Electrochemical Conversion of Glucosamine Monosaccharides into Variety of Linear and Cyclic Oligosaccharides

Md Azadur Rahman

Graduate School of Engineering

Tottori University

Declaration

This is Md Azadur Rahman, declaring that the thesis is an original report of my research, has been written by me and has not been submitted to any other degree. I confirm that the work submitted is my own, except where work which has formed part of jointly authored publications have been included; the collaborative contributions have been indicated clearly and acknowledged. Due references have been provided on all supporting literatures and resources; moreover, I confirm that appropriate credit has been given within this thesis where reference has been made to the work of others.

I dually admit that the work in this dissertation is performed in between October 2019 to January 2023 in the department of chemistry and biotechnology, graduate school of engineering, Tottori university under the supervision of Prof. Toshiki Nokami.

Date: 31st January 2023

Place: Tottori University, Tottori. Name: Md Azadur Rahman

Acknowledgments

First, I would like to express my sincere gratitude to my advisor Prof. Toshiki Nokami for the continuous support of my PhD study and related research, for his patience, motivation, and immense knowledge. His guidance helped me in all the time of research and writing of this thesis. I could not have imagined having a better advisor and mentor for my PhD study.

I find myself highly grateful to Prof. Toshiki Nokami for allowing me to pursue my doctoral study in his group in graduate school of engineering of Tottori university.

Besides my advisor, I would like to present my sincere thanks to Prof. Jun-ichi Tamura and Prof. Shinsuke Ifuku, for their kindly agreeing to review this thesis.

I am thankful to my present and former lab mates in for the stimulating discussions, for the good times we were working together before deadlines, and for all the fun we have had in the last three and half years. Specially I would like to acknowledge my former lab mate Mr. Kunpei Yano for his great support for teaching me the lab work in safer way and the delicacy to follow to get best result in experiments. Several times I followed several lab mates to learn new things or new ways of experiments. I would like to thank Hirofumi Endo for his constructive help in building up the experimental work and generous support.

I am highly grateful to Asst. Prof. Norihiko Sasaki for his great support during the publication of my first research paper. I am also thankful to Asst. Prof. Shuichi Hayase.

I deeply acknowledge Ms. Kana Horio for her great support during my admission, although I never met her. The great support of Mr. Yuta Isoda and Ms. Emi Clarke helped me a lot as well.

I am also thankful to Prof. Tsuyoshi Ichiyanagi and Prof. Tamura for their valuable suggestions in the sugar seminar. Moreover, I would like to thank all the past and present members of Prof. Tamura and Prof. Ichiyanagi laboratories for keeping a healthy atmosphere in the sugar seminar and for their wonderful presentations which helped me to elaborate my view.

I keep my humble respect to the International Affairs division of Tottori University. They are much more than helpful and life in Tottori was easier with their valuable suggestions for academic and outside of academic as well. I acknowledge all the staff of graduate school of engineering for their direct or indirect support. Especially I want to thank Prof. Otachi, Prof. Itoh, Prof. Oshima and other members, for teaching me Japanese language, which helped me a lot specially in the starting days in Japan. I dually appreciate their hard work of teaching Japanese language to foreigners.

I want to express my gratitude to Koganei Corporation for their contribution to develop and continuous support for betterment of automated electrochemical synthesizer.

Finally, I would like to thank my family: my parents and my brothers and sister for supporting me spiritually throughout writing this thesis and my life in general.

Dedicated to my teachers and Supervisors who enlightened my soul.

Table of Contents

Table of Contents 3

List of Publications 4

Acronyms and Abbreviations 5

1. General Introduction

I. General Introduction 7
II. References 10

2. Chapter 1.
Synthesis of TMG-chitotriomycin analogues by automated electrochemical assembly

I. Abstract 12
II. Introduction 13
III. Results and Discussion 14
IV. Conclusion 20
V. References 20
VI. Experimental Section 21

3. Chapter 2.
Synthesis of Protected Precursors of Chitin Oligosaccharides by Electrochemical Polyglycosylation of Thioglycosides

I. Abstract 45
II. Introduction 46
III. Results and Discussion 46
IV. Conclusion 52
V. References 52
VI. Experimental Section 53

4. Chapter 3.
Synthesis of Cyclic Oligosaccharides of PNAG by Electrochemical Polyglycosylation

I. Abstract 64
II. Introduction 65
III. Results and Discussion 65
IV. Conclusion 69
V. References 70
VI. Experimental Section 70

List of Publications

Original article

Synthesis of Protected Precursors of Chitin Oligosaccharides by Electrochemical Polyglycosylation of Thioglycosides.
Rahman, M. A.; Kuroda, K.; Endo, H.; Sasaki, N.; Hamada, T.; Sakai, H.; Nokami, T.
Beilstein J. Org. Chem. **2022**, *18*, 1133–1139.

Other Original article

Synthesis of Cyclic α-1,4-Oligo-N-acetylglucosamine 'Cyclokasaodorin' via One-Pot Electrochemical Polyglycosylation–Isomerization–Cyclization Process
Endo, H.; Ochi, M.; Rahman, M. A.; Hamada, T.; Kawano, T.; Nokami, T.
Chem. Commun. **2022**, *58*, 7948-7951.

Book chapters

1. Electrochemical Synthesis of Oligosaccharides as Middle-Sized Molecules
   Rahman, M. A.; Yano, K.; Manmode, S.; Isoda, Y.; Sasaki, N.; Itoh T.; Nokami, T.
   Middle Molecular Strategy, Fukase K.; Doi T. Eds. Springer, Singapore, 2021, pp127-137.

2. Electrochemical Activation of Glycosyl Donors
   Rahman, M. A.; Nokami T.
   Comprehensive Glycoscience, 2nd edition, Barchi, Jr. J. Ed. Elsevier, Oxford, 2021, Vol. 2, pp313-326.

3. A Sugar Machine
   Endo, H.; Rahman, M. A.; Nokami, T.
   Sustainable and Functional Redox Chemistry, Inagi, S. Ed. RSC, pp.80-98.

Acronyms and Abbreviations

[α]: specific rotation expressed without units.

Ac: acetyl

AEA: Automated Electrochemical Assembly

Ar: aryl

Bn: benzyl

Bu: butyl

Bz: benzoyl

°C: degrees Celsius

ClAc: chloroaccetyl

CSA: camphorsulfonic acid

COSY: correlation spectroscopy

δ: chemical shift in parts per million downfield from tetramethylsilane

d: doublet (in NMR)

DCM: dichloromethane

DMAP: 4,4'-dimethylaminopyridine

DMF: dimethylformamide

DMSO: dimethyl sulphoxide

Et: ethyl

EtOAc: ethyl acetate

Fmoc: 9-fluorenylmethyloxycarbonyl

g: gram(s)

GPC: gel permeation chromatography

h: hour(s)

Hex: Hexane

HMQC: Heteronuclear single quantum coherence spectroscopy

HRMS: high resolution mass spectrometry

Hz: hertz

J: coupling constant (in NMR)

Lev: levulinoyl

LevOH: levulinic acid

m: multiplet (in NMR)

Me: methyl

mol: mole(s)

MOM: methoxymethyl

MS: mass spectrometry

NIS: *N*-iodosuccinimide

NMR: nuclear magnetic resonance

PBB: 4-bromobenzyl

Ph: phenyl

PhthN: *N*-phthalimide

Piv: pivaloyl

q: quartet (in NMR)

Rf: retention factor in thin layer chromatography

rt: room temperature

s: singlet (in NMR), second(s)

t: triplet (in NMR)

TBS: *tert*-butyldimethylsilyl

Tf: (trifluoromethyl)sulphonyl (triflyl)

TFA: trifluoroacetic acid

TfOH: triflic acid

THF: tetrahydrofuran

TLC: thin layer chromatography

TMSOTf: trimethylsilyl triflate

General introduction

The word "chitin" came from the Greek etymology, meaning "tunic" or "envelope". Antoine Odier was the first to use the chitin two hundred years ago. Chitin was first isolated from mushrooms by Professor Henri Braconnot of France in 1811 and the name chitin was introduced in the 1830s after isolation from insects (Figure 1). In 1859 "chitosan" was introduced by Professor C. Rouget. The 1930s and 1940s were the development period of polymers and during this time polymers attract considerable attention as evidenced by about 50 patents. But the commercial development was restricted because of the lack of adequate manufacturing facilities and cutthroat competition. In the 1970s the interest was revived and encouraged the need to better utilize shellfish shells. Chitin and chitosan are used in several countries worldwide in a variety of applications, and today there are more than 2000 applications of chitin and its derivatives. (Global industry analysis since 2004).

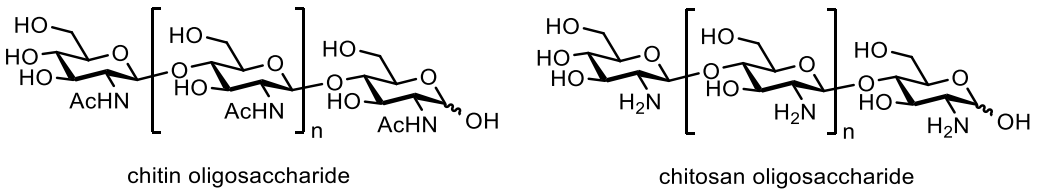


Figure 1. Structures of chitin and chitosan oligosaccharides.

Modern biomedical research for health care largely focuses on the biomaterials obtained from natural polymers which have biocompatible and biodegradable nature. Among the natural polymers, chitin and chitosan have received attention as functional biopolymers due to their cationic nature. Chitin is the most abundant glucosamine polysaccharide in nature and is the building material that gives strength to the exoskeletons of crustaceans, insects, and the cell walls of fungi.[1] Through enzymatic or chemical deacetylation, chitin can be converted to its most well-known derivative, chitosan.

Chitin and chitosan have been widely used as biopolymers because of their low cost, high availability, biocompatibility, biodegradability, and nontoxicity. The main natural sources of chitin are shrimp and crab shells, which are abundant byproducts of the food-processing industry provide large quantities of this biopolymer to be used in biomedical applications. Chitin nanofibers are prepared from the exoskeletons of crabs and prawns by a simple mechanical treatment after the removal of proteins and minerals.[2] It is highly useful in making filter, hair and skin care products, sensors, protective clothing. This chitin nanofiber can be used in tissue engineering, drug delivery, functional materials, energy storage and wound healing.[2]

As a characteristic of polymer, the molecular weight of the natural chitin can reach up to several million Daltons. Higher molecular weight and degree of polymerization limit the water solubility of these polymers.[3–5] This property has restricted their usage, particularly in medicine and the food industry.[2] Based on molecular weight, chitosan can be grouped into low molecular weight (<100 kDa), medium molecular weight (100–1000 kDa), and high molecular weight (>1000 kDa).[6] The high molecular weight and the high degree of polymerization (DP) of chitosan result in low solubility at neutral pH. Chitosan produces viscous solution in neutral pH water. The high viscosity in solution is the main limitation in the food, cosmetics, agriculture, and health industry.[7] Therefore, to obtain chitosan with a more uniform molecular size and easy solubility, it is necessary to convert chitosan into oligomers. Chitosan with DP <20 and a molecular weight less than 3.9 kDa is called chitosan oligomers, chitooligomers, or chitooligosaccharides (COS).[8] Chitooligosaccharides are the products of the hydrolysis of chitosan, and because they are soluble in water, they have several applications, such as antioxidant, anti-tumor, and agricultural purposes. Moreover it

exhibit numerous biological functions like metastasis suppression, antifungal, antibacterial, anticoagulant, enzyme inhibition, and wound healing acceleration.[5] Chitosan is also used in the production of injectable thermosensitive carriers and can be easily blended with other polymers or fabricated into nanoparticles, microspheres, gels, or fibers for drug delivery applications.[9,10] Based on the different degree of polymerization, degree of *N*-acetylation, and pattern of acetylation chitooligosaccharides perform different biological processes.[11] The derivatives of chitin and chitosan also play critical role in a variety of biological functions. Lipochtiooligosaccharides are found to be nodulation signal molecule in the symbiosis of *Rhizobium* bacteria and leguminous plants.[8] Another well-known derivative TMG chitotriomycin is a selective inhibitor of GlcNAcases. This is highly selective in the inhibition of the enzyme of fungus and bacteria but has not inhibitory activity against the enzyme of plants and mammals.[12] If the glycosidic oxygen is replaced by sulfur from chitooligosaccharides then it became thiochitooligosacchrdies and this is resistance to enzymatic degradation.[13]

Chitooligosaccharides can be synthesized from the chemical or enzymatic degradation of chitin of chitosan.[14] But the limited availability of high enzyme-yielding microbial resources, along with the high cost of enzyme/product extraction and purification processes and low yields restricted the development of chitin bioconversion processes at a commercial scale.[15] Moreover resulting chitooligosaccharides from the chemical or enzymatic degradation of chitin of chitosan are heterogeneous.

Therefore, the scientific community is currently focusing on the development of highly effective and environmentally friendly methods. Preparation of structurally well-defined and pure oligosaccharides has remained a challenge for researchers for many years. The earlier and pioneering synthesis of chitin oligosaccharide was introduced by professor Shinji Ikeshita and his coworkers.[16] They synthesized "Nod factors NodRm-IV" in a stereo controlled manner.[16] Now a days Biologically important oligosaccharides can be synthesized by the conventional method of oligomerization or one-pot synthesis. The automated synthesis of oligosaccharide synthesis in solid phase was developed by Seeberger and co-workers.[17] The group developed an automated approach, "the first fully automated solid-phase oligosaccharide synthesizer".[18] But the solid phase synthesis of oligosaccharide has some draw as well.

