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Automated Electrochemical Assembly of the Protected Potential TMG-chitotriomycin Precursor Based on Rational Optimization of the Carbohydrate Building Block

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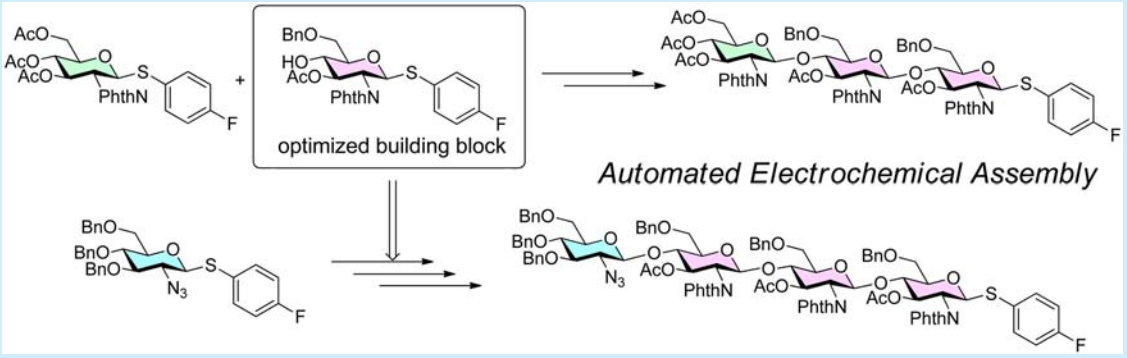
S *Supporting Information*



ABSTRACT: The anomeric arylthio group and the hydroxyl-protecting groups of thioglycosides were optimized to construct carbohydrate building blocks for automated electrochemical solution-phase synthesis of oligoglucosamines having 1,4-β-glycosidic linkages. The optimization study included density functional theory calculations, measurements of the oxidation potentials, and the trial synthesis of the chitotriose trisaccharide. The automated synthesis of the protected potential *N*,*N*,*N*-trimethyl-D-glucosaminylchitotriomycin precursor was accomplished by using the optimized building block.

Over the last few decades, oligosaccharides have attracted much attention as drug candidates because of their crucial roles in diseases.[1] Consequently, the development of practical methods for the chemical synthesis of biologically active oligosaccharides and their derivatives in an automated manner has been highly desired.[2] Solid-phase automated synthesis of oligosaccharides has already been achieved, but both the structures of carbohydrate building blocks and the reaction conditions have to be optimized in the solution phase prior to applying them to the solid-phase synthesis.[3] In the case of thioglycosides, which are one of the most popular building blocks for oligosaccharide synthesis,[4] the choice of anomeric arylthio group and the nature of the hydroxyl-protecting groups significantly affect both reactivity and selectivity in glycosylations.[5] To utilize thioglycosides as building blocks for automated synthesis, careful optimization of both building blocks and the reaction conditions in solution phase is necessary. Therefore, the automated synthesizer that is used for solution-phase synthesis of oligosaccharides could be used for both rational optimization of building blocks and preparative-scale production of oligosaccharides.

Recently, we have developed an electrochemical method[6,7] for the one-pot, solution-phase synthesis of oligosaccharides and have demonstrated the automated electrochemical solution-phase synthesis of oligoglucosamines with β-1,6-glycosidic linkages by using the automated electrochemical synthesizer.[8] Because of the abundance of oligoglucosamines with β-1,4-glycosidic linkages in nature, we became interested in the synthesis of β-1,4-oligoglucosamines as target oligosaccharides for automated synthesis (Figure 1).

N,*N*,*N*-Trimethyl-D-glucosaminyl (TMG)-chitotriomycin, which has only β-1,4-glycosidic linkages, is a tetrasaccharide that was isolated by Kanzaki and co-workers from a culture filtrate of *Streptomyces anulatus* NBRC13369 strain; the compound shows selective inhibitory activities against β-*N*-acetylglucosaminidase (GlcNAcases) of insects, bacteria, and fungi (Figure 2).[9] Yu and co-workers reported the total synthesis of this compound and a revision of the initially proposed structure of TMG-chitotriomycin in 2009.[10] In their

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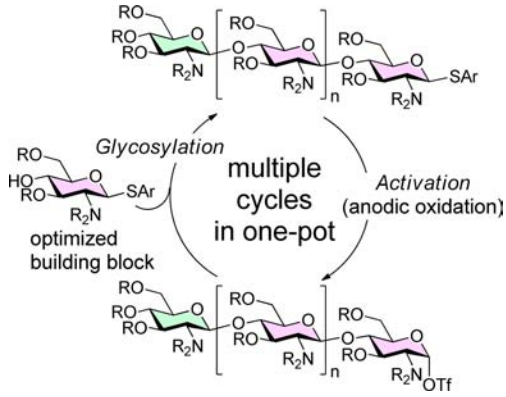


Figure 1. Automated electrochemical solution-phase synthesis of 1,4-β-oligoglucosamines.

