Electrochemical Glycosylation as an Enabling Tool for the Stereoselective Synthesis of Cyclic Oligosaccharides

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Electrochemical glycosylation of a linear oligosaccharide with a protecting-group-free primary hydroxyl group afforded cyclic oligo-saccharides, up to hexasaccharides, in high yields. Precursors of the cyclic oligosaccharides were prepared by automated electro-chemical assembly—a method for the automated electrochemical solution-phase synthesis of oligosaccharides. We demonstrated that electrochemical glycosylation is useful not only for intermolecular glycosylation but also for intramolecular glycosylation to synthesize cyclic oligosaccharides.

Cyclic oligosaccharides such as cyclodextrins, which involve 1,4-α-glycosidic linkages of D-glucopyranose, have attracted the interest of researchers for more than a century because of their unique structures and properties.[1,2] Both chemical[3] and enzymatic[4] approaches have been developed to prepare natural and unnatural cyclodextrins. Chemical modification of the exocyclic hydroxyl groups of cyclodextrins alters the fundamental properties of cyclic oligosaccharides.[5] Cyclic oligosaccharides composed of monosaccharides other than glucose have also been investigated.[6–11] Recently, Nifantiev and co-workers[12] reported the synthesis of cyclic 1,6-β-D-glucosamines using thioglycosides as precursors and N-iodosuccinimide (NIS) and triflic acid (TfOH) as a promoter system in their pioneering works. However, the scope of this system was found to be limited. It was unable to afford the stereochemically pure β-isomer of oligosaccharide as a single product, even in the presence of a N-phthalimide group at the C-2 position, which secures β stereoselectivity in glycosylation. To address the issue of stereoselectivity and yield of cyclic oligosaccharides, further improvements in the synthetic methodology were considered desirable.

During our study of electrochemical glycosylation,[13] we developed automated electrochemical assembly—a method for the automated electrochemical solution-phase synthesis of oligosaccharides. The method has already been successfully applied to the synthesis of linear oligoglucosamines,[14a] TMG-Chitotriosymycin,[14b,c] GPI anchor tri saccharides[14d] and 1,3-1,6-β-linked oligoglucosides.[14e] Thus, we envisioned that precursors of cyclic oligosaccharides, especially oligoglucosamines, could also be readily prepared using this method, and that subsequent electrochemical glycosylation might be an alternative method for the chemical and enzymatic synthesis of cyclic oligosaccharides. Here, we demonstrate that electrochemical glycosylation is useful not only for intermolecular glycosylation but also for intramolecular glycosylation to synthesize cyclic oligosaccharides.

We commenced our study by developing the terminal building block with a temporary protecting group at the C-6 hydroxyl group (6-OH). We created a series of thioglycosides 1a–e equipped with a C-2 N-phthalimide group, to ensure a stereochemical outcome in the glycosylation, and various substituents at the remaining hydroxyl groups. With these thioglycosides in hand, these compounds were evaluated as building blocks by synthesizing disaccharide (Table 1). The electro-chemical glycosylation of terminal building blocks 1a–e and building block 3, was performed under the same conditions

| Building block | R1 | R2 | Product Yield (%) | Selectivity |
|---------------|----|----|--------------------|-------------|
| 1a            | Ac | Ac | 4a 92°             | β only      |
| 1b            | Fmoc | Bn | 4b (R1 = H) 59°    | β only      |
| 1c            | MOM | Bn | 4c ND°             | –           |
| 1d            | CIC | Bn | 4d 45°             | β only      |
| 1e            | CIAC | Ac | 4e 57°             | β only      |

[a] Reference 13a, [b] Glycosylation at −40 °C and yield after Fmoc deprotection (R1 = H). [c] Not detected.

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as reported previously for the synthesis of oligoglucosamines. To our delight, glycosylation of 1b afforded the desired product 4b in 59% yield with perfect \( \beta \)-selectivity (entry 2); however, the yield was much lower than that achieved with 1a (entry 1). Building block 1c with methoxymethyl (MOM) protection at 6-OH and benzyl (Bn) protection at the C-4 hydroxyl group (4-OH) equipped donor did not afford even a trace amount of the desired disaccharide 4c. Building block 1d bearing chloroacetyl (ClAc) and Bn groups at 6-OH and 4-OH, respectively, gave a decreased glycosylation yield of 45% (entry 4). Finally, building block 1e bearing ClAc and Bn groups at 6-OH and 4-OH, respectively, afforded disaccharide 4e in 57% yield (entry 5). These results may be attributed to tolerance of the protecting groups under the electrochemical conditions and stability of the glycosyl triflate formed during the reactions.