In this regard, automated electrochemical solution phase oligosaccharide synthesis comes up as a promising alternative.[19] This method can produce oligosaccharides in one pot by the generation of glycosyl triflate intermediate at low temperatures and the addition of an acceptor solution as a single cycle.[19] This is still time-consuming and very delicate in operation.

In this study, we developed electrochemical polyglycosylation by using a second-generation electrochemical synthesizer that can produce longer oligosaccharides in less time. With the development of electrochemical polyglycosylation, we tried to synthesize linear and cyclic oligosaccharides which are biologically important.[20]

Automated electrochemical assembly is an advanced tool for synthetic chemistry (Figure 2). For synthesis this advanced tool is a great combination of time bound, cost effective, electrochemistry-based instrument. Electrochemical glycosylation was first reported by Noyori and Kurimoto in 1986.[21] The last two decades variety of instruments was developed to synthesize glycoconjugates in automated manner. For example, Seeberger and co-workers developed automated solid phase synthesizer[17] where "Denchenko"[22] and "Wang"[23] group reported HPLC-assisted automated synthesizer independently.

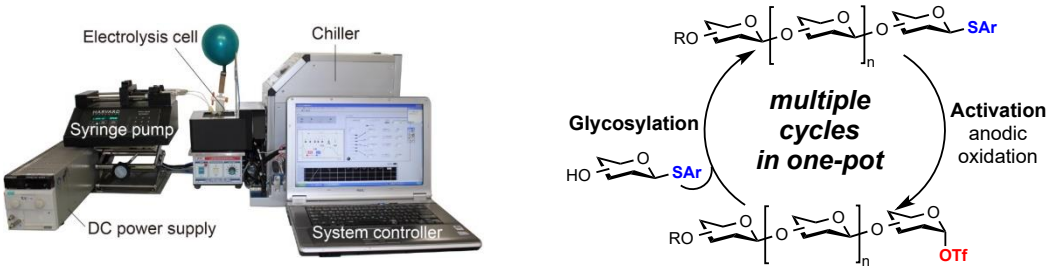


Figure 2. Electrochemical synthesizer and the working principle.

The main advantage of using automated electrochemical synthesizer is that it works in solution phase and can be used for large scale production of oligosaccharides. It is different from the solid phase synthesis by its principle as well as it activates donor, so no need to run common protection and deprotection sequence. Moreover, electrochemical assembly doesn't need to use hazardous activator to activate the donor. Becoming cost effective, user friendly and the combination of reliable instruments is getting popularity for the synthesis of longer carbohydrate molecules.

Abstract of this thesis

Chapter 1

Oligoglucosamins are abundant and important oligosaccharides for living particles including plants, fungi, insects, and other organisms. **TMG-chitotriomycin** with the *N*, *N*, *N*-Trimethyl-D-glucosaminyl (TMG) unit showed potent and selective inhibition of insect and fungal GlcNAcases with no inhibition of mammalian and plant GlcNAcases. We already achieved the total synthesis of TMG-chitotriomycin using an automated electrochemical synthesizer. We have been interested in the inhibitory activity of oligoglucosamine analogs with the TMG unit. We synthesized three different analogues of TMG-chitotriomycin including a pentasaccharide.

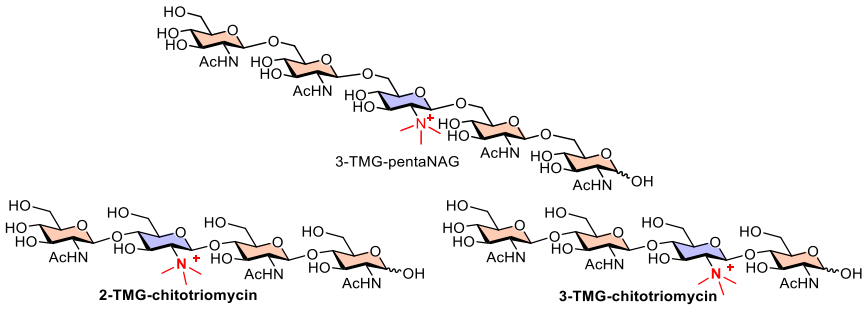


Figure 3. Analogues of TMG-chitotriomycin

Chapter 2

Chitin and its derivative chitosan are biopolymers with excellent bioactive properties, such as biodegradability, non-toxicity, biocompatibility, hemostatic activity, and antimicrobial activity. A wide variety of biomedical applications for chitin and chitin derivatives have been reported, including wound-healing applications. In this study we described the synthesis of protected precursor of chitin oligosaccharides by electrochemical polyglycosylation of thioglycoside as a monomer. We synthesized up to hexasaccharide under optimized condition of reaction which was elongated till octasaccharide by the modification of polyglycosylation reaction. This modification

of the reaction was based on selective activation of monosaccharide, and we described the mechanism in this poster.

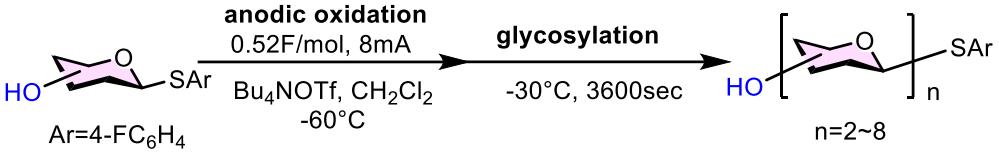


Figure 4. Synthesis of chitin oligosaccharide precursor by electrochemical polyglycosylation

Chapter 3

Electrochemical polyglycosylation is already reported as an effective tool for the synthesis of longer precursor of chitin oligosaccharides. We applied the same strategy for the synthesis of PNAG oligosaccharide. Here we obtained both linear and cyclic oligosaccharide as products. In this study we investigated over protecting groups of different hydroxy groups and amino groups for the best outcome. We described the structure of obtained hetero oligosaccharides here as well.

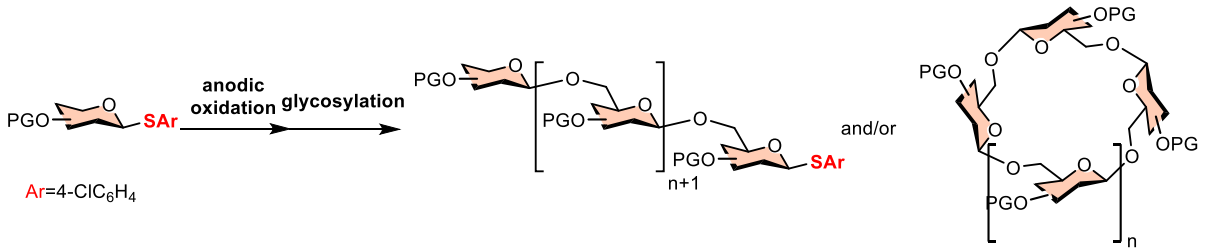


Figure 5. Synthesis of cyclic and linear PNAG oligosaccharide precursor by electrochemical polyglycosylation

References

(1) Tharanathan, R. N.; Kittur, F. S. *Crit. Rev. Food Sci. Nutr.* **2003**, *43*, 61–87.
(2) Ifuku, S.; Saimoto, H. *Nanoscale* **2012**, *4*, 3308–3318.
(3) Aranaz, I.; Alcántara, A. R.; Civera, M. C.; Arias, C.; Elorza, B.; Caballero, A. H.; Acosta, N. *Polymers*. 2021, 3256–3283.
(4) Berezina, N. *Phys. Sci. Rev.* **2016**, *1*, 1-8.
(5) Jeon, Y. J.; Shahidi, F.; Kim, S. K. **2007**, *16*, 159–176.
(6) Santoso, J.; Adiputra, K. C.; Soerdirga, L. C.; Tarman, K. In *IOP Conference Series: Earth and Environmental Science*; Institute of Physics Publishing, **2020**; Vol. 414. 012021.
(7) Xia, W.; Liu, P.; Zhang, J.; Chen, J. *Food Hydrocoll.* **2011**, *25*, 170–179.
(8) Mourya, V. K.; Inamdar, N. N.; Choudhari, Y. M. *Polym. Sci. Ser.* **2011**, *53*, 583–612.
(9) Parhi, R. *Environmental Chemistry Letters* **2020**, 577–594.
(10) Li, J.; Cai, C.; Li, J.; Li, J.; Li, J.; Sun, T.; Wang, L.; Wu, H.; Yu, G. *Molecules*, **2018**, 2661.
(11) Vander, P.; Våain, K. M.; Domard, A.; El Gueddari, N. E.; Moerschbacher, B. M. *Plant Physiol.* **1998**, *118*, 1353–1359.
(12) Usuki, H.; Nitoda, T.; Ichikawa, M.; Yamaji, N.; Iwashita, T.; Komura, H.; Kanzaki, H. *J. Am. Chem. Soc.* **2008**, *130*, 4146–4152.
(13) Fettke, A.; Peikow, D.; Peter, M. G.; Kleinpeter, E. *Tetrahedron* **2009**, *65*, 4356–4366.
(14) Aam, B. B.; Heggset, E. B.; Norberg, A. L.; Sørlie, M.; Vårum, K. M.; Eijsink, V. G. H. *Mar. Drugs* **2010**, *8*, 1482–1517.
(15) Kumar et al; *Latest Advances in Glycoengineering - Google Books* **2020**, *8*, 128.
(16) Ikeshita, S.; Sakamoto, A.; Nakahara, Y.; Nakahara, Y.; Ogawa, T. *Tetrahedron Lett.* **1994**, *35*,

3123–3126.
(17) Plante, O. J.; Palmacci, E. R.; Seeberger, P. H. *Science* **2001**, *291*, 1523–1527.
(18) Hahm, H. S.; Schlegel, M. K.; Hurevich, M.; Eller, S.; Schuhmacher, F.; Hofmann, J.; Pagel, K.; Seeberger, P. H. *Proc. Natl. Acad. Sci. U. S. A.* **2017**, *114*, E3385–E3389.
(19) Nokami, T.; Hayashi, R.; Saigusa, Y.; Shimizu, A.; Liu, C. Y.; Mong, K. K. T.; Yoshida, J. *Org. Lett.* **2013**, *15*, 4520–4523.
(20) Rahman, M. A.; Kuroda, K.; Endo, H.; Sasaki, N.; Hamada, T.; Sakai, H.; Nokami, T. *Beilstein J. Org. Chem.* **2022**, *18*, 1133–1139.
(21) Noyori, R.; Kurimoto, I. *J. Org. Chem.* **1986**, *51*, 4320–4322.
(22) Ganesh, N. V.; Fujikawa, K.; Tan, Y. H.; Stine, K. J.; Demchenko, A. V. *Org. Lett.* **2012**, *14*, 3036–3039.
(23) Wen, L.; Edmunds, G.; Gibbons, C.; Zhang, J.; Gadi, M. R.; Zhu, H.; Fang, J.; Liu, X.; Kong, Y.; Wang, P. G. *Chem. Rev.* **2018**, *118*, 8151–8187.

Chapter 1.

Synthesis of TMG-chitotriomycin analogues by automated electrochemical assembly

Abstract

TMG-chitotriomycin is well known for its selective enzymatic inhibition against insects and fungus. The derivatives of TMG-chitotriomycin made us curious whether these molecules are also selective inhibitors of endo type GlcNAcases or not. From that curiosity we synthesized three different analogues of TMG-chitotriomycin which is shown here in this study. The key tool for the analogues were two different β-selective disaccharide building blocks which were synthesized with the description of optimized condition.

Introduction

Chitin is a linear polysaccharide of β-1,4-linked *N*-acetylglucosamine (GlcNAc). This is the second largest natural biopolymer after cellulose which remains mainly in the insect exoskeleton and fungal cell wall.[1] The chitin degradation into oligoglucosamine and glucosamine is an important biological process where several enzymes such as chitinases and glucosaminidases are involveds.[2] Therefore, inhibitors of these enzymes are expected to be potential pesticides or fungicides.[3] Several inhibitors like PUGNAc[4], nagastatin[5], NAGthiazoline[6], and pochonicine[7] have already been developed. These are small molecules and strong molecular inhibitors and show a very wide spectrum towards enzymes of various species including animals' enzyme. Therefore, TMG-chitotriomycin has the potential as a leading compound for safe insecticides and pesticides because it selectively inhibits the enzymes of insects and fungi but has no activity against mammal's enzymes (Figure 1-1).[3] Recently, the total synthesis of TMG-chitotriomycin initiated from building blocks that were prepared by the degradation of chitin has been reported by Beau[8]; but, practical synthetic methods to provide TMG-chitotriomycin and its derivative in preparative scale are still highly desirable. Our group have already achieved the total synthesis of TMG-chitotriomycin via automated electrochemical assembly.[2] TMG-chitotriomycin shows inhibition activity towards exo-type GlcNAcase. Thus, we are interested in the analogues of TMG-chitotriomycin which can inhibit endo-type GlcNAcase.

In the 1st chapter, I show the total synthesis of three analogues of TMG-chitotriomycin including 2 and 3-TMG-chitotriomycin as possible inhibitors of endo-type GlcNAcases and 3-TMG pentaNAG as a potential inhibitor of Pga-B. These syntheses are also followed by automated electrochemical assembly like the synthesis of TMG chitotriomycin. Pga-B is one of the essential enzymes for biofilm production.[9] Biofilm is made by pathogens and a protected niche for them, where they are safe from antibiotic treatment and can create a source of persistent infection. Biofilm is formed in the cell membrane of pathogens and inside the cell building block is assembled to form PNAG polysaccharide by the enzyme located at the inner cell membrane. Then Pga-B, which remains in periplasm and catalyzes deacetylation of NHAc group, makes this polysaccharide more flexible and the polysaccharide goes to outside of the cell by Pga-A which is also a membrane protein.[10] The inhibition of Pga-B may prevent transport of PNAG and this 3-TMG-pentaNAG may work as a pathogen killer.