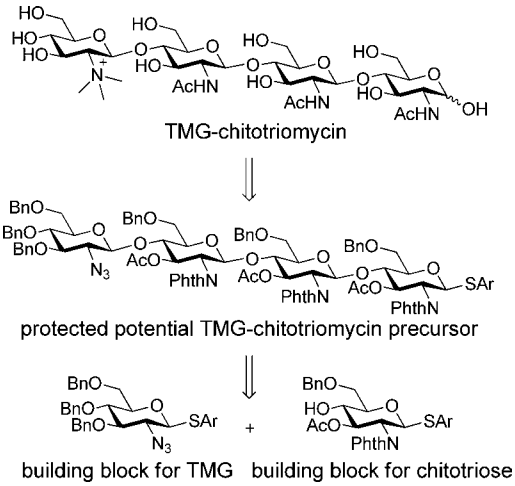
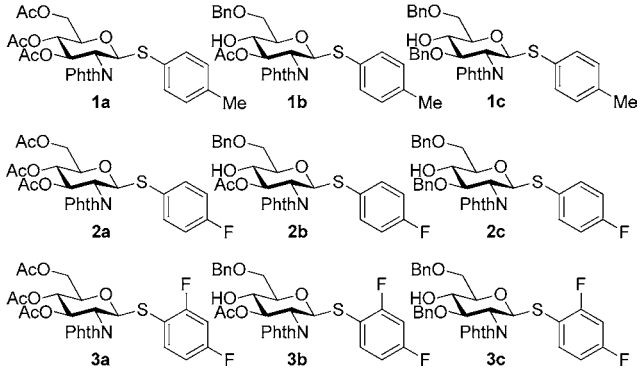


Figure 2. Retrosynthesis of TMG-chitotriomycin.

convergent synthesis of the tetrasaccharide, four different building blocks were required to accomplish the synthesis.[11,12] However, because TMG-chitotriomycin can be divided into two structural units, that is, the chitotriose trisaccharide part and the TMG part, it is reasonable to synthesize TMG-chitotriomycin by using two building blocks: a block for the chitotriose and a terminal TMG block. To achieve the automated synthesis of the protected potential TMG-chitotriomycin precursor, it is essential to optimize the carbohydrate building block for β-1,4-glycosidic linkages of oligoglucosamines. With this aim, we attempted a rational optimization of the building block based on both density functional theory (DFT) calculations[13] and electrochemical analysis. The synthesis of the chitotriose trisaccharide was then performed by using the automated electrochemical synthesizer for further optimization of the building block in solution phase. In the final step, automated solution-phase synthesis of the protected potential TMG-chitotriomycin precursor was performed using the optimized building block.

We initiated our study by conducting a rational optimization of the building block for the chitotriose trisaccharide. Oxidation potentials (E_{ox}) of thioglycosides are the most important parameters to predict their reactivity. A lower oxidation potential is preferable for the anodic oxidation (activation step) of the building block; however, a higher oxidation potential is also preferable for the glycosylation (coupling step) between an accumulated glycosyl triflate and a building block to prevent side reactions.[8] To optimize the structure of the building block, both electrochemical analysis and DFT calculations of thioglycosides were performed (Table 1).

Table 1. Difference of HOMO Energies and Oxidation Potentials of Carbohydrate Building Blocks



building block	oxidation potential E_{ox} (V vs SCE)[a]	ΔE_{ox} (V vs SCE)	ΔE_{HOMO} (eV)
1a	1.65	0	0
1b	1.47	−0.18	−0.25
1c	1.39	−0.26	−0.26
2a	1.73	+0.08	+0.18
2b	1.70	+0.05	−0.08
2c	1.58	−0.07	−0.12
3a	1.79	+0.14	+0.24
3b	1.73	+0.08	−0.02
3c	1.68	+0.03	−0.02

[a]Oxidation potentials were measured on glassy carbon rotating disk electrode (RDE) in 0.1 M Bu_4NOTf/CH_2Cl_2.

Although we reported that the ionization potentials obtained by ab initio calculations (HF/LANL2DZ) correlate with the oxidation potentials of chalcogenoglycosides,[6j] we have found that the HOMO energies of thioglycosides obtained by DFT calculations are also useful in estimating the oxidation potentials of building blocks. Relative HOMO energies of the building blocks (ΔE_{HOMO}) calculated by DFT (B3LYP/6-31G*) were compared with the corresponding relative oxidation potentials (ΔE_{ox}) (Table 1).

