

Automated Solution-Phase Synthesis of Oligosaccharides via Iterative Electrochemical Assembly of Thioglycosides

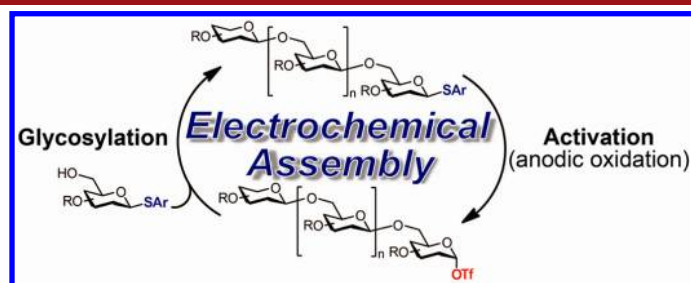
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ABSTRACT



A new iterative one-pot sequential method for the solution-phase synthesis of oligosaccharides has been devised on the basis of the electrochemical oxidation of a propagating thioglycoside terminus to generate the corresponding triflate, followed by the reaction with a thioglycoside building block having a free hydroxyl group. A practical automated synthesizer was developed for the method and was effectively used for assembling up to six thioglycoside building blocks to synthesize partial structures of poly- β -D-(1-6)-N-acetylglucosamine.

The iterative assembly of small building blocks¹ by the integration of reactions² in a one-pot sequential manner serves as a powerful method for constructing oligosaccharides.³ Although solid-phase oligosaccharide synthesis is advantageous from the viewpoint of separation of intermediates and final products from excess reagents and

wastes derived from them,⁴ it often suffers from limited scale, a high price for the solid supports, a low reactivity of substrates on the resin-bound media, and difficulties in the analysis of intermediates. To solve such problems, a one-pot sequential solution-phase method has been developed. However, at least two orthogonal activation protocols are necessary because a glycosyl donor (precursor of reactive intermediate) is usually activated in the presence of a

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glycosyl acceptor (building block).⁵ A method consisting of activation of a precursor in the absence of a building block, followed by the addition of a building block, is beneficial because one activation protocol can be used iteratively,^{1d,6} and therefore the glycosylation can be easily repeated in a one-pot sequential manner enabling automated synthesis of oligosaccharides (Figure 1).⁷

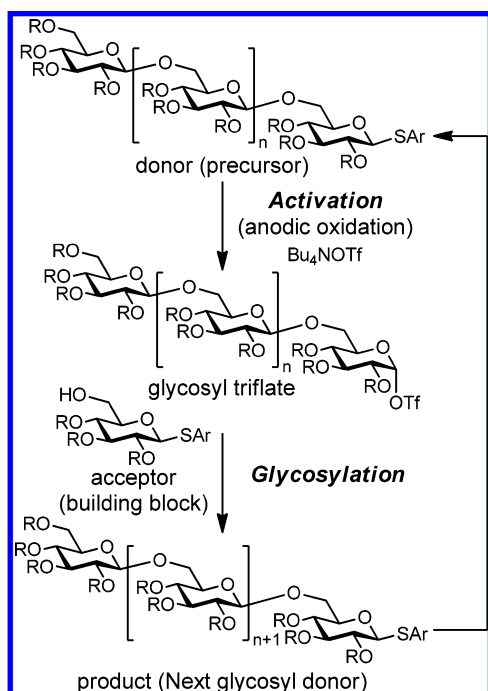


Figure 1. Iterative synthesis of oligosaccharides based on the activation of a thioglycoside donor in the absence of a glycosyl acceptor.

Herein, we report a new method for automated solution-phase synthesis of oligosaccharides based on electrochemical activation⁸ of thioglycosides to generate glycosyl triflates in the absence of a building block.⁹ This method enables us to make up oligosaccharides in the different

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direction of assembly from Seeberger method based on the acceptor-bound solid-support approach. If the present method would be a solid-support approach, it would be the donor-bound version. We also report the synthesis of partial structures of poly- β -D-(1-6)-*N*-acetylglucosamine (PNAG), by assembling six thioglycosides in a one-pot sequential manner using an automated synthesizer developed for the method.

We focused on the synthesis of oligoglucosamines,¹⁰ because PNAG has received significant research interest as the major component of the biofilm formed by pathogens.¹¹ We initiated our study by optimizing the structure of the aryl group in arylthioglycosides both as precursors of glycosyl triflates and building blocks (Table 1). Arylthioglycoside **1a** (Ar = 4-CH₃C₆H₄-, E_{ox} = 1.65 V vs SCE) was electrochemically oxidized to generate glycosyl triflate **2** according to our previous procedure (see the Supporting Information). The reaction with building block **3a** (Ar = 4-CH₃C₆H₄-, E_{ox} = 1.54 V vs SCE), which has the same aryl group, gave the corresponding disaccharide **4a** in 84% yield. The 1,6-anhydrosugar **5** was also obtained in 13% yield, which was produced by the intramolecular glycosylation of **3a**.¹² To prevent the formation of **5**, the reaction with a building block bearing a bulky aryl group (**3b**, Ar = 2,6-(CH₃)₂-C₆H₃-, E_{ox} = 1.50 V vs SCE) was examined.¹³ Although the yield of **5** decreased slightly to 8%, the yield of the desired **4b** also decreased. However, the use of a building block bearing an electron-withdrawing fluorine atom on the phenyl ring (**3c**, Ar = 4-FC₆H₄-, E_{ox} = 1.67 V vs SCE) resulted in the formation of the corresponding disaccharide **4c** in 84% yield. Now, the 1,6-anhydrosugar **5** was produced only in a trace amount. The structure of the aryl group in the precursor proved important. The use of building block **3c** with precursor **1c** gave the best result among those examined (**4c**: 92% yield). Therefore, the remaining syntheses of oligoglucosamines were carried out using thioglycosides having the 4-FC₆H₄- group on sulfur.