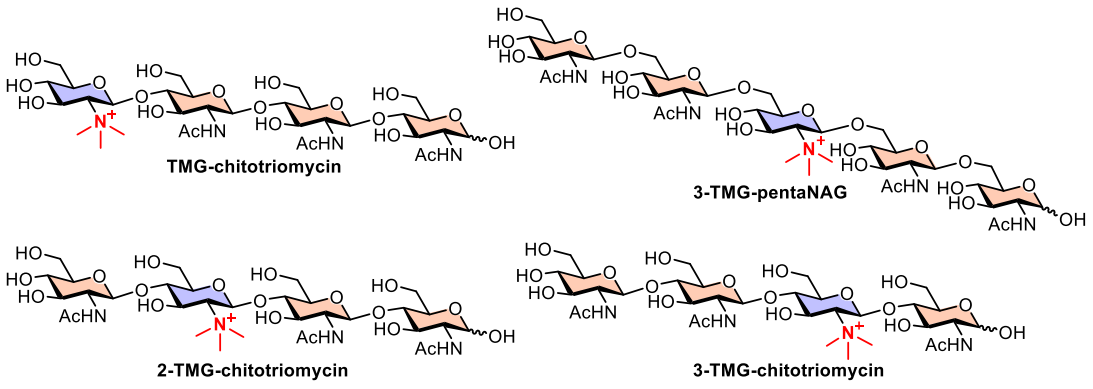


Figure 1-1. Structures of TMG-chitotriomycin and its analogues.

Results and Discussion

We carried out the total synthesis of 3-TMG-pentaNAG **1** based on the retrosynthetic analysis as shown in Figure **1-2**. To complete the total synthesis of 3-TMG-pentaNAG **1** we designed two pentasaccharide precursors **2a** (X = SPhF) and **2b** (X = OBn) which were equipped with conventional protecting groups such as *O*-benzyl (Bn), *O*-acetyl (Ac), and *N*-phthalimide (Phth). Precursor **2** can be divided into two monosaccharide building blocks **3** and **5**, and one disaccharide building block **4** which can be prepared from two different monosaccharide building blocks **6** and **5**. The monosaccharide with the 2-deoxy-2-azido group was planned to introduce as a part of disaccharide building block **4** because of a potential problem in the stereoselectivity of glycosylation of **6**.

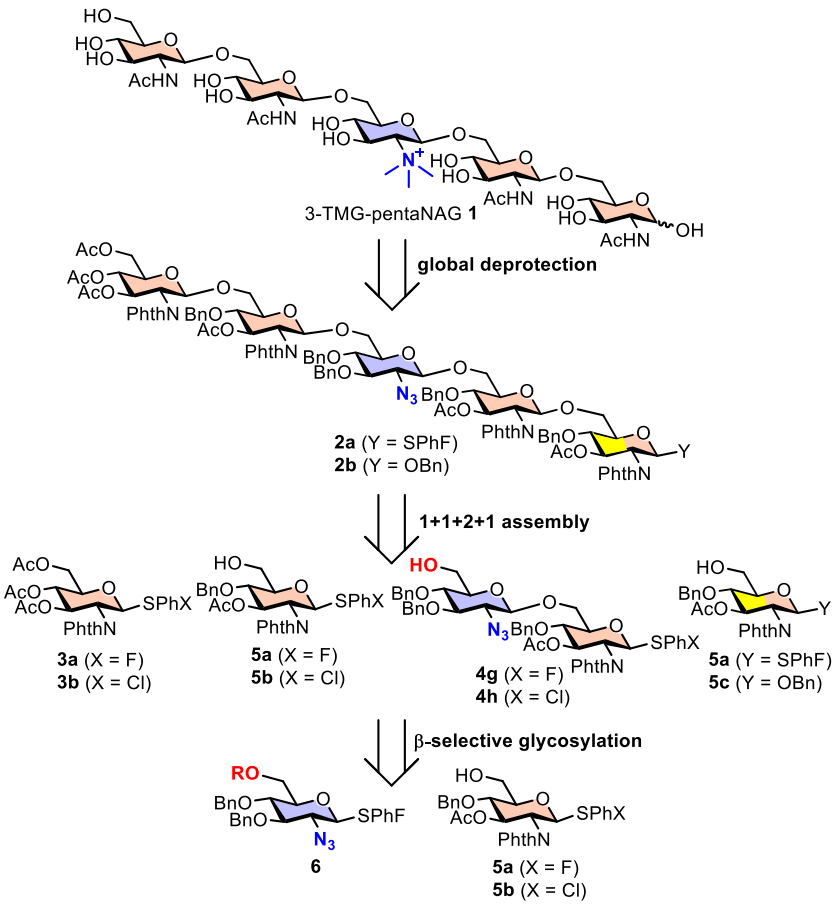
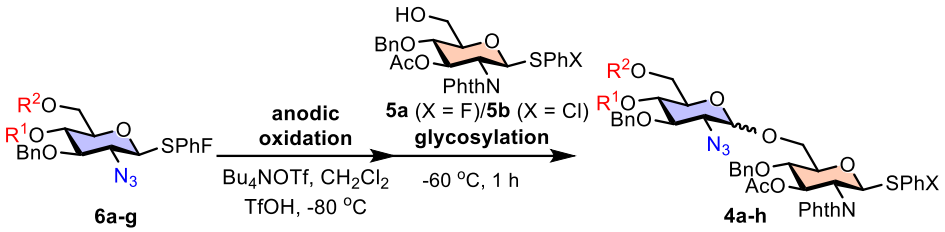


Figure 1-2. Retrosynthetic analysis of 3-TMG-pentaNAG.

The stereoselective synthesis of disaccharide building block **4** is a key challenge to synthesize the precursor of **2**. For the β-selective synthesis of disaccharide **4,** we tested several building blocks with different protecting groups at 4-OH (R^1) and 6-OH (R^2) (Table 1-1). The protecting group R^2 at 6-OH should be a temporary protecting group. From several protecting groups 4,6-*O*-benzylidene acetal induced reasonably high β-selectivity but the yield of **4a** was moderate (entry 1). Acetyl (Ac) protecting group gave disaccharide **4b** in moderate yield and β-selectivity (entry 2). Both chloroacetyl (ClAc) and *tert*-butyldimethylsilyl (TBS) protecting groups also induced moderate β-selectivity, but yields were very low

(entries 3 and 4). By introducing *tert*-butyldiphenylsilyl (TBDPS) and allyl protecting group we obtained disaccharide **4e** and **4f** in moderate yields (entries 5 and 6), but β-selectivity was 93% by introducing the allyl group (entry 6). We observed the highest yield and selectivity using the Fmoc group as a temporary protecting group (entry 7). In this case disaccharide **4g** (R^2 = H) was ready for the next glycosylation because the Fmoc group at 6-OH was removed in one pot by adding an excess amount of triethylamine after glycosylation. Although the reason has not been revealed yet, disaccharide **4h** was obtained in complete β-selectivity by using **5b** (X = Cl) as a coupling partner (entry 8).

Table 1-1. Stereoselective synthesis of disaccharide building block **4**



entry	**6**	R^1	R^2	**5**	X	**4**	yield (α:β ratio)
1	**6a**	PhCH (acetal)		**5a**	F	**4a**	30% (11:89)
2	**6b**	Bn	Ac	**5a**	F	**4b**	56% (17:83)
3	**6c**	Bn	ClAc	**5a**	F	**4c**	7% (17:83)
4	**6d**	Bn	TBS	**5a**	F	**4d**	8% (15:85)
5	**6e**	Bn	TBDPS	**5a**	F	**4e**	57% (25:75)
6	**6f**	Bn	Allyl	**5a**	F	**4f**	48% (7:93)
7	**6g**	Bn	Fmoc (H)	**5a**	F	**4g**	54% (12:88)[a]
8	**6g**	Bn	Fmoc (H)	**5b**	Cl	**4h**	58% (β only)[a]

[a]2 steps in one pot.

After optimization of the synthesis of disaccharide building block **4**, we assembled the building blocks to prepare pentasaccharide precursors **2a** and **2b** by using automated electrochemical assembly (Figures 1-3 and 1-4).

First I started synthesizing **2a** (Figure 1-3). The process is a combination of three-cycle electrochemical assembly reaction. Monosaccharide building blocks **5a** coupled with donor **3a** in the first cycle, disaccharide building block **4h** in the second cycle and monosaccharide building block **5a** again to prepare pentasaccharide precursors **2a**. In pentasaccharide precursor synthesis the yield was 20% and the average was 58%.

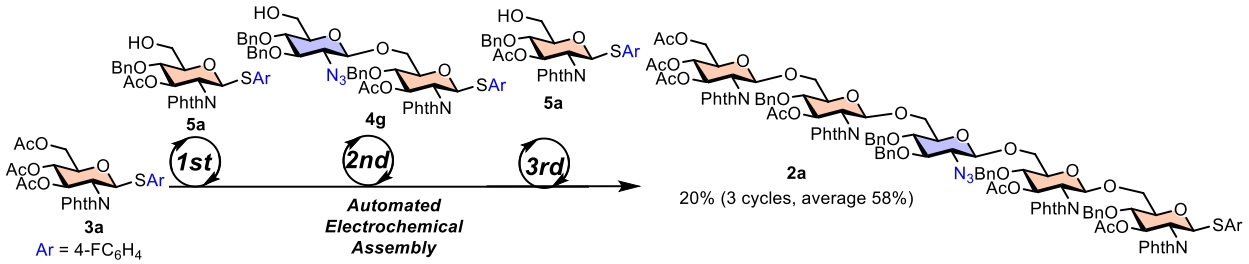


Figure 1-3. Automated electrochemical assembly of 3-TMG-pentaNAG precursor **2a**.

After obtaining **2a** we started the assembly of **2b** using building block **3b** as the donor (Figure 1-4). Monosaccharide building blocks **5b** and disaccharide building block **4h** were added at the first and the second cycles, respectively. In the third cycle of the assembly, we used benzyl group-protected building block **5c**. In these three cycles of reaction, the yield was 33% and the average was 69%.

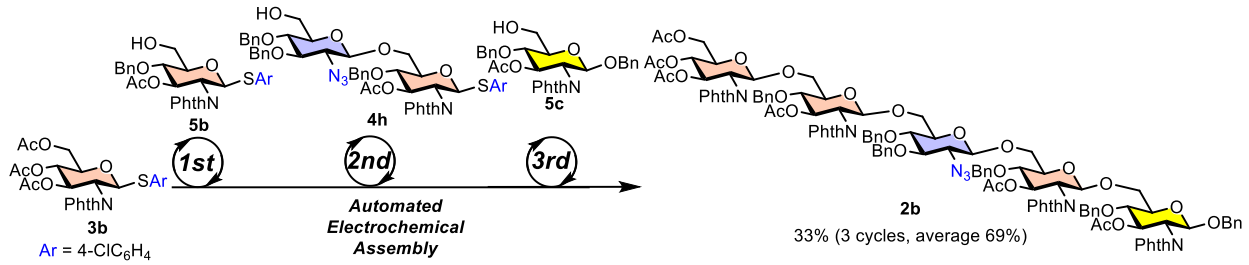


Figure 1-4. Automated electrochemical assembly of 3-TMG-pentaNAG precursors **2b**.

The global deprotection of pentasaccharide precursors **2a** and **2b** were performed according to the reported procedure for the total synthesis of TMG-chitotriomycin (Figure 1-5).[2] For phthalimide deprotection, we refluxed precursor **2a**/**2b** with ethylene diamine and then we acetylated the compound to obtain **7a**/**7b** where the yield was 93% and 81% respectively in two steps. Then we reduced the azido group using 1,3-propane dithiol and triethyl amine. The reduced compound was treated with methyl iodide to get TMG product **8a**/**8b**. This time we obtained 53% and 44% yields respectively using two steps. The final deprotection was started by deacetylation with potassium carbonate to get the deacetylated product. The deacetylated pentasaccharide was treated with palladium hydroxide on activated carbon to get completely deprotected 3-TMG-pentaNAG where the yields were 61% and 50% respectively. The yield was 12.2% for using the benzyl group at anomeric position **2a** and 17.4% from the synthesis of pentasaccharide precursor which had the thioaryl group at its anomeric position **2b**.

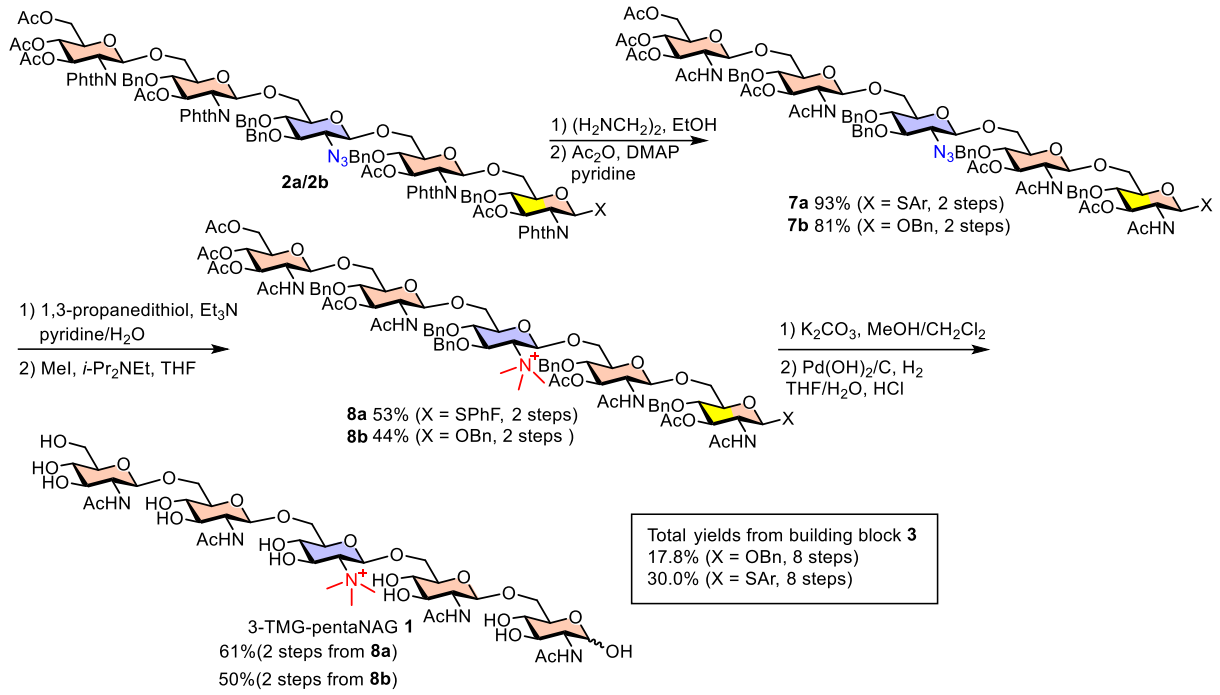


Figure 1-5. Global deprotection of 3-TMG-pentaNAG.