Oxidation potentials depend on both the hydroxyl-protecting groups and the substituent on the anomeric sulfur atom. Actually, building block **1c**, with a 3-*O*-benzyl group, shows the lowest oxidation potential (E_{ox} = 1.39 V vs SCE), and building blocks **1a**, **2a**, and **3a**, with 3,4,6-tri-*O*-acetyl-protecting groups, show higher oxidation potentials than those with other protecting groups. Substituents on the phenyl group of the anomeric sulfur atom also affect the oxidation potentials of the building blocks, and fluorine substituents are quite effective in raising the oxidation potentials. The relative oxidation potentials ΔE_{ox} are in good agreement with the respective relative HOMO energy ΔE_{HOMO}. In the previous study,[8] we found that oxidation potentials of the building blocks above 1.6 V vs SCE are suitable. Thus, among these building blocks, **2b** (E_{ox} = 1.70 V vs SCE), **3b** (E_{ox} = 1.73 V vs SCE), and **3c** (E_{ox} = 1.68 V vs SCE), with reasonably high oxidation potentials, were investigated for further optimization studies.

Further optimization of the building blocks by automated synthesis of the chitotriose trisaccharide was considered reasonable because synthesis of the disaccharide is not sufficient

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to identify small differences in reactivity. The scheme and the schedule for the synthesis of the trisaccharide are shown in Figure 3. Thioglycosides **2a** and **3a**, with a 4-fluorophenyl and a

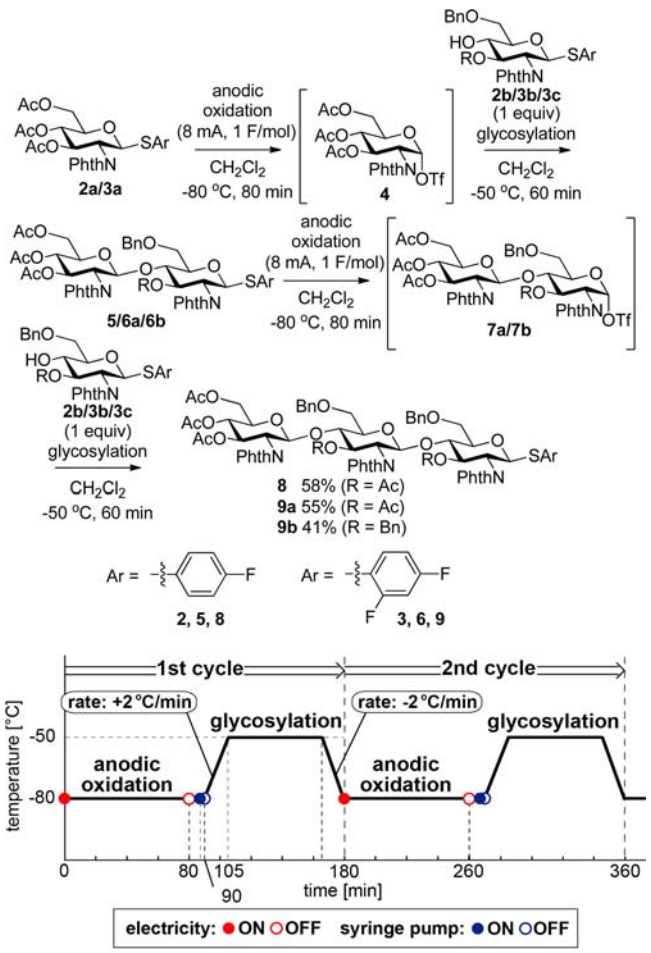

Figure 3. Automated synthesis of the chitotriose trisaccharide for optimization of the building block.

2,4-difluorophenyl group, respectively, on the anomeric sulfur atom were used as the starting building blocks. Their anodic oxidations to generate and accumulate the same glycosyl triflate intermediate **4** were carried out at −80 °C, and the subsequent glycosylations with building blocks **2b**, **3b**, and **3c** were performed at −50 °C to obtain disaccharides **5**, **6a**, and **6b**, respectively. Disaccharides **5**, **6a**, and **6b**, thus obtained, were activated by anodic oxidation in the same pot at −80 °C, and subsequent glycosylations with the corresponding building block at −50 °C afforded chitotriose trisaccharides **8**, **9a**, and **9b** with only *β*-glycosidic linkages in 58, 55, and 41% yield, respectively (the average yields for two elongation cycles were 76, 74, and 64% yield, respectively). The reason why building block **3c** gave the corresponding trisaccharide **9b** in significantly lower yield is not clear. Moreover, the yields of trisaccharides derived from other building blocks are lower than that of **9b** (see Figure S2 of the Supporting Information for details). We chose building block **2b** as an optimized building block for the synthesis of the protected potential TMG-chitotriomycin precursor.