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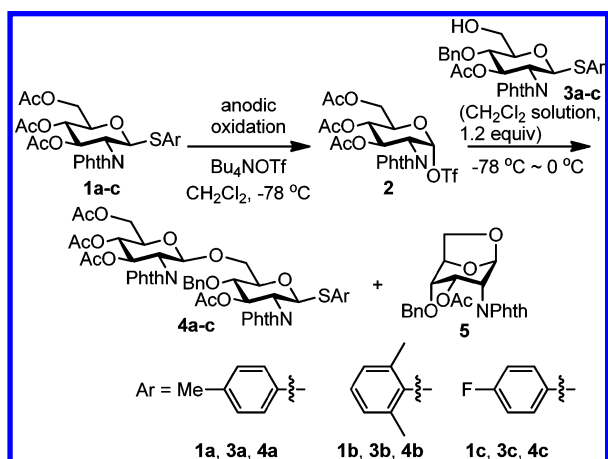
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Table 1. Optimization of the Structure of the Aryl Group in Thioglycosides



precursor (E_{ox}) ^a	building block (E_{ox}) ^a	product (yield %) ^b
1a (1.65 V)	3a (1.54 V)	4a (84)/ 5 (13)
1a	3b (1.50 V)	4b (76)/ 5 (8)
1a	3c (1.67 V)	4c (84)/ 5 trace
1b (1.52 V)	3b	4b (76)/ 5 trace
1c (1.73 V)	3c	4c (92)/ 5 – ^c

^aOxidation potentials V vs SCE. ^bNMR yields based on 1,1,2,2-tetrachloroethane as an internal standard. ^c**5** was not detected by ¹H NMR.

With the optimized aryl group for both the precursor and the building block identified, we next performed the stepwise synthesis of oligoglucosamines manually as shown in Scheme 1. The electrochemical oxidation of thioglycoside precursors **4c**, **6**, **7**, and **8** ($n = 1-4$) to generate the corresponding glycosyl triflates and the subsequent reaction with 1.2 equiv of building block **3c** was performed to obtain the corresponding oligoglucosamines **6-9** ($n = 1-4$), respectively. Oxidation potentials of oligoglucosamines were around 1.7 V vs SCE, which is almost the same as that of thioglycoside **1c** (1.73 V vs SCE). Therefore, the length of the oligoglucosamines did not affect the current efficiency of the electrochemical oxidation and chemical yields of the oligoglucosamines except for hexaglucosamine **9**.¹⁴ The successful result of manual operation prompted us to develop an automated synthesizer for the method.

An automated synthesizer was developed by assembling commercially available devices such as a syringe pump, a DC power supply, a temperature controlling system, a magnetic stirrer, an electrochemical reaction system equipped with a divided electrolysis cell, and system controller (Figure 2). The system controller,¹⁵ which is connected to a DC power supply, a syringe pump, and a chiller, controls the schedule of the electrochemical oxidation (1.0 F/mol, $-80\text{ }^{\circ}\text{C}$, 40 min),

(14) We assume that reactivity of glycosyl triflates of pentasaccharide might be low, because starting pentasaccharide **8** was consumed after the electrolysis.

(15) The system controller is a Windows PC equipped with our original program, which was written in LabVIEW (National Instrument Corporation) to control instruments.

the addition of a building block (CH_2Cl_2 solution, 1.0 equiv, 1 min), and the reaction block (CH_2Cl_2 solution, 1.0 equiv, 1 min), and the reaction temperature. It takes 20 min to change the temperature of the cooling bath from -80 to $-60\text{ }^{\circ}\text{C}$ or vice versa (rate of temperature change = $\pm 1\text{ }^{\circ}\text{C}/\text{min}$). The reaction with a building block was carried out at $-60\text{ }^{\circ}\text{C}$ for 30 min.