Successful introduction of the TMG part to the pentasaccharide of PNAG encouraged us to prepare TMG-chitotriomycin analogues 2-TMG-chitotriomycin and 3-TMG-chitotriomycin by the same strategy using disaccharide building block (Figure 1-6). Protected precursors of two analogues can be prepared by changing the order of assembling building blocks. The stereoselective synthesis of disaccharide building block is again the key step of total synthesis of the analogues.

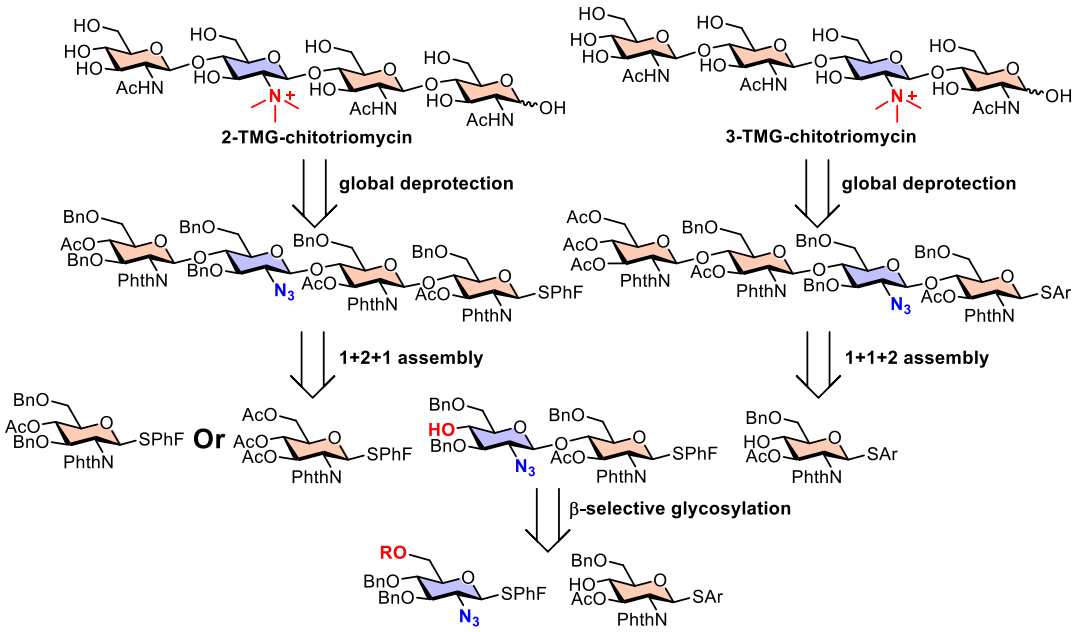
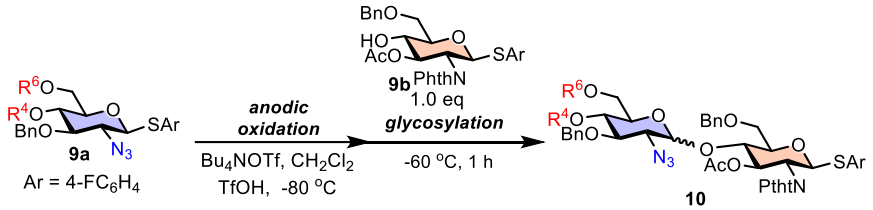


Figure 1-6. Retrosynthetic analysis of 2-TMG-chitotriomycin and 3-TMG-chitotriomycin.

Stereoselective synthesis of disaccharide building block was examined using glycosyl donor with a temporary protecting group at 4-OH (Table 1-2). Initially 4,6-*O*-benzylidene protecting group was used for the reaction; however, the desired disaccharide was obtained in high α-selectivity (entry 1). Then we tested several temporary protecting groups (R^4) at 4-OH of glycosyl donor with benzyl group (R^6 = Bn) at 6-OH (entries 2-5). Although the Fmoc group is useful temporary protecting group which can be removed in one-pot, β-selectivity and yields were moderate (entry 2). Both acetyl and allyl groups afforded the disaccharide in moderate yields and β-selectivity (entries 3 and 4). Among these protecting groups, the methoxymethyl (MOM) group was found to be the best protecting group for R^4 (entry 5). Thus, we also tested the mixed electrolyte system to improve β-selectivity and obtained the desired disaccharide building block **10** up to 90% β-selectivity (entry 6).[11.]

Table 1-2. Stereoselective synthesis of disaccharide building block **3**



entry	R^4	R^6	**9**	yield (%)	**10**	ratio (α:β)
1	PhCH (acetal)		**9b**	34	**10a**	97:3
2	Fmoc	Bn	**9b**	32[a]	**10b**	42:58
3	Ac	Bn	**9b**	66	**10c**	33:67
4	Allyl	Bn	**9b**	57	**10d**	33:67
5	MOM	Bn	**9b**	65[a]	**10e**	23:77
6[b]	MOM	Bn	**9b**	57[a]	**10e**	10:90

[a]Determined by ^{1}H NMR, [b]Mixed electrolyte conditions (Bu_4NOTf/Bu_4NTf_2N = 3:1).

2-TMG-chitotriomycin tetrasaccharide precursor **12a** was synthesized in two cycles of automated electrochemical assembly with the [1+2+1] strategy (Figure 1-7). In this strategy, disaccharide building block **10** and monosaccharide building block **11b** were assembled with monosaccharide building block **11a** sequentially.

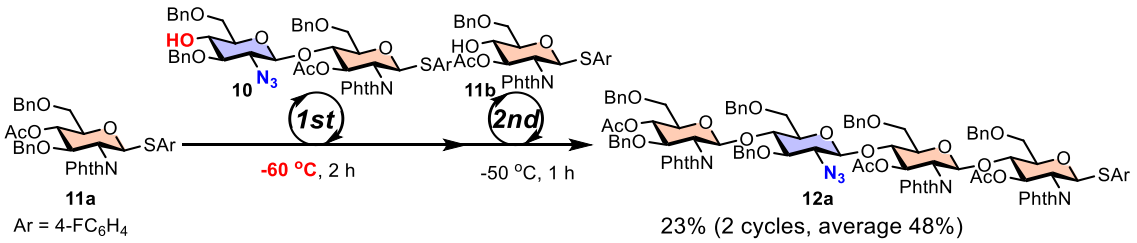


Figure 1-7. Automated electrochemical assembly of 2-TMG-chitotriomycin precursor.

3-TMG chitotriomycin tetrasaccharide precursor **12b** was also synthesized in the same procedure but the disaccharide building block **10** was assembled in the second cycle (Figure 1-8). So, the assembly of the building block followed the 1+1+2 assembling policy. After the automated electrochemical assembly of the precursor of 2-TMG-chitotriomycin and 3-TMG-chitotriomycin, we started global deprotection.

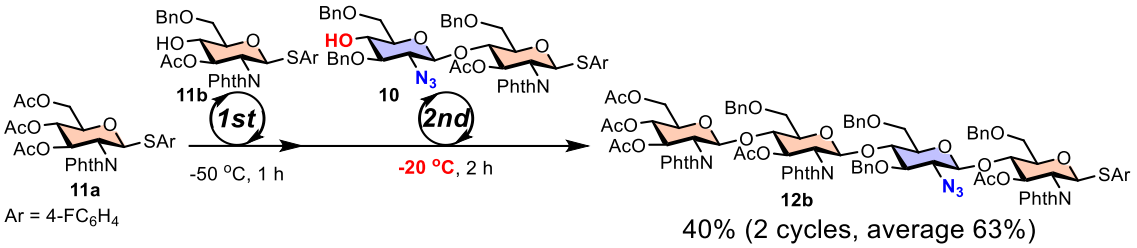


Figure 1-8. Automated electrochemical assembly of 3-TMG-chitotriomycin precursor.

Global deprotection was started with phthalimide deprotection (Figure 1-9). Phthalimide was deprotected by the reflux with ethylene diamine. Then acetylation was done by DMAP, and acetic anhydride produced **12c** and **12d**. The acetylated product was treated with triethyl amine and 1,3-propane dithiol to reduce the azido group. The reduced azido group was methylated to get TMG compounds **12e** and **12f**. The compound was deacetylated using potassium carbonate and after deacetylation, we deprotected the benzyl group and anomeric thio-aryl group by catalytic hydrogenation reaction using palladium hydroxide in activated carbon. Our yields were 21.5% for 2-TMG chitotriomycin from disaccharides and 13.4% for 3-TMG chitotriomycin from disaccharide building block **10**.

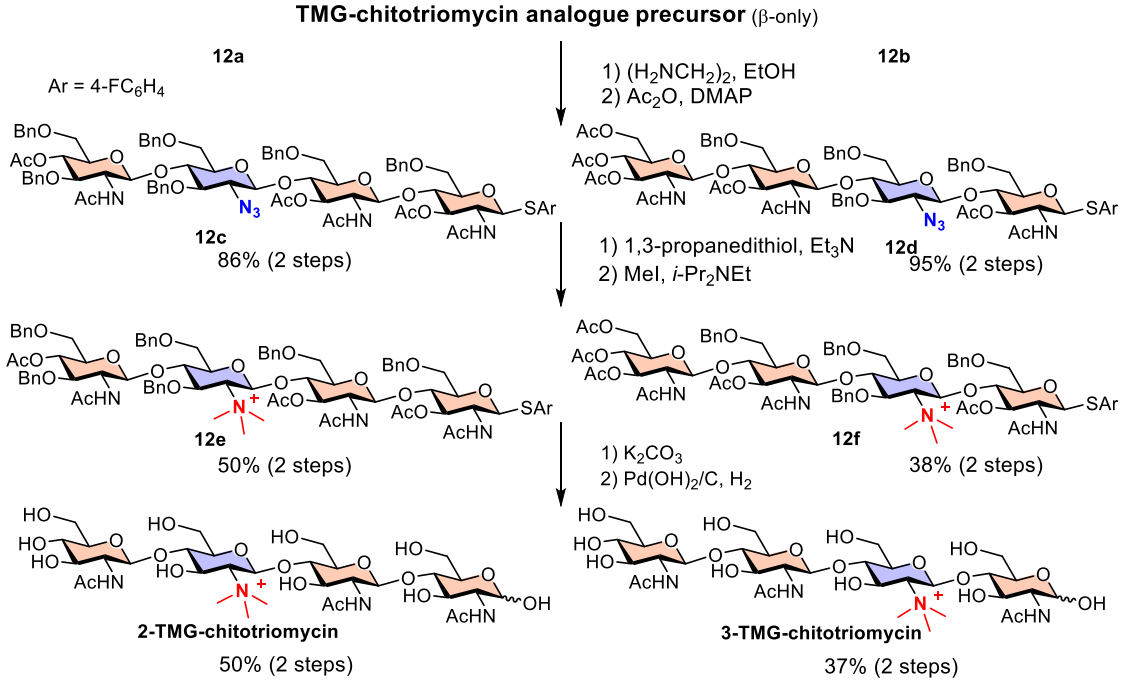


Figure 1-9. Global deprotection of 2-TMG-chitotriomycin and 3-TMG-chitotriomycin.

Conclusion

In conclusion, we have synthesized three analogues of TMG-chitotriomycin by the automated electrochemical assembly. The synthesis of the precursor was confirmed with a good amount of yield and stereoselectivity by the synthesis of two different disaccharide building blocks. Thus, synthesized structurally well-defined pentasaccharide and tetrasaccharide analogues of TMG-chitotriomycin after manipulations of the amino groups and global deprotection. Further investigations into biological activity and a large-scale synthesis are in progress in our laboratory.

References:

(1) Elieh-Ali-Komi, D.; Hamblin, M. R.; Daniel, E.-A.-K. *HHS Public Access*; **2016**; *4*, 411-427.

(2) Isoda, Y.; Sasaki, N.; Kitamura, K.; Takahashi, S.; Manmode, S.; Takeda-Okuda, N.; Tamura, J. ichi; Nokami, T.; Itoh, T. *Beilstein J. Org. Chem.* **2017**, *13*, 919–924.

(3) Usuki, H.; Nitoda, T.; Ichikawa, M.; Yamaji, N.; Iwashita, T.; Komura, H.; Kanzaki, H. *J. Am. Chem. Soc.* **2008**, *130*, 4146–4152.

(4) Horsch, M.; Hoesch, L.; Vasella, A.; Rast, D. M. *Eur. J. Biochem.* **1991**, *197*, 815–818.

