

(様式 2)

学位論文の概要及び要旨

氏 名 Sujit Manmode 印

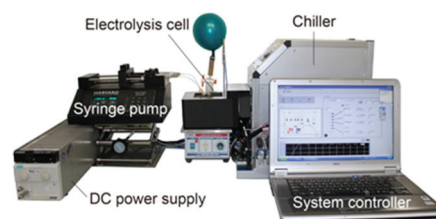
題 目 Automated Electrochemical Assembly of β -Glucans and its Application to Synthesis of Cyclic Oligosaccharides

(β -グルカンの液相自動合成とその環状オリゴ糖合成への応用)

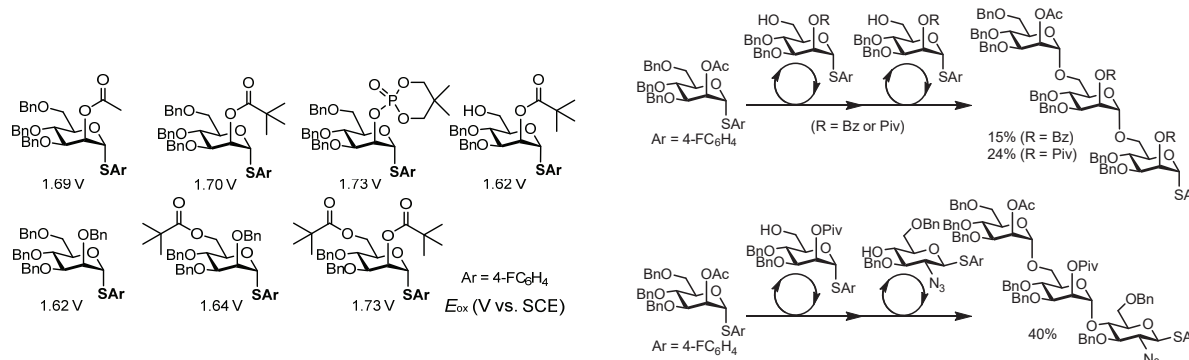
学位論文の概要及び要旨

1. Automated Electrochemical Assembly for oligomannosides and GPI anchor core trisaccharide synthesis.

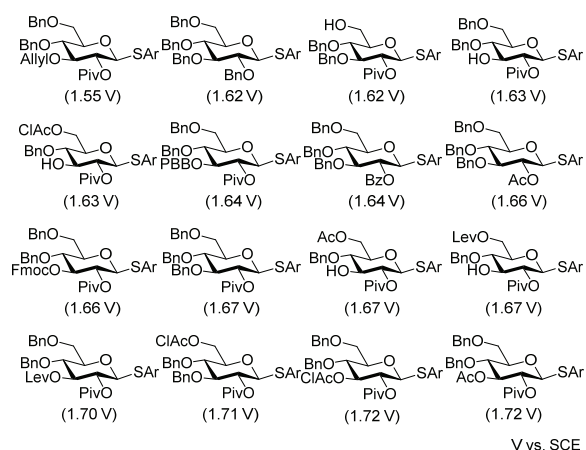
Major classes of macromolecules found in living systems are nucleic acids, proteins and carbohydrates. The later class is the most complex with respect to structural and stereochemical diversity. These carbohydrate macromolecules not only compose of a massive “information” content but also plays a vital role in many biological processes. Access of sufficient amount of structurally well-defined and pure oligosaccharides remains a challenging hurdle to chemist and biologist for many years. To get the access of these biologically important oligosaccharides, automated electrochemical oligosaccharide synthesizer come up as a promising alternative. The automated electrochemical synthesizer has been successfully employed in synthesis of oligoglucosamine and total synthesis of TMG-chitotriomycin. In the endeavors of expanding the scope of this method, we focus on biologically important oligosaccharides including oligomannosides.