The best building block 1b with 9-fluorenylmethoxycarbon- yl (Fmoc) at 6-OH was then used in the automated electrochemical assembly with building block 3 for chain elongation for the synthesis of tetrasaccharides (Scheme 1). Subsequent one-pot deprotection of Fmoc group afforded the desired tetrasaccharide 5b in 19% overall yield, which was much lower than that of the tetrasaccharide assembled from the terminal building block 1a. Therefore, improvement in the choice of terminal building block is highly desirable in efforts to address the precursors of cyclic oligosaccharides.

To reduce the negative influence of the protecting group at 6-OH, we then examined the synthesis of tetrasaccharide from disaccharide terminal building blocks (Table 2). The di-saccharide building block 4f with the Fmoc group afforded tetrasaccharide 5b without the Fmoc group in 47% yield. Although fewer elongation cycles were required for the synthesis of tetrasaccharide 5b from 4f than from the monosaccharide building block 1b, the average yield was >10% higher than from 1b. We also considered other disaccharide building blocks 4 equipped with temporary protecting groups, such as MOM, ClAc and levulinoyl (Lev). However, the yields of the corresponding tetrasaccharides 5 were lower than those obtained from using the terminal building block 4f with the Fmoc group.

We then carried out the intramolecular cyclization of tetrasaccharide 5b under conventional chemical glycosylation conditions (Scheme 2). Tetrasaccharide 5b was treated with NIS and TFOH at −15°C. The reaction afforded both \( \alpha \)- and \( \beta \)-isomers of cyclic oligoglucosamine 6a and 6b in 12% and 30% yields, respectively. This result is in agreement with the finding reported by Nifantiev and coworkers. The choice of protecting groups at 4-OH and 3-OH is not crucial for obtaining cyclic oligoglucosamine stereoselectively.

To improve the efficiency of intramolecular glycosylation of tetrasaccharide 5b, we optimized the reaction conditions of electrochemical glycosylation (Table 3). Tetrabutylammonium tetrafluoroborate (Bu4NBF4), a typical electrolyte for electrochemical reactions, afforded the corresponding \( \beta \)-isomer of cyclic oligosaccharide 6b stereoselectively, but the yield was moderate and the corresponding glycosyl fluoride of the tetrasaccharide was observed by ESI-MS (m/z calc. for C22H18F9O11K [M + K]+, 1751.4966; found, 1751.4931). Tetrabutyl bis(trifluoro- omethanesulfonfylimide) (Bu4NNTf2) led to an improved yield of 6; however, selectivity was not perfect. Finally, we determined tetrabutylammonium triflate (Bu4NOTf) to be the best choice of electrolyte; it afforded 6b in high yield and with high stereo-selectivity. To investigate the influence of reaction temperature, we also performed the reaction at elevated temperature at −15°C. Although the yield was decreased, complete \( \beta \)-selectivity was observed. Therefore, the lower reaction temperature was not crucial to obtain cyclic oligosaccharide 6b stereoselectively. We propose the same reaction mechanism as the glycosyl triflate pool, which is an electrochemical con-
version of a thioglycoside into the glycosyl triflate at low temperature. The reaction is initiated by single electron transfer from the linear oligosaccharide and thus-generated glycosyl cation is immediately trapped by triflate anion to afford the corresponding glycosyl triflate intermediate. The intramolecular glycosylation may occur from the glycosyl triflate gradually. Therefore, lower temperature is important to obtain the desired cyclic oligosaccharides in better yields.

Next the intramolecular cyclization of longer oligosaccharides under anodic oxidation conditions was investigated (Table 4). The following model reaction was carried out. An electrochemical cell charged with 0.008 M linear tetrasaccharide 5b was electrochemically activated at –60 °C by means of 1.6 F/mol electricity. The reaction temperature was gradually increased to 0 °C over a period of 2.5 h. The reaction was finally quenched with Et,N (See the Supporting Information for the detailed procedure). Purification of the reaction products by simple extraction with EtOAc avoiding tedious chromatographic methods, resulted cyclic tetrasaccharide 6β as a single compound in 81% yield. Increasing the concentration of substrate and then using a fourfold higher concentration than that used in the standard reaction conditions did not lead to a significant change in the glycosylation yield. This indicated high efficiency of the intramolecular glycosylation (Table 4). Similarly, cyclic pentasaccharide 9 and hexasaccharide 10 were also efficiently synthesized; yields were 93% and 78%, respectively.

The excellent results obtained for electrochemical cyclization encouraged us for detailed investigation of reactivity based selective cyclization in the presence of both primary and secondary sugar alcohols (Figure 1). We first prepared tetrasaccharide 5e from 1a. Subsequent treatment with hydrochloric acid afforded the partially protected tetrasaccharide 5f in quantitative yield. This method was then used for the partially protected tetrasaccharide 5f. After acetylation the cyclic tetra-saccharide 11 with complete stereoselectivity was obtained in 84% yield (3 steps from 5e). Although further improvement of the procedures is necessary, global deprotection of the cyclic tetrasaccharide was also achieved in 24% (6 steps from 5e, See the Supporting Information for the details).