(5) Suda, H.; Uotani, K.; Kojima, F.; Aoyama, T.; Horiguchi, K.; Hamada, M.; Takeuchi, T.; Aoyagi, T. *J. Antibiot. (Tokyo).* **1992**, *45*, 1404–1408.

(6) Knapp, S.; Vocadlo, D.; Gao, Z.; Kirk, B.; Lou, J.; Withers, S. G. *J. Am. Chem. Soc.* **1996**, *118* , 6804–6805.

(7) Usuki, H.; Toyo-oka, M.; Kanzaki, H.; Okuda, T.; Nitoda, T. *Bioorg. Med. Chem.* **2009**, *17*, 7248–7253.

(8) Despras, G.; Alix, A.; Urban, D.; Vauzeilles, B.; Beau, J. M. *Angew. Chem. Int. Ed.* **2014**, *53*, 11912–11916.

(9) Bossé, J. T.; Sinha, S.; Li, M. S.; O'Dwyer, C. A.; Nash, J. H. E.; Rycroft, A. N.; Kroll, J. S.; Langford, P. R. *J. Bacteriol.* **2010**, *192*, 2414–2423.

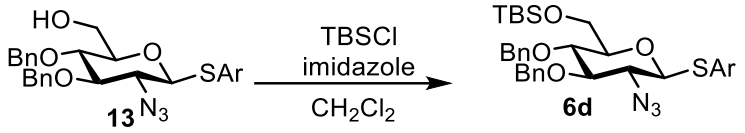
(10) Wang, X.; Preston, J. F.; Romeo, T. *J. Bacteriol.* **2004**, *186*, 2724-2734.

(11) Isoda, Y.; Kitamura, K.; Takahashi, S.; Nokami, T.; Itoh, T. *ChemElectroChem* **2019**, *6*, 4149–4152.

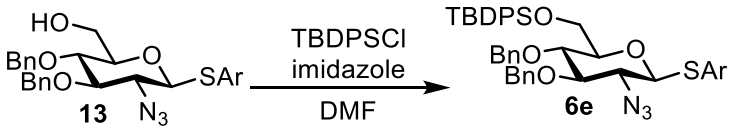
Experimental Section

General

All reactions were carried out under argon atmosphere except notice. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AVANCE II 600 (600 MHz for ^{1}H and 150 MHz for ^{13}C), JEOL JNM-ECZ600 (600 MHz for ^{1}H and 150 MHz for ^{13}C) and JEOL JNM-ECA800 (800 MHz for ^{1}H and 200 MHz for ^{13}C). ESI-MS spectra were recorded on Thermo Scientific Exactive spectrometer. MALDI-TOF MS spectra were recorded on Bruker Ultraflextreme spectrometer. Optical rotation data was recorded on JASCO DIP-370 digital polarimeter. Merck TLC (silica gel 60 F_{254}) was employed for TLC analysis. Gel permeation chromatography (GPC) was used with JAI Labo Ace LC-5060 recycling preparative HPLC (eluent: $CHCl_3$). Kanto silica gel (spherical, neutral, 63-210 μm) and Sephadex LH-20 were used for Silica gel chromatography and gel filtration chromatography, respectively. Compounds **3a**, **3b**, **5a**, **5b**, **5c**, **13** & **15** were synthesized according to the reported[1,2] procedures. Unless otherwise mentioned, all reagents were obtained from commercial suppliers and used without extra purification.

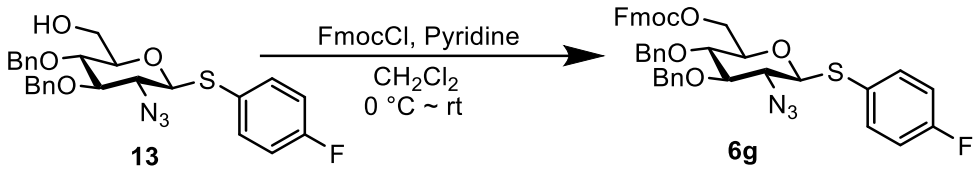


Added imidazole (1.03 mmol, 68 mg), TBSCl (1.5 mmol, 225 mg) and **13** (1.0 mmol, 496 mg) in room temperature for one day. TLC was checked by DCM: MeOH 5:1 eluent and quenched using Na_2CO_3 washed with water and organic part was isolated and dehydrated by Na_2SO_4. Pure 542mg 6d, (0.899 mmol, 89.9%) was obtained by column chromatography (Hexane/EtOAc 5:1) **4-Fluorophenyl-2-azido-3,4-di-*O*-benzyl-6-*O*-(*tert*-butyl)dimethylsilyl-2-deoxy-1-thio-β-D-glucopyranoside (6d)** $[\alpha]_D$ = -7.2807 (*c* = 0.415, $CHCl_3$); TLC (Hexane/EtOAc 5:1) R_f = 0.66; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.60 (dd, *J* = 9.0, 5.4 Hz, 2 H), 7.36–7.26 (m, 10 H), 7.00 (*pseudo*-t, *J* = 9.0 Hz, 2 H), 4.82 (s, 2 H), 4.81 (d, *J* = 10.8 Hz, 1 H), 4.69 (d, *J* = 10.8 Hz, 1 H), 4.32 (d, *J* = 9.6 Hz, 1 H), 3.88 (dd, *J* = 11.4, 12.0 Hz, 1 H), 3.62 (*pseudo*-t, *J* = 9.6 Hz, 1 H), 0.926 (s, 9 H), 0.85 (s, 3 H), 0.11 (s, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 163.2 (d, *J* = 247.64 Hz), 138.1, 137.6, 136.4 (d, *J* = 7.5 Hz), 128.6 128.55, 128.3, , 128.1, 127.8, 125.9 (d, *J* = 2.85 Hz), 116.1 (d, *J* = 21.75 Hz), 80.3, 77.1, 76.0, 75.0, 64.9, 61.8, 25.9, 18.3; HRMS (ESI) *m/z* calculated for $C_{32}H_{40}FN_3O_4SSi$ $[M+Na]^+$,632.2385 , found, 632.2311.

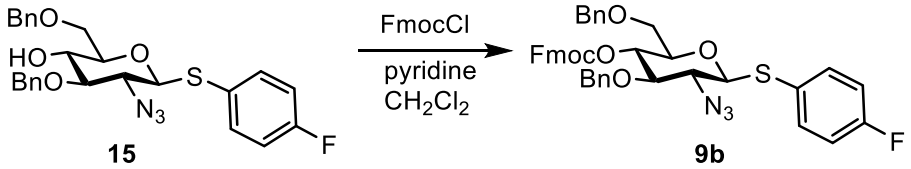


DMF (2.5 mL) was added to compound **13** (0.75 mmol, 375 mg), the temperature was adjusted to 0 °C, TBDPSCl (1.12 mmol, 0.29 mL) and imidazole (1.5 mmol, 110 mg) were added, and the mixture was stirred at room temperature for one day. After that, ethyl acetate was added, and the mixture was separated (1 N-HCl, $NaHCO_3$, H_2O), dehydrated, concentrated, purified by silica gel column chromatography (Hexane/EtOAc 5:1), concentrated, and dried under reduced pressure. As a result, the desired compound **6e** was obtained in a yield of 511 mg, (0.69 mmol, and a yield of 93%) **4-Fluorophenyl-2-azido-2-deoxy-3,4-di-*O*-benzyl-6-*O*-(*tert*-butyl)diphenylsilyl-1-thio-β-D-glucopyranoside (6e)** $[\alpha]_D$ = -62.538 (*c* = 0.308, $CHCl_3$); TLC (Hexane/EtOAc 5:1) R_f = 0.66; ^{1}H NMR ($CDCl_3$, 600 Hz) δ 7.77 (d, *J* = 7.8, Hz, 2 H), 7.69 (d, *J* = 7.8, Hz, 2 H), 7.60 (dd, *J* = 8.4, 6.6 Hz, 2 H), 7.44–7.28 (m, 14 H), 7.15–7.14 (m, 2 H), 6.91

(*pseudo*-t, 8.4 Hz, 2 H), 4.85 (d, *J* = 8.4 Hz, 3 H), 4.68 (d, *J* = 10.8 Hz, 1 H), 4.36 (d, *J* = 10.2 Hz, 1 H), 4.00 (d, *J* = 11.4 Hz, 1 H), 3.94 (dd, *J* = 11.4, 3.0 Hz, 1 H), 3.76 (*pseudo*-t, *J* = 9.0 Hz, 1 H), 3.53 (*pseudo*-t, *J* = 9.0, 1 H), 3.35–3.31 (m, *J* = 9.0 Hz, 2 H), 1.08 (s, 9 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 163.1 (d, *J* = 247.4 Hz), 137.9, 137.6, 136.2 (d, *J* = 8.4 Hz), 135.9, 135.7, 133.3, 132.9, 129.88, 129.86, 128.7, 128.6, 128.4, 128.2, 127.92, 127.89, 127.8, 127.78, 126.1 (d, *J* = 3.2 Hz), 116.2 (d, *J* = 21.6 Hz), 86.0, 85.2, 80.2, 77.2, 75.2, 62.3, 60.4, 26.9, 19.4; HRMS (ESI) *m/z* calculated for $C_{42}H_{44}FN_3O_4SSi$ $[M+Na]^+$, 756.2698, found, 756.2634.

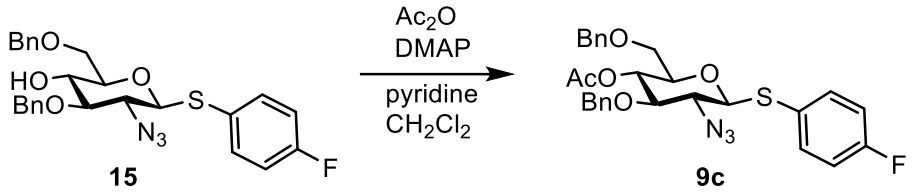


In a stirring solution of compound **13** (3.44 g, 6.94 mmol), dichloromethane (31.3 mL) and pyridine (6.8 ml) FmocCl (2.7 g) was added under argon atmosphere at 0 °C then brought to room temperature. The reaction was quenched after 15 hours. The mixture was diluted with additional dichloromethane (25 mL) and washed with $NaHCO_3$ solution, water, and brine by sequence. The organic layer was dried over Na_2SO_4, and solvent was removed under reduced pressure. The crude product was purified by column chromatography (Hexane/EtOAc 5:1) to obtain **6g** (6.24 mmol, 4.48 g) as white solid in 90% yield. **4-Flurophenyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-6-(9*H*-fluoren-9-ylmethylcarbonate)-1-thio-β-glucopyranoside (6g)** TLC (Hexane/EtOAc 3:1) R_f = 0.60; $[\alpha]_D$ = -32.3199 (*c* = 1.0, $CHCl_3$, 27 °C); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.79–7.77 (m, 2 H), 7.62–7.61 (m, 2 H), 7.59–7.56 (*m*, 2 H), 7.43–7.40 (m, 2 H), 7.35–7.29 (m, 9 H), 7.27–7.25 (m, 3 H), 6.98–6.95 (2 H), 4.89 (d, *J*=10.3 Hz, 1 H), 4.83 (d, *J* = 10.3 Hz, 2 H), 4.57 (d, *J* = 10.9 Hz, 1 H), 4.45–4.43 (m, 3 H), 4.34 (d, *J* = 10.1 Hz, 1 H), 4.28–4.25 (m, 2 H), 3.55–3.51 (m, 2 H), 3.47 (dd, *J* = 14.0, 8.9 Hz, 1 H), 3.27 (dd, *J* = 9.7, 9.2 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 164.1, 162.5, 154.9, 143.33, 143.27, 141.4, 137.4, 137.3, 136.57, 136.52, 128.7, 128.6, 128.3, 128.1, 128.0, 127.3, 125.6, 125.15, 125.12, 120.2, 116.2, 116.1, 85.9, 84.9, 76.0, 75.2, 69.9, 66.3, 64.8, 46.8; HRMS (ESI) *m/z* calcd for $C_{41}H_{36}FN_3O_6S$ $[M+K]^+$, 756.19404; found, 756.1907.