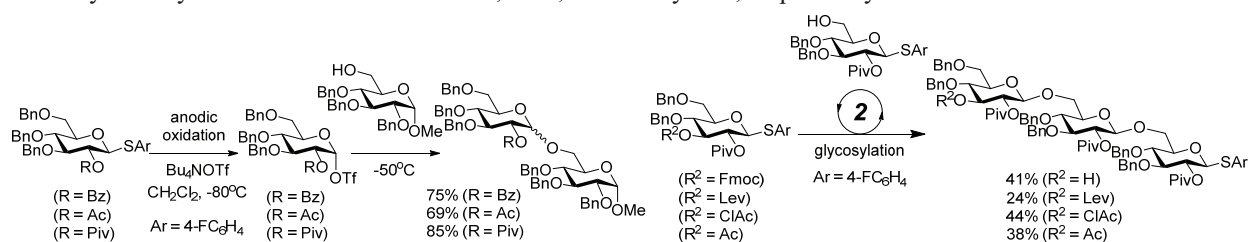
Mannosides are abundant in nature and also found in cell wall of *Mtb*. In order to synthesize oligomannosides using an automated electrochemical synthesizer it is necessary to optimize structure of carbohydrate building blocks in advance. We have prepared series of thioglycosides as a potential building block of oligomannosides and measured their oxidation potentials. Electron withdrawing groups such as acetyl and pivaloyl groups raise oxidation potentials of building blocks. Further, we extended scope of our methodology for synthesis of 1,6- α -linked trimannosides, where the first cycle of the process was initiated by anodic oxidation of terminal building block at -80 °C, followed by the coupling with building blocks (R = Bz or Piv) which has a free 6-OH. The second cycle was performed in one pot and the subsequent purification of the crude product by preparative gel-permeation chromatography (GPC) afforded desired trisaccharides 15% and 24% yields, respectively. We finally performed automated synthesis of the core trisaccharide of GPI anchor oligosaccharides in 40% overall yield in two steps.



2. Automated electrochemical assembly for β -(1,3)-(1,6) linked glucans

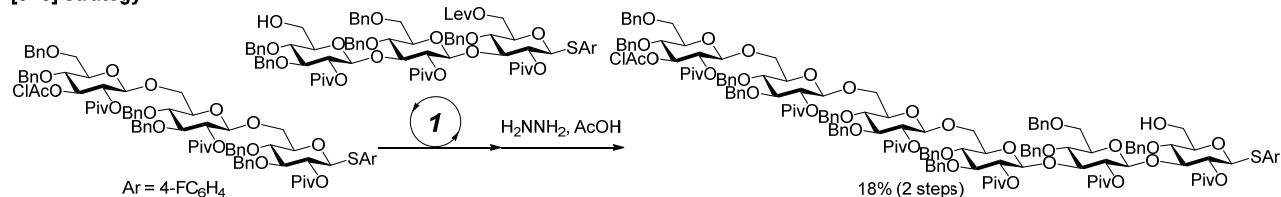


Having done with the successful synthesis of GPI anchor core trisaccharide, further, we became interested in automated electrochemical synthesis of oligoglucosides. Herein too, thioglucosides equipped with variety of protecting group were synthesized. Furthermore, oxidation potentials were measured to confirmed their feasibility in anodic oxidation condition. Comparison of closely related building blocks, such as 3-OH Fmoc protection of thioglycoside (1.66V) with that of 3-OH protection of Allyl (1.55 V), ClAc (1.72 V) and Ac (1.72 V) shows a distinct effect of protecting group on the oxidation potential. Introduction of allyl group at the 3-OH position lowers the oxidation potential (1.55 V), whereas introduction of acetyl (Ac) or chloroacetyl (ClAc) group at the same position increases the oxidation potential to 1.72 V. Next, we verified our hypothesis, for which we chose the thioglucosides having an ester protecting group such as Benzoyl (Bz), Acetyl (Ac), and Pivaloyl (Piv) at the 2-OH position and benzyl (Bn) groups at remaining hydroxyl groups. Thioglucosides are then electrochemically activated to form the corresponding glucosyl triflates at $-80\text{ }^{\circ}\text{C}$ and accumulated. In the glycosylation step, the subsequent addition of solution of glycosyl acceptor afforded a desired disaccharide. The donor having the 2-OH acetyl group gave 69% of the desired disaccharide whereas glycosyl donors containing 2-OBz and 2-OPiv gave 75% and 86% of disaccharides, respectively. Further we tested the established protocol for trisaccharide synthesis. As a result of this trisaccharide synthesis, we found that glycosyl donor, having the ClAc group as a temporary protecting group at 3-OH gave best yield of trisaccharide in 44% yield whereas, Fmoc, Ac and Lev gave relatively lower yields of trisaccharide in 41%, 38%, and 24% yields, respectively.