For the automated synthesis of protected potential TMG-chitotriomycin precursor **16**, we chose thioglycoside **10** as a starting building block (Figure 4). The oxidation potential of

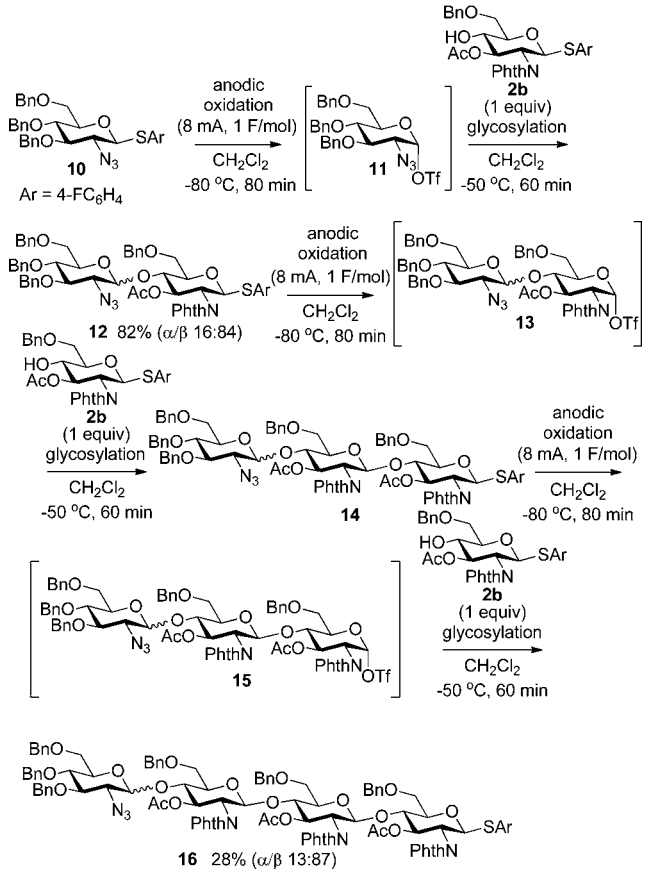

Figure 4. Automated synthesis of protected potential TMG-chitotriomycin precursor.

building block **10** (E_{ox} = 1.68 V vs SCE) was found to be slightly lower than that of building block **2b** (E_{ox} = 1.70 V vs SCE). The automated synthesis of precursor **16** was achieved by three elongation cycles in one pot. After purification by using preparative recycling gel permeation chromatography (GPC), the desired precursor **16** was obtained as an anomeric mixture of the terminal glycosidic linkage (210 mg, 28%, **16*α***/**16*β*** ratio 13:87) together with both shorter and longer oligosaccharides as byproducts (Figure 5).[14] Although we also

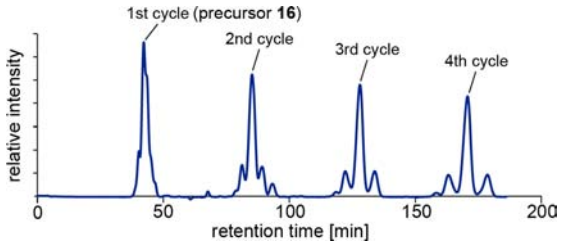

Figure 5. Preparative recycling GPC trace of the protected potential TMG-chitotriomycin precursor.

performed the glycosylation at −80 °C, the observed *β*-selectivity did not change. The selectivity was determined kinetically in the first glycosylation step because the anomeric ratio of the disaccharide (**12*α***/**12*β*** ratio 16:84)[15] was almost the same as that of precursor **16**, and glycosidic linkages thus formed are stable under the reaction conditions. Precursor **16** can be used for further transformations, including the

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introduction of a linker at the anomeric position to immobilize oligosaccharides on a surface for biological tests.

In summary, we have achieved the automated solution-phase synthesis of the protected potential TMG-chitotriomycin precursor by using a rationally optimized carbohydrate building block. DFT calculations of the HOMO energies of thioglycosides as building blocks are useful in estimating the oxidation potentials of the building blocks, and the introduction of fluorine substituents is an effective way to control their oxidation potentials. This methodology can be applied to synthesize TMG-chitotriomycin derivatives. Further investigations focused on the preparation of their derivatives for biological applications are in progress in our laboratory.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures, results of DFT calculations, and spectroscopic data of compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(13) DFT calculations were performed with Gaussian 09. See the Supporting Information for the complete reference.

(14) As to the formation of longer oligosaccharides, we consider the following two possibilities. (1) The glycosyl triflate intermediate works as an activator for the desired product. (2) The radical cation of diaryl disulfide which is generated during the anodic oxidation works as an activator. The reaction of the activated product with a building block leads to the formation of longer oligosaccharides.

(15) Disaccharide **12** was obtained in 82% isolated yield (α/β = 16:84) by the single cycle glycosylation using the automated synthesizer. Pure β-isomer **12β** was obtained after purification using silica gel chromatography See the Supporting Information for details.

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