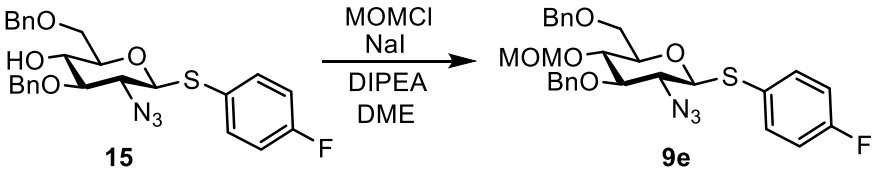


Dry pyridine (1.5 mL) and dichloromethane (6.75 mL) were added to a flask containing **15** (665.4 mg, 1.50 mmol) and stirred. FmocCl (2.27 mmol, 587 mg) was added dropwise at 0 °C. Two hours later, the mixture was turned to room temperature, and one day later, a TLC check (Hexane/EtOAc 5:2) was performed, and sodium bicarbonate (aq) was added to quench the reaction. The organic layer was separated and purified by gel filtration chromatography. After concentration and drying under reduced pressure, the desired **9b** was obtained with a yield of 73% (807 mg, 1.10 mmol).**4-Fluorophenyl-2-azido-3,6-di-*O*-benzyl-4-*O*-fluorenylmethoxycarbonyl-2-deoxy-1-thio-β-D-glucopyranoside (9b)** $[\alpha]_D$ = -4.2881 (*c* = 1.18, $CHCl_3$); TLC (Hexane/EtOAc 2:1) R_f = 0.63; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.74 (dd, *J* = 1.8, 7.8 Hz, 2 H), 7.56 (d, *J* = 7.8 Hz, 2 H), 7.51 (d, *J* = 4.2 Hz, 1 H), 7.39 (*pseudo*-t, *J* = 7.8 Hz, 2 H), 7.25–7.27 (m, 10 H), 6.93

(*pseudo*-t, J = 8.4 Hz, 2 H), 4.79 (*pseudo*-t, J = 9.0 Hz, 1 H), 4.76 (d, J = 11.4 Hz, 1 H), 4.66 (d, J = 10.8 Hz, 1 H), 45.3 (d, J = 12.0 Hz, 1 H), 4.50 (d, J = 11.4 Hz, 1 H), 4.36 (d, J = 10.2 Hz, 2 H), 4.36 (dd, J = 7.2, 10.8 Hz, 2 H), 4.30 (dd, J = 7.8, 10.8 Hz, 2 H), 4.10–4.14 (m, 1 H), 3.60–3.64 (m, 3 H), 3.55 (*pseudo*-t, J = 9.6 Hz, 1 H), 3.31 (*pseudo*-t, J = 9.6 Hz, 2 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 163.2 (d, J = 247.8 Hz), 154.2, 143.2, 143.1, 141.33, 141.30, 137.8, 137.1, 136.3 (d, J = 8.1 Hz), 128.42, 128.40, 128.03, 127.98, 127.8, 127.6, 127.2 (d, J = 7.8 Hz), 125.1, 125.0, 120.1, 116.2 (d, J = 21.6 Hz), 85.9, 82.3, 75.6, 75.0, 73.6, 70.2, 69.2, 64.5, 60.4, 46.7, 21.1, 14.2; HRMS (ESI) *m/z* calcd for $C_{41}H_{37}FN_3O_6S$ $[M+Na]^+$, 740.2201; found, 740.2202.



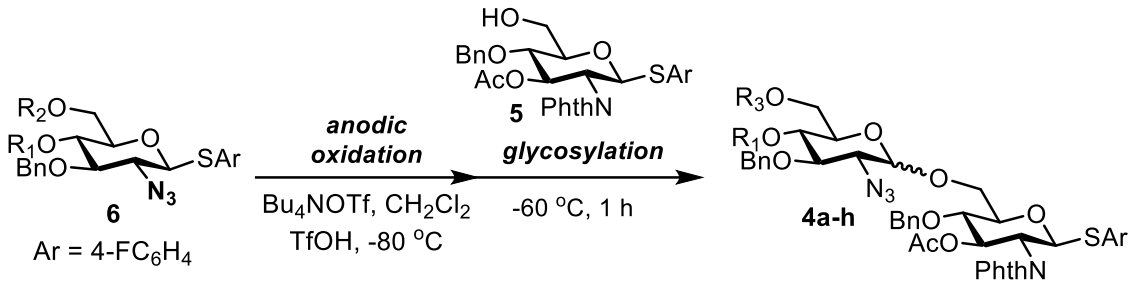
DMAP (20.8 mg, 0.17 mmol) was added to an eggplant flask containing **15** (665 mg, 1.34 mmol), pyridine (1.6 mL) and DCM (7.6 mL) at 0° C. Ac_2O (1.0 mL) was added dropwise. After returning to room temperature, the mixture was stirred for 1 day. After azeotropic distillation, recrystallization was performed with hexane and ethyl acetate. After drying under reduced pressure, the desired **9c** was obtained with a yield of 75% (537.9 mg, 1.0 mmol).**4-Fluorophenyl 4-acety-2-azido-3,6-di-*O*-benzyl-2-deoxy-1-thio-β-D-glucopyranoside (9c)** $[\alpha]_D$ = -6.48 (c = 1.05, $CHCl_3$); TLC (Hexane/EtOAc 2:1) R_f = 0.54; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.56 (dd, J = 5.4, 9.0 Hz, 2 H), 7.26–7.37 (m, 10 H), 6.93 (*pseudo*-t, J = 9.0 Hz, 2 H), 4.899–4.941 (m, 2 H), 4.80 (d, J = 10.8 Hz, 1 H), 4.63 (d, J = 11.4 Hz, 1 H), 4.53 (d, J = 11.4 Hz, 1 H), 4.50 (d, J = 11.4 Hz, 1 H), 4.37 (d, J = 10.2 Hz, 1 H), 3.51–3.56 (m, 3 H), 3.49 (*pseudo*-t, J = 9.0 Hz, 1 H), 3.33 (*pseudo*-t, J = 9.6 Hz, 1 H), 1.87 (s, 1 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 166.8 (d, J = 8.4 Hz), 137.8, 137.3, 136.3 (d, J = 8.6 Hz), 128.5, 128.4, 128.06, 128.02, 127.8, 125.5 (d, J = 3.5 Hz), 116.2 (d, J = 21.8 Hz), 85.8, 82.5, 77.6, 75.4, 70.5, 69.4, 64.6, 20.8; HRMS (ESI) *m/z* calcd for $C_{28}H_{28}FN_3O_5S$ $[M+Na]^+$, 560.1626, found, 560.1618.



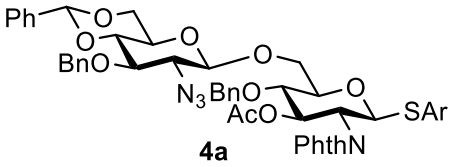
DIPEA (6.0 mL) was added to eggplant flask containing **15** (743 mg, 1.50 mmol), DME (6.0 mL) was added, and the mixture was stirred for 1 hour, NaI (899 mg, 6.0 mmol) and MOMCl (0.57 mL, 7.5 mmol) again DME (2.1 mL) was added, and the mixture was stirred for 10 min. TLC check was performed (Hexane/EtOAc 5:2) and sodium carbonate was added to stop the reaction. The organic layer was separated and after dehydration, the residue was filtered, concentrated, and subjected to column chromatography (Hexane/EtOAc 2:1). After concentration and drying under reduced pressure, the desired product **9e** was obtained with a yield of 88% (718 mg, 1.33 mmol). **4-Fluorophenyl 2-azido- 3,6-di-*O*-benzyl-4-methodxymethyl-2-deoxy-1-thio-β-D-glucopyranoside (9e)** $[\alpha]_D$ = -2.17 (c = 0.92, $CHCl_3$); TLC (Hexane/EtOAc 2:1) R_f = 0.57; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.58 (dd, J = 4.8, 8.4 Hz, 2 H), 7.37-7.29 (m, 10 H), 6.91 (*pseudo*-t, J = 9.0 Hz, 2 H), 4.85 (d, J = 10.2 Hz, 1 H), 4.79 (d, J = 6.0 Hz, 1 H), 4.76 (d, J

= 10.8 Hz, 1 H), 4.64 (d, J = 6.0 Hz, 1 H), 4.61 (d, J = 12.0 Hz, 1 H), 4.57 (d, J = 11.4 Hz, 1 H), 4.34 (d, J = 9.6 Hz, 1 H), 3.81 (dd, J = 1.8, 10.8 Hz, 1 H), 3.69 (dd, J = 4.8, 10.8 Hz, 1 H), 3.52 (*pseudo*-t, J = 9.6 Hz, 1 H), 3.46 (ddd, J = 1.8, 4.8, 9.6 Hz, 1 H), 3.43 (*pseudo*-t, J = 9.6 Hz, 1 H), 3.29 (s, 3 H), 3.25 (*pseudo*-t, J = 9.6 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 163.1 (d, J = 247.5 Hz) 138.2, 137.4, 136.2 (J = 8.4 Hz) 128.5, 128.4, 128.2, 128.1, 127.7, 127.6, 125.8 (d, J = 3.2 Hz), 116.1 (d, J = 21.0 Hz), 98.4, 85.7, 84.8, 79.0, 75.82, 75.76, 73.4, 69.0, 64.8, 56.5; HRMS (ESI) *m/z* calcd for $C_{28}H_{30}FN_3O_5S$ $[M+Na]^+$, 562.1782; found, 562.1785.

Disaccharide synthesis was carried out according to the following procedure.

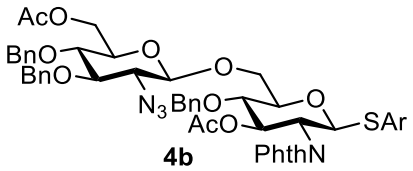


The dried MS4A was put into 100 mL and 10 mL eggplant flasks respectively, 40 mL of CH_2Cl_2 was added to the 100 mL eggplant flask, and 1.2 mL of CH_2Cl_2 and building block **5** (0.25 mmol) were added to the 15 mL eggplant flask. A supporting salt (1.0 mmol) was added to both electrodes and building block **6** (0.25 mmol) was added to the anode. CH_2Cl_2 (10 mL) was added to each electrode, and TfOH (0.25 mmol) was added to the cathode. 1.0 mL of CH_2Cl_2 was sucked up from the 10 mL eggplant-flask with the gas-tight syringe (A), and 1 mL of CH_2Cl_2 was sucked up from the 100 mL eggplant-flask with the gas-tight syringe (B). (A) was set as the anode and (B) was set as the cathode, stirred, and 1 cycle of electric current was applied. Et_3N was added in an amount of 0.3 mL to each.



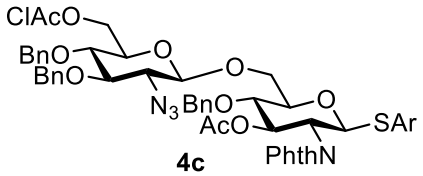
4-Fluorophenyl-2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl-(1→6)-3-*O*-acetyl-4-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (4a) $[\alpha]_D$ = -21.739 (c = 0.92 , $CHCl_3$); TLC (Hexane/EtOAc 2:1) R_f = 0.278; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.88–7.87 (m, 2 H), 7.76–7.74 (m, 2 H), 7.44–7.29 (m, 14 H), 7.28 (d, J = 6.6 Hz, 1 H), 7.24 (d, J = 7.8 Hz, 2 H), 6.97 (*pseudo*-t, J = 8.4 Hz, 2 H), 5.80 (td, J = 10.8, 1.2 Hz, 1 H), 5.68 (dd, J = 10.8, 1.8 Hz, 1 H), 5.6 (s, 1 H), 4.95 (d, J = 10.8 Hz, 1 H), 4.82 (d, J = 11.4 Hz, 1 H), 4.67 (s, 2 H), 4.37 (dd, J = 10.8, 4.8 Hz, 1 H), 4.35 (dd, J = 7.8, 1.2 Hz, 1 H), 4.21 (td, J = 10.8, 1.8 Hz, 1 H), 4.15 (d, J = 11.4 Hz, 1 H), 3.85 (dd, J = 11.4, 4.8 Hz, 1 H), 3.82–3.78 (m, 2 H), 3.73 (*pseudo*-t, J = 9.0 Hz, 2 H), 3.60 (td, J = 9.0, 1.2 Hz, 1 H), 3.50 (td, J = 9.6, 1.2 Hz, 1 H), 3.34 (td, J = 9.6, 3.6 Hz, 1 H), 1.76 (s, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.1, 167.9, 167.3, 163.0 (d, J = 247.7 Hz), 137.8, 137.1, 136.0 (d, J = 7.95 Hz), 134.5, 134.2, 131.8, 131.2, 129.12, 128.54, 128.45, 128.3, 128.28, 127.97, 127.9, 127.5, 126.2 (d, J = 3.15 Hz), 126.0, 116.1 (d, J = 21.6 Hz), 102.7, 101.4,

83.1, 81.6, 79.2, 78.7, 76.5, 75.0, 74.6, 74.1, 68.8, 68.6, 66.3, 66.2, 50.0, 20.6; HRMS (ESI) *m/z* calcd for $C_{49}H_{45}FN_4O_{11}S$ $[M+Na]^+$, 939.2681; found, 939.2695.



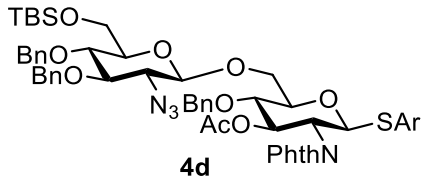
4b

4-Fluorophenyl 6-*O*-acetyl-3,4-di-*O*-benzyl-2-deoxy-2-adido-β-D-glucopyranosyl-(1→6)-3-*O*-acetyl-4-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (4b) $[\alpha]_D$ = 18.837 (*c* = 0.86, $CHCl_3$); TLC (Hexane/EtOAc 2:1): R_f = 0.397; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.87–7.83 (m, 2 H), 7.77–7.72 (m, 2 H), 7.44–7.41 (m, 2 H), 7.39–7.24 (m, 15 H), 6.92 (*pseudo*-t, *J* = 9.0 Hz, 2 H), 5.83–5.75 (m, 1 H), 5.72 (dd, *J* = 9.0, 10.2 Hz, 1 H), 5.62 (d, *J* = 10.2 Hz, 1 H), 5.17 (dd, *J* = 17.4, 1.8 Hz, 1 H), 5.13 (s, 1 H), 5.09 (dd, *J* = 10.8, 1.8 Hz, 1 H), 4.79 (d, *J* = 8.4 Hz, 1 H), 4.76 (d, *J* = 10.8 Hz, 1 H), 4.69 (d, *J* = 12.0 Hz, 1 H), 4.555 (d, *J* = 12.0 Hz, 1 H), 4.552 (s, 1 H), 4.45 (d, *J* = 11.4 Hz, 1 H), 4.41 (d, *J* = 12.0 Hz, 1 H), 4.21–4.16 (m, 3 H), 4.02–3.93 (m, 4 H), 3.87 (dd, *J* = 10.8, 1.2 Hz, 1 H), 3.82 (d, *J* = 4.2 Hz, 1 H), 3.79–3.76 (m, 2 H), 3.72 (d, *J* = 10.2 Hz, 1 H), 3.82–3.78 (m, 2 H), 3.73 (*pseudo*-t, *J* = 9.0 Hz, 2 H), 3.60 (td, *J* = 9.0, 1.2 Hz, 1 H), 3.50 (td, *J* = 9.6, 1.2 Hz, 1 H), 3.34 (td, *J* = 9.6, 3.6 Hz, 1 H), 1.76 (s, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.72, 170.10, 167.89, 167.33, 163.1 (d, *J* = 247.2 Hz), 137.78, 137.73, 137.46, 136.0 (d, *J* = 8.55 Hz), 134.49, 134.23, 131.74, 131.22, 128.62, 128.59, 128.55, 128.16, 128.14, 128.06, 127.94, 127.55, 126.1 (d, *J* = 3.15 Hz), 123.74, 123.59, 116.0 (d, *J* = 21.75 Hz), 102.17, 83.35, 82.99, 78.7, 77.3, 76.7, 75.7, 75.2, 74.6, 74.1, 73.1, 68.5, 66.3, 62.7, 54.0, 20.9, 20.6; HRMS (ESI) *m/z* calcd for $C_{51}H_{49}FN_4O_{12}S$ $[M+Na]^+$, 983.2944; found, 983.2973.