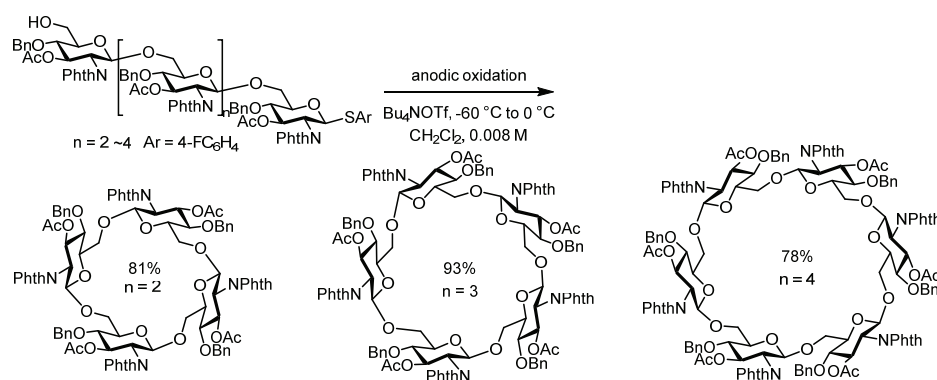
We choose [3+1+2] strategy as a model strategy for hexasaccharide synthesis. Application of the [3+1+2] strategy using standard protocol of automated synthesis, unable to furnish hexasaccharide and we end up with hydration of a glycosyl triflate intermediate and unreacted glycosyl acceptor. To overcome this problem, we modified our strategy from [3+1+2] to [3+2+1], where we thought that the problematic β -(1,3)-linkages should be synthesized in advance, and more reactive 6-OH primary alcohol, is allowed to react in the first cycle, followed by the reaction with the 3-OH of disaccharide glycosyl acceptor, in the second cycle. The strategy seemed to be promising; however, we got an only 6 mg of the desired hexasaccharide. Finally, sufficient quantity of desired hexasaccharide was accomplished using [3+3] strategy.

[3+3] strategy



3. Electrochemical synthesis of cyclic oligosaccharide

Low temperature NMR studies of electrochemically generated glycosyl triflate reveals that it can be efficiently accumulated at low temperature, taking the clue from these studies we hypothesize that if we generate glycosyl triflate of oligosaccharide having one of the hydroxyl free from protecting group at low concentration and at low temperature then generated glycosyl triflate may undergo intramolecular reaction to form cyclic oligosaccharide.



To validate the theory of our hypothesis we synthesized oligoglucosamine units of tetra-, penta- and hexasaccharides using automated electrochemical assembly with reasonable yields. Now, the stage was set for automated electrochemical intramolecular glycosylation. As a model reaction, electrochemical cell charged with 0.008 M linear tetrasaccharide was electrochemically activated at $-60\text{ }^\circ\text{C}$ by means of 1.6 F/mole electricity and further the reaction temperature gradually brought to $0\text{ }^\circ\text{C}$ over a period of 2.5 h and finally reaction is quenched with Et_3N . Purification of compound by simple extraction with EtOAc avoiding tedious chromatographic methods, resulted cyclic tetrasaccharide in 81% as a single compound. Further, increasing the concentration of substrate up to 0.032 M doesn't make a significant change in the glycosylation yield, suggesting only intramolecular reaction are favored under these concentrations. Similarly, cyclic pentasaccharide and hexasaccharides were also efficiently synthesized exploiting generality of methodology with an excellent 93 and 78% yield respectively. Excellent results of electrochemical cyclisation encourage us for detailed investigation of reactivity based selective cyclisation in presence of primary as well as secondary sugar alcohols, thus previously reported tetrasaccharide having 3,4,6-triacetylated terminal sugar moiety have been effectively reproduced using AEA. Successive treatment of hydrochloric acid resulted partially protected tetrasaccharide in quantitative yield. Aforementioned electrochemical protocol is then employed for partially protected tetrasaccharide, followed by base catalyzed acetylation resulted cyclic tetrasaccharide with complete β selectivity in an excellent 84% yield over two steps as a single product. To established the selectivity in the cyclization is solely govern by the primary alcohol, global deprotection have been performed following sequential reactions of dephthaloylation, acetylation, acid mediated acetate hydrolysis and finally hydrogenation over $\text{Pd}(\text{OH})_2/\text{C}$ gives fully deprotected cyclic tetrasaccharide in an excellent yield. The ^1H NMR and mass spectral analysis is in total agreement in those of literature report not only confirms identity of the molecule but also concludes that selectivity in cyclization is govern by primary alcohols.

In summary, we have developed a carbohydrate building block of mannosides based on DFT calculations, electrochemical analysis and automated solution-phase synthesis. The optimized thiomannosides was used to prepare the core trisaccharide of GPI anchor oligosaccharides. Furthermore, automated electrochemical assembly of β -glucans was explored with the design, synthesis, and rational optimization of carbohydrate building blocks of glucosides. Oxidation potentials of building blocks with various types of protecting groups of hydroxyl groups were measured to estimate their reactivity under anodic oxidation conditions. Building blocks for both β -1,3- and β -1,6-glycosidic linkages were optimized by automated electrochemical assembly of disaccharides and trisaccharides. Several synthetic attempts were also made for the synthesis of the hexasaccharide repeating unit in a macrocyclic β -glucan tridecasaccharide. Finally, cyclic oligoglucosamines have been successfully synthesized with excellent cyclization yields. Oligomer concentration effect study and sugar alcohols reactivity-based cyclization also put forward to explore inherent features of electrochemical glycosylation.