In summary, we have developed a protocol for the stereo-selective synthesis of cyclic oligoglucosamines, up to the hexasaccharides based on the automated electrochemical assembly of oligosaccharide precursors. The choice of electrolyte is crucial to obtain the desired cyclic oligosaccharides in reasonable yield and with complete stereoselectivity. A simplified protocol to obtain cyclic oligosaccharides, which is based on higher reactivity of the primary hydroxyl group of oligosaccharides, was demonstrated. Thus, we believe that this

Table 3. Effect of electrolyte on intramolecular electrochemical glycosylation.

| Electrolyte  | Yield (%) | Selectivity |
|--------------|-----------|-------------|
| Bu₄NBF₄     | 17        | 6β only     |
| Bu₄NNTf₂     | 66        | 6α/6β 15 : 85 |
| Bu₄NOTf     | 65        | 6β only     |
| Bu₄NHTf     | 52        | 6β only     |

[a] The reaction was performed at –15 °C.

Table 4. Effect of concentration and chain length on intramolecular electrochemical glycosylation.

| Oligosaccharide | Conc. [M] | Yield (%) | Product | Selectivity |
|-----------------|-----------|-----------|---------|-------------|
| 5b (n = 2)      | 0.008     | 81        | 6β      | β only      |
| 5b (n = 2)      | 0.032     | 78        | 6β      | β only      |
| 7 (n = 3)       | 0.008     | 93        | 9       | β only      |
| 8 (n = 4)       | 0.008     | 78        | 10      | β only      |

Figure 1. Selective intramolecular electrochemical glycosylation of the 6-OH primary alcohol.
Experimental Section

The electrochemical synthesis of cycloletatrakis-(1→6)-(3-O-acetyl-4-O-benzyl-2-deoxy-2-phthalimido-D-glucopyranosyl) (6β) was carried out in an H-type divided cell (40 glass filter) equipped with a carbon felt anode (Nippon Carbon JF-20P) and a platinum plate cathode (10 mm × 20 mm). In the anodic chamber were placed 4-Fluorophenyl-3-O-acetyl-4-O-benzyl-2-deoxy-2-phthalimido-D-glucopyranosyl-(1→6)-(3-O-acetyl-4-O-benzyl-2-deoxy-2-phthalimido-D-glucopyranosyl)-(1→6)-(3-O-acetyl-4-O-benzyl-2-deoxy-2-phthalimido-D-glucopyranosyl) (6β) (0.072 g, 0.039 mmol), Bu₄NOTf (0.195 g, 0.50 mmol) and CH₂Cl₂ (5.0 mL). In the cathodic chamber were placed trifluoromethanesulfonic acid (3.5 μL), Bu₄NCl (0.195 g, 0.50 mmol) and CH₂Cl₂ (5.0 mL). The current constant electrolysis (8.0 mA; current density: 2.0 mA/cm²), 43 V (electrode distance: 4.5 cm) was carried out at −80 °C with magnetic stirring until 1.6 F/mol of electricity was consumed. After the electrolysis, the temperature was raised to 0 °C and Et₂N (0.5 mL) was added and the mixture was evaporated under reduced pressure and extracted five times with ethyl acetate. The extract is then evaporated under reduced pressure and kept under strong vacuum for extreme dryness to furnish desired cyclic oligosaccharide 6β in 81% isolated yield (54 mg, 0.031 mmol). ¹H NMR (pyridine-d₅, 600 MHz) δ 1.61 (s, 3 H), 3.94 (pseudo-d, J = 7.8 Hz, 1 H), 4.08 (t, J = 7.8 Hz, 1 H), 4.31 (d, J = 10.8 Hz, 1 H, 4.38 (pseudo-d, J = 10.8 Hz, 1 H), 4.47 (pseudo-d, J = 10.8 Hz, 1 H, 4.60 (pseudo-d, J = 9.6 Hz, 1 H), 4.76 (td, J = 11.4, 5.4 Hz, 1 H), 6.04 (d, J = 7.8 Hz, 1 H), 6.13 (td, J = 12.0, 3.0 Hz, 1 H), 7.00 (bd, J = 2.4 Hz, 2 H), 7.14 (bd, J = 1.8 Hz, 2 H), 7.35 (s, 1 H), 7.42 (s, 1 H), 7.58 (s, 1 H), 7.64 (s, 1 H), 7.77 (s, 1 H); ¹³C NMR (pyridine-d₅, 150 MHz) δ 170.9, 169.1, 168.4, 138.6, 136.1, 128.9, 128.3, 124.2, 99.1, 77.5, 75.5, 75.3, 74.2, 69.6, 56.0, 20.6; HRMS (ESI) m/z calcd for C₂₀H₁₈N₂O₆·AK [M+K⁺], 1731.4904; found, 1731.4893.

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Conflict of Interest

The authors declare no conflict of interest.

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