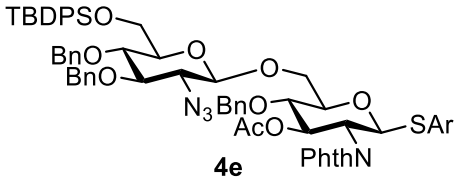


4c

4-Fluorophenyl-2-azido-3,4-di-*O*-benzyl-6-chloroacetyl-2-deoxy-β-D-glucopyranosyl-(1→6)-3-*O*-acetyl-4-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (4c) $[\alpha]_D$ = 22.151 (*c* = 0.95 , $CHCl_3$); TLC (Hexane/EtOAc 2:1) R_f = 0.29; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.88–7.87 (m, 2 H), 7.76–7.75 (m, 2 H), 7.43–7.27 (m, 18 H), 7.24 (d, *J* = 6.6 Hz, 2 H), 6.97 (*pseudo*-t, *J* = 8.4 Hz, 2 H), 5.79 (dd, *J* = 10.2, 9.0 Hz, 1 H), 5.68 (d, *J* = 10.8 Hz, 1 H), 4.94 (d, *J* = 10.8 Hz, 1 H), 4.88 (d, *J* = 10.8 Hz, 1 H), 4.84 (d, *J* = 10.8 Hz, 1 H), 4.65 (s, 1 H), 4.61 (d, *J* = 10.8 Hz, 1 H), 4.45 (dd, *J* = 12.0, 1.8 Hz, 1 H), 4.29 (d, *J* = 7.8 Hz, 1 H), 4.23 (dd, *J* = 11.4, 4.8 Hz, 1 H), 4.20 (*pseudo*-t, *J* = 10.2 Hz, 1 H), 4.15 (d, *J* = 9.6 Hz, 1 H), 3.82–3.79 (m, 2 H), 3.68 (*pseudo*-t, *J* = 9.0 Hz, 1 H), 3.55 (*pseudo*-t, *J* = 8.4 Hz, 1 H), 3.46–3.41 (m, 3 H), 1.76 (s, 3 H), 1.56 (s, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.1, 167.0, 163.1 (d, *J* = 248.1 Hz), 137.7, 137.6, 137.4, 135.9 (d, *J* = 7.95 Hz), 134.5, 134.2, 131.2, 128.64, 128.59, 128.54, 128.22, 128.16, 128.09, 127.94, 127.5, 126.2 (d, *J* = 3.4 Hz), 123.7, 123.6, 116.0 (d, *J* = 21.75 Hz), 102.2, 83.2, 83.1, 78.7, 76.76, 76.67, 75.7, 74.6, 74.1, 72.8, 68.6, 66.3, 64.2, 54.05, 40.7, 20.53; HRMS (ESI) *m/z* calcd for $C_{51}H_{48}ClFN_4O_{12}S$ $[M+Na]^+$,1017.2554; found, 1017.2582.

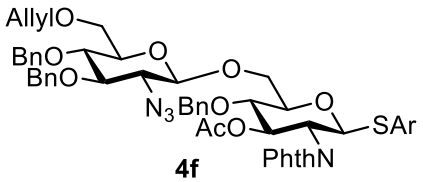


4-Fluorophenyl-2-azido 3,4-*O*-benzyl-6-*O*-(tert-butyl)dimethylsilyl-2-deoxy-β-D-glucopyranosyl-(1→6)-3-*O*-acetyl-4-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (4d) $[\alpha]_D$ = 0.999 (*c* = 1.0, $CHCl_3$); TLC (Hexane/EtOAc 2:1) R_f = 0.428; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.81–7.78 (m, 2 H), 7.68–7.66 (m, 2 H), 7.36–7.16 (m, 17 H), 6.891 (*pseudo*-t, *J* = 8.4 Hz, 2 H), 5.74–5.71 (m, 1 H), 5.60 (d, *J* = 10.2 Hz, 1 H), 4.781 (d, *J* = 10.8 Hz, 1 H), 4.776 (d, *J* = 10.8 Hz, 1 H), 4.66 (d, *J* = 10.8 Hz, 1 H), 4.61 (d, *J* = 13.2 Hz, 1 H), 4.58 (d, *J* = 13.2 Hz, 1 H), 4.19 (dd, *J* = 6.0, 3.0 Hz, 1 H), 4.13 (*pseudo*-t, *J* = 10.8 Hz, 1 H), 4.10 (d, *J* = 10.2 Hz, 1 H), 3.83 (d, *J* = 3.0 Hz, 1 H), 3.72–3.70 (m, 3 H), 3.64 (*pseudo*-t , *J* = 9.6 Hz, 1 H), 3.35 (d, *J* = 3.6 Hz, 1 H), 3.34 (d, *J* = 2.4 Hz, 1 H), 3.16 (d, *J* = 3.0 Hz, 1 H), 1.67 (s, 3 H), 0.84 (s, 9 H), 0.01 (s, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 169.1, 166.8, 166.3, 162.1 (d, *J* = 247.05 Hz) 137.2, 136.9, 134.9 (d, *J* = 8.25 Hz) 133.4, 133.1, 130.7, 130.2, 127.49, 127.45, 127.2, 126.9, 126.5, 125.1 (d, *J* = 3.3 Hz) 122.7, 122.5, 114.9 (d, *J* = 21.6 Hz), 101.0, 82.1, 82.0, 77.7, 76.4, 74.9, 74.7, 74.1, 73.6, 73.1, 67.1, 65.4, 60.8, 53.0, 28.7, 24.90, 19.5, 17.3; HRMS (ESI) *m/z* calcd for $C_{55}H_{61}FN_4O_{11}SSi$ $[M+Na]^+$, 1055.3703; found, 1055.3750.



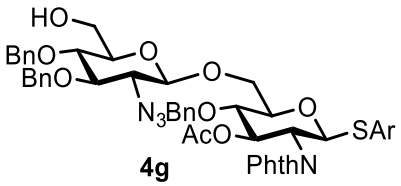
4-Fluorophenyl-2-azido-3,4-di-*O*-benzyl-6-*O*-(tert-butyl)diphenylsilyl-2-deoxy-β-D-glucopyranosyl-(1→6)-3-*O*-acetyl-4-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (4e)

$[\alpha]_D$ = -2.52 (*c* = 0.87, $CHCl_3$); TLC (Hexane/EtOAc 2:1) R_f = 0.44; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.88–7.85 (m, 2 H), 7.75-7.65 (m, 7 H), 7.44–7.18 (m, 31 H), 6.92 (*pseudo*-t, *J* = 8.4 Hz, 2 H), 5.83 (td, *J* = 9.0, 1.2 Hz, 1 H), 5.74 (dd, *J* = 10.8, 1.2, Hz, 1 H), 4.93–4.90 (m, 3 H), 4.87 (d, *J* = 10.8 Hz, 1 H), 4.76 (d, *J* = 10.8 Hz, 1 H), 4.69 (d, *J* = 11.4 Hz, 1 H), 4.46 (d, *J* = 11.4 Hz, 1 H), 4.28–4.25 (m, 2 H), 4.21 (*pseudo*-t, *J* = 10.8 Hz, 1 H), 3.98–3.81 (m, 6 H), 3.76 (*pseudo*-t, *J* = 9.0 Hz, 2 H), 3.73 (dd, *J* = 10.8, 1.6 Hz, 1 H), 3.51 (dd, *J* = 9.6, 2.4 Hz, 1 H), 3.45 (*pseudo*-t, *J* = 9.6 Hz, 1 H), 3.28 (d, *J* = 8.4 Hz, 1 H), 1.74 (s, 3 H), 1.06 (s, 9 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.1, 168.0, 167.3, 163.0 (d, *J* = 247.2 Hz), 138.1, 138.0, 137.9, 137.8, 136.8 (d, *J* = 8.1 Hz), 136.2 (d, *J* = 8.25 Hz), 135.93, 135.89, 135.6, 134.5, 134.2, 133.61, 133.55, 133.1, 132.8, 131.8, 131.3, 129.8, 129.7, 128.59, 128.54, 128.51, 128.48, 128.46, 128.3, 128.03, 128.01, 127.89, 127.85, 127.82, 127.80, 127.77, 127.74, 127.67, 127.55, 127.4, 125.9 (d, *J* = 5.85 Hz), 116.1 (d, *J* = 21.75 Hz), 116.0 (d, *J* = 21.6 Hz), 101.99, 97.8, 83.3, 83.0, 82.4, 80.3, 79.1, 78.7, 78.3, 77.6, 76.6, 76.4, 76.0, 75.8, 75.6, 75.22, 75.19, 74.7, 74.5, 74.22, 74.16, 72.2, 67.5, 67.4, 63.8, 62.5, 62.4, 54.1, 54.0, 26.9, 26.8, 20.5, 19.4, 19.3; HRMS (ESI) *m/z* calcd for $C_{65}H_{65}FN_4O_{11}SSi$ $[M+Na]^+$, 1179.4016; found, 1179.4058.

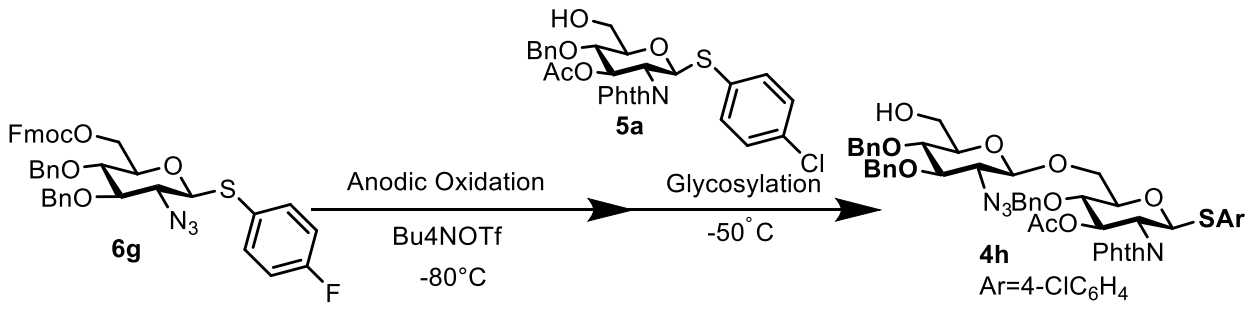


4-fluorophenyl 6-allyl-2azido-3,4-di-*O*-benzyl-2-deoxyβ-D-glucopyranosyl-(1→6)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy2-phthalimido-1-thio-β-D-glucopyranoside (4f)

TLC (Hexane/EtOAc 2:1): R_f 0.397; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.84 (s, 2 H), 7.73–7.72 (m, 2 H), 7.44 (dd, *J* = 8.4, 5.4 Hz, 2 H), 7.42–7.24 (m, 15 H), 5.91–5.86 (m, 1 H), 5.82 (dd, *J* = 9.6, 2.4 Hz, 1 H), 5.70 (d, *J* = 10.2 Hz, 1 H), 5.28 (ddd, *J* = 18.6, 3.0, 1.2 Hz, 1 H), 5.17 (dd, *J* = 10.8, 1.8 Hz, 1 H), 4.91 (d, *J* = 10.8 Hz, 1 H), 4.87 (d, *J* = 10.8 Hz, 1 H), 4.84 (d, *J* = 10.8 Hz, 1 H), 4.68 (d, *J* = 11.4 Hz, 1 H), 4.67 (d, *J* = 10.8 Hz, 1 H), 4.65 (d, *J* = 12.0 Hz, 1 H), 4.29 (d, *J* = 7.2 Hz, 1 H), 4.21 (d, *J* = 7.2 Hz, 1 H), 4.21 (*pseudo*-t, *J* = 10.2 Hz, 1 H), 4.14 (d, *J* = 6.6 Hz, 1 H), 3.84 (dd, *J* = 11.4, 5.4 Hz, 1 H), 3.83 (dd, *J* = 15.6, 6.0 Hz, 1 H), 3.80 (dd, *J* = 5.4, 1.8 Hz, 1 H), 3.74 (*pseudo*-t, *J* = 9.6 Hz, 1 H), 3.71–3.67 (m, 3 H), 3.46 (*pseudo*-t, *J* = 7.8 Hz, 1 H), 3.43 (*pseudo*-t, *J* = 9.6 Hz, 1 H), 3.38 (ddd, *J* = 12.0, 4.8, 2.4 Hz, 1 H), 1.74 (s, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 171.13, 170.10, 167.9, 167.3, 163.1 (d, *J* = 247.5 Hz), 138.1, 137.98, 137.90, 136.1 (d, *J* = 9.0 Hz), 134.6, 134.5, 134.2, 132.3, 131.7, 131.3, 128.5, 128.11, 128.08, 127.94, 127.88, 127.6, 126.0 (d, *J* = 3.0 Hz), 123.7, 123.6, 117.3, 116.0 (d, *J* = 21.0 Hz), 102.4, 83.3, 82.7, 78.8, 77.8, 76.6, 75.6, 75.2, 75.1, 74.6, 74.2, 72.6, 68.6, 68.5, 66.4, 60.4, 54.1, 20.5; HRMS (ESI) *m/z* calcd for $C_{52}H_{51}ClFN_4O_{11}S$ $[M+Na]^+$, 997.2891; found, 997.2824.