Scheme 1. Stepwise Synthesis of Oligoglucosamines

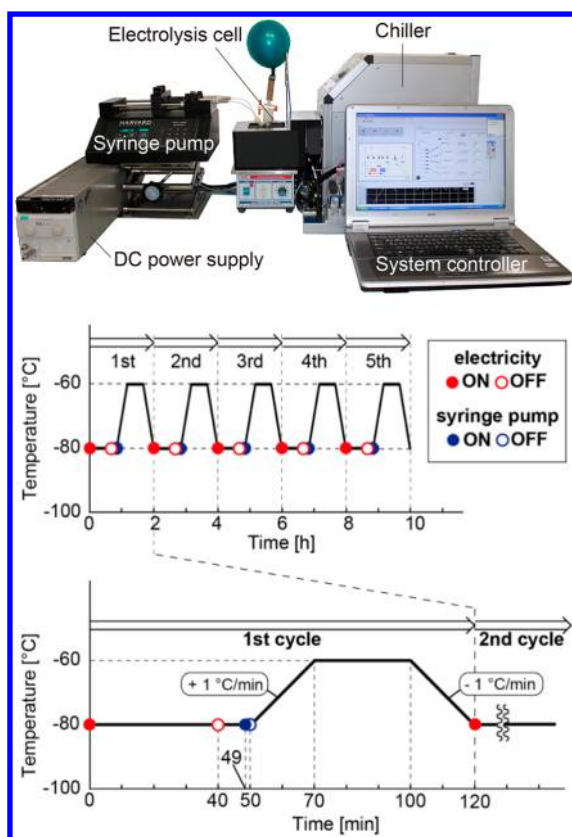
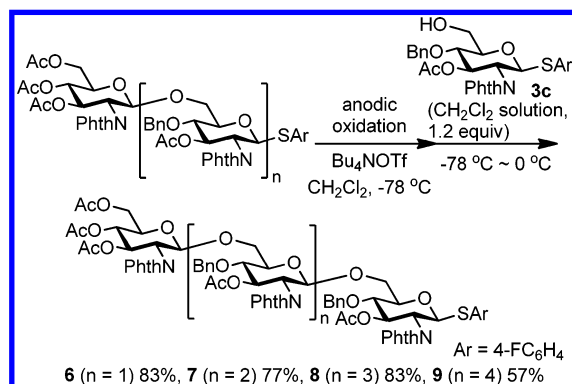
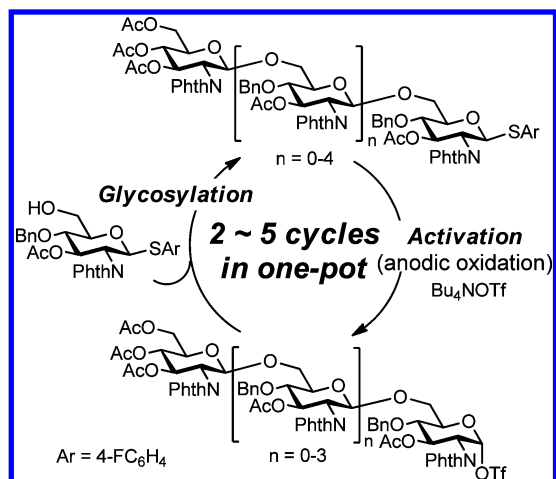


Figure 2. The automated oligosaccharide synthesizer and the schedule of the automated process.

As shown in Table 2, several oligoglucosamines were successfully synthesized using the automated synthesizer. One of the advantages of solution-phase syntheses is that

Table 2. Automated Iterative Synthesis of Oligoglucosaminesyields %^a (average yields % per cycle)

cycles	oligoglucosamine	condition A ^b	condition B ^c
2	6 ($n = 1$)	50 (71)	69 (82)
3	7 ($n = 2$)	34 (70)	52 (81)
4	8 ($n = 3$)	17 (64)	31 (75)
5	9 ($n = 4$)	9 (62)	15 (68)

^a Isolated yield. ^b Condition A (glycosylation temp: -60 °C, changing rate: ± 1 °C/min). ^c Condition B (glycosylation temp: -50 °C, changing rate: ± 2 °C/min).

the reactions in each cycle can be easily monitored by conventional analytical methods. Indeed, we observed the corresponding molecular ions of oligoglucosamines in every cycle by analyzing the reaction mixture with MALDI-TOF MS (see the Supporting Information).

Further optimization of the reaction conditions showed that glycosylation at slightly higher temperature (-50 °C) afforded the corresponding oligoglucosamines **6–9** in

better yields, and average yields were improved by around 10%. Although the targeted oligoglucosamines were contaminated with byproducts such as 1,6-anhydrosugar **5** and shorter oligoglucosamines, such byproducts were easily separated at the end by preparative recycling gel permeation chromatography (PR-GPC). Thus, partial structures of PNAG were easily prepared. Repeating the cycle, in principle, leads to the synthesis of PNAG of higher molecular weight.

In summary, we have developed an iterative method for automated solution-phase synthesis of oligosaccharides based on the electrochemical method. The electrochemical method avoids the use of strong activators, which often causes the formation of byproducts derived from them. A practical automated synthesizer has been developed for the method, and it was effectively used for assembling up to six thioglycoside building blocks to synthesize partial structures of PNAG. Further studies to obtain PNAGs of higher molecular weight, and their modification for biological tests, and the application of this method to the synthesis of other oligosaccharides is in progress in our laboratory.

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Supporting Information Available. Procedures and data for all new compounds. This material is available free of charge via Internet at <http://pubs.acs.org>.

The authors declare no competing financial interest.