle. To proof our concept, we run the electrochemical polyglycosylation under the optimized conditions and one equivalent of monosaccharide **1a** was added before the second anodic oxidation. After the second cycle we could isolate heptasaccharide **7a** (3%) together with the increasing amount of hexasaccharide **6a** (5%). We run the process up to the

third cycle and isolated octasaccharide **8a** (3%) which was never isolated after the first cycle and the second cycle. These results also supported the reaction mechanism as proposed path a of Figure 7.

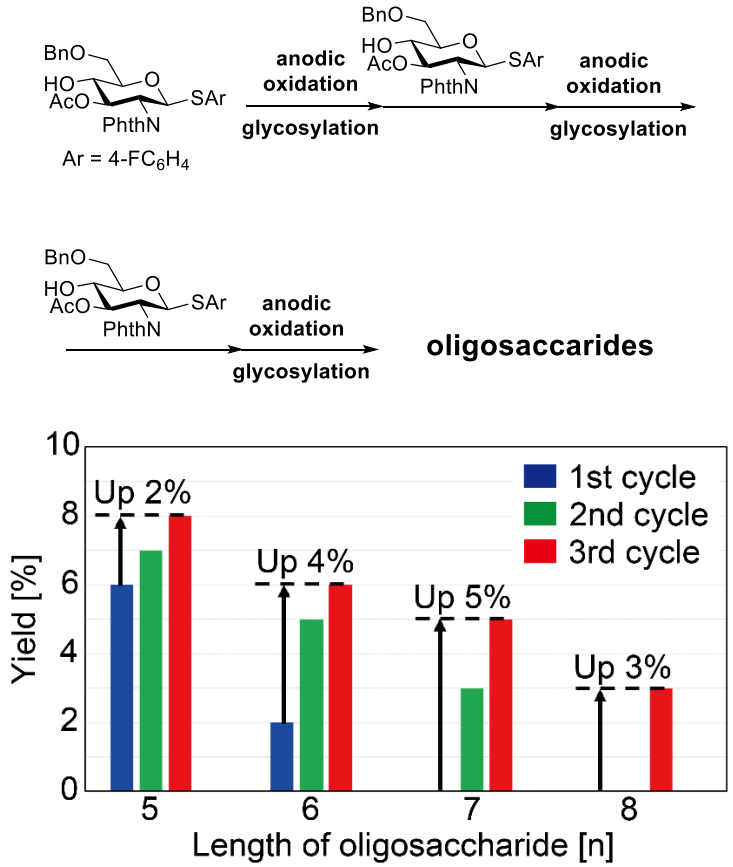


Figure 9: Increasing yields of longer oligosaccharide by the increasing number of cycles.

Conclusion

In conclusion, we have developed a practical method of synthesizing longer chain oligosaccharides within a short period of time through electrochemical polyglycosylation. Rational reaction mechanism was proposed based on oxidation potentials of oligosaccharides and further modification of the protocol was examined. The modified method by repeating cycles in one pot enabled us to prepare longer

oligosaccharides up to octasaccharide. Further optimization of the modified method and deprotection of oligosaccharides thus obtained are in progress in our laboratory.

Experimental

Electrochemical polyglycosylation has been performed using our second-generation automated electrochemical synthesizer equipped with the H-type electrolysis cell. Thioglycoside **1a** (0.20 mmol, 109 mg), Bu_4NOTf (1.0 mmol, 393 mg), and dry CH_2Cl_2 (10 mL) were added to the anodic chamber. Triflic acid (0.2 mmol, 17.6 μL) and CH_2Cl_2 (10 mL) were added to the cathodic chamber. Electrolysis was performed at -60 °C under the constant current condition until 0.52 F/mol of electricity was consumed. Then the reaction temperature was elevated to -30 °C and the temperature was kept for 1 h. The reaction was quenched with Et_3N (0.30 mL), and the reaction mixture was diluted with Et_2O and EtOAc and washed with water to remove electrolyte. The combined organic layer was dried with Na_2SO_4, and solvent was removed under reduced pressure. Thus-obtained crude product (110 mg) was purified by preparative GPC using $CHCl_3$ as eluent.

Supporting Information

Supporting Information File 1:

File Name: Electrochemical Polyglycosylation-SI

File Format: PDF

Title: Supporting Information of Synthesis of Protected Precursors of Chitin Oligosaccharides by Electrochemical Polyglycosylation of Thioglycosides

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