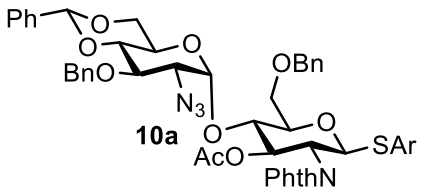


β-D-Glucopyranoside, 4-Fluorophenyl 6-*O*-[2-azido-2-deoxy-3,4-bis-*O*-(phenylmethyl)-β-D-glucopyranosyl]-2-deoxy-2-(1,3-dihydro-1,3-dioxo-2*H*-isoindol-2-yl)-6-*O*-(phenylmethyl)-1-thio-3-acetate. (4g); TLC (Hexane/EtOAc 2:1) R_f = 0.35; $[\alpha]_D$ = 22.15 (*c* = 0.105, $CHCl_3$, 27 °C); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.86 (dd, *J* = 5.4, 3.0 Hz, 2 H), 7.75 (d, *J*= 5.4 Hz, 2 H), 7.43–7.26 (m, 19 H), 6.97 (*pseudo*-t, *J* = 8.4Hz, 2 H), 5.79 (dd, *J* = 10.2, 8.4Hz, 1 H), 5.69 (d, *J* = 10.2 Hz, 1 H), 4.91 (d, *J*= 10.8 Hz, 1 H), 4.86 (*pseudo*-t, *J* = 10.8 Hz, 2 H), 4.67 (d, *J* = 10.2 Hz, 3 H), 4.32 (dd, *J* = 5.4, 2.4 Hz, 1 H), 4.22 (*pseudo*-t, *J* = 10.2 Hz, 1 H), 3.86 (d, *J* = 10.8 Hz, 1 H), 3.85 (d, *J* = 11.4 Hz, 1 H), 3.78 (ddd, *J* = 9.6, 4.2, 1.2 Hz, 1 H), 3.74 (d, *J* = 8.4 Hz, 1 H), 3.72 (d, *J* = 6.0 Hz, 1 H), 3.62–3.59 (m, 1 H), 3.45 (d, *J* = 6.0 Hz, 1 H), 3.44 (d, *J* = 6.0 Hz, 1 H), 3.32 (ddd, *J* = 9.6, 3.6, 1.2 Hz, 1 H), 1.76 (s, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 171.2, 170.4, 170.1, 167.9, 167.3, 163.1 (d, *J*= 247.4 Hz), 137.83, 137.77, 137.7, 137.5, 135.8 (d, *J*= 8.4 Hz), 134.5, 134.2, 131.7, 131.2, 128.7, 128.6, 128.5, 128.13, 128.07, 128.0, 127.9, 127.8, 127.6, 123.7 (d, *J* = 24.9 Hz), 116.1 (d, *J* = 21.8 Hz), 102.3, 101.9, 83.3, 83.0, 81.7, 78.8, 77.5, 76.5, 76.4, 75.7, 75.4, 75.1, 74.6, 74.1, 71.8, 70.9, 70.8, 68.7, 67.8, 66.5, 61.8, 60.4, 56.1, 54.1, 31.9, 31.0, 30.4, 27.0, 26.5, 21.1, 20.7, 20.5, 14.2; HRMS (ESI) *m/z* calcd for $C_{49}H_{47}ClKN_{44}O_{11}S$ $[M+K]^+$, 957.2542; found, 957.2577.



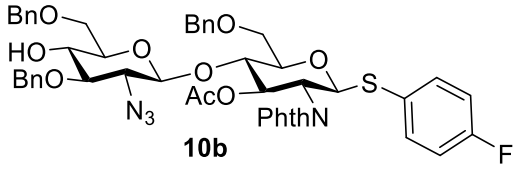
0.287 g **6g** was taken into the cell anode of an electrolysis cell (H-type) and 0.626 g Electrolyte (Bu_4NOTf) was taken to both parts. The cell was vacuumed overnight and then taken under argon atmosphere. After vacuuming solvent dichloromethane (16 mL) was added to both anode and cathode and TfOH (36 μL) was added to cathode. An acceptor solution was made by this time with (0.227 g **5a** in 1 mL of dichloromethane).

The synthesizer was started for one cycle with 12 mA and 1.05 F/mol. Acceptor solution (0.227 g **5a** in 1ml of dichloromethane) was injected by the synthesizer. After the completion of synthesizer 1.0 mL triethylamine was added to both anode and cathode to quench the reaction. Completion of the reaction was confirmed by TLC. Taken the whole solution and then removed all the solvent by rotary evaporator and vacuumed line. The weight of the crude product was 2.02 g. Column chromatography was done for purification. The weight of the pure product is 0.217 g (0.232 mmol, 58%). **β-D-Glucopyranoside, 4-Chlororophenyl 6-*O*-[2-azido-2-deoxy-3,4-bis-*O*-(phenylmethyl)-β-D-glucopyranosyl-2-deoxy-2-(1,3-dihydro-1,3-dioxo-2*H*-isoindol-2-yl)-6-*O*-(phenylmethyl)-1-thio-,3-acetate (4h);** TLC (Hexane/EtOAc 2:1) R_f = 0.33; $[\alpha]_D$ = 1.071 (*c* = 1.12, $CHCl_3$, 26 °C); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.86–7.84 (m, 2 H), 7.74–7.73 (m, 2 H), 7.40–7.27 (m, 14 H), 7.24–7.22 (m, 5 H), 5.81 (*pseudo*-t, *J* = 10.2 Hz, 1 H), 5.74 (d, *J*= 10.2 Hz, 1 H), 4.91 (d, *J*=10.8 Hz, 1 H), 4.85 (dd, *J* = 10.8, 4.8 Hz, 2 H), 4.68 (*pseudo*-t, *J* = 9.6 Hz, 3 H), 4.32 (dd, *J*= 3.8, 1.5 Hz, 1 H), 4.25 (*pseudo*-t, *J* = 10.5 Hz, 1 H), 4.11 (dd, *J*= 12.0, 1.5 Hz, 1 H), 3.86 (dd, *J*= 11.4, 4.8 Hz, 2 H), 3.81–3.78 (m, 1 H), 3.75 (d, *J* = 8.7 Hz, 1 H), 3.73–3.70 (m, 1 H), 3.61–3.58 (m, 1 H), 3.44–3.43 (m, 2 H), 3.32–3.30 (m, 1 H), 1.95 (*pseud*o-t, *J* = 6.0 Hz, 1 H), 1.76 (s, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 169.9, 169.8, 167.2, 137.8,137.7, 137.6, 134.7, 134.6, 134.5, 134.3 (d, *J* = 1.9 Hz), 134.1, 131.6, 131.1, 129.8, 129.1, 129.0, 128.51, 128.46, 128.45, 128.0, 127.91, 127.89, 127.88, 127.7, 127.5, 123.6, 123.5, 102.2, 82.9, 78.7, 77.3, 76.3, 75.5, 75.3, 75.0, 74.5, 74.0, 68.5, 66.3, 61.6, 53.9, 20.4; HRMS (ESI) *m/z* calcd for $C_{49}H_{47}ClN_{44}O_{11}S$ $[M+Na]^+$, 957.2543; found, 957.2554.



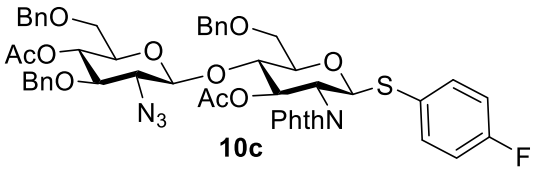
4-Fluorophenyl-2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-α-D-glucopyraniside (10a) $[\alpha]_D$ = 0.7407 (*c* = 0.81, $CHCl_3$); TLC (Hexane/EtOAc 5:3) R_f = 0.53; ^{1}H NMR ($CDCl_3$, 600MHz) δ 7.90 (d, *J* = 6.0 Hz, 1 H), 7.86 (d, *J* = 7.2 Hz, 1 H), 7.73–7.78 (m, 2 H), 7.49 (dd, *J* = 7.8 ,1.8 Hz, 2 H), 7.49 (dd, *J* = 7.8, 1.8 Hz, 2 H), 7.37–7.44 (m, 9 H), 7.27–7.35 (m, 6 H), 6.89 (*pseudo*-t, *J* = 9.0 Hz, 2 H), 5.8 (*pseudo*-t, *J* = 9.0 Hz, 2 H), 5.70 (d, *J* = 10.2 Hz, 2 H), 5.56 (s, 1 H), 5.12 (d, *J* = 3.6 Hz, 1 H), 4.92 (d, *J* = 10.8 Hz, 1 H), 4.74 (d, *J* = 10.8 Hz, 1 H), 4.63 (s, 2 H), 4.16 (*pseudo*-t, *J* = 10.8 Hz, 1 H), 4.15 (d, *J* = 10.2 Hz, 1 H), 4.00 (*pseudo*-t,

J = 9.0 Hz, 1 H), 3.97 (*pseudo*-t, *J* = 9.6 Hz, 1 H), 3.86 (d, *J* = 3.6 Hz, 1 H), 3.80 (dd, *J* = 2.4, 9.6 Hz, 1 H), 3.68 (*pseudo*-t, *J* = 10.2 Hz, 2 H), 3.37 (dd, *J* = 4.2, 10.2 Hz, 1 H), 1.88 (s, 1 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.1, 168.0, 167.4, 163.3 (d, *J* = 247.2 Hz), 138.1, 137.6, 137.1, 136.4 (d, *J* = 7.95 Hz), 134.5, 134.2, 131.8, 131.2, 129.1, 128.5, 128.33, 128.27, 128.0, 127.7, 127.5, 126.0, 125.5 (d, *J* = 2.88 Hz), 123.8, 123.5, 116.0 (d, *J* = 21.9 Hz), 101.4, 99.7, 82.6, 82.3, 78.4, 76.1, 75.7, 75.1, 74.4, 73.6, 69.0, 68.6, 63.7, 63.0, 53.6, 20.6; HRMS (ESI) *m/z* calcd for $C_{49}H_{45}FN_4O_{11}S$ $[M+Na]^+$, 939.2682; found, 939.2680.



10b

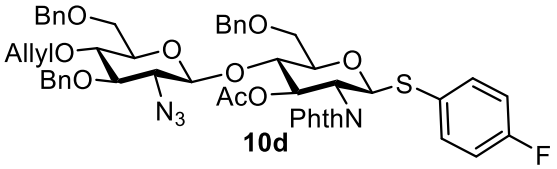
4-Fluorophenyl-2-azido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (10b) $[\alpha]_D$ = -2.4230 (*c* =1.04, $CHCl_3$); TLC (Hexane/EtOAc 2:1) R_f = 0.44; ^{1}H NMR ($CDCl_3$, 600Hz) δ 7.84–7.87 (m, 2H), 7.73–7.66 (m, 2H), 7.42 (dd, *J* = 8.4 , 5.4 Hz, 2 H), 7.49 (dd, *J* = 1.8, 7.8 Hz, 2 H), 7.27–7.39 (m, 15H) 6.92 (*pseudo*-t, *J* = 8.4 Hz, 2 H), 5.70 (dd, *J* = 9.6, 10.2 Hz, 1 H), 5.61 (d, *J* = 10.8 Hz, 1 H), 4.85 (d, *J* = 11.4 Hz, 1 H), 4.77 (d, *J* = 11.4 Hz, 1 H), 4.70 (d, *J* = 12.0 Hz, 1 H), 4.55 (d, *J* = 12.0 Hz, 1 H), 4.49 (d, *J* = 11.4 Hz, 1 H), 4.45 (d, *J* = 12.0 Hz, 1 H), 4.23 (*pseudo*-t, *J* = 10.2 Hz, 1 H), 4.22 (d, *J* = 8.4 Hz, 1 H), 3.99 (*pseudo*-t, *J* = 9.6 Hz, 1 H), 3.97 (dd, *J* = 3.6, 12.0 Hz, 1 H), 3.87 (dd, *J* = 1.2, 10.8 Hz, 1 H), 3.67 (dd, *J* = 4.8, 10.2 Hz, 1 H), 3.58–3.61 (m, 1 H), 3.22 (dd, *J* = 9.6, 8.4 Hz, 1 H), 5.61 (d, *J* = 10.8 Hz, 1 H), 3.11 (dd, *J* = 9.6, 9.0 Hz, 1H), 2.63 (d, *J* = 2.4 Hz, 1 H), 1.77 (s, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.3, 167.8, 167.2, 163 (d, *J* = 247.2 Hz), 138.1, 138.0, 137.3, 136.0 (d, *J* = 8.6 Hz), 134.4, 134.2, 131.7, 131.2, 128.6, 128.5, 127.99, 127.96, 127.87, 127. 84, 127.6, 125.9 (d, *J* = 3.3 Hz), 123.8, 123.6, 116.0 (d, *J* = 21.8 Hz), 101.0, 82.8, 82.6, 78.9, 75.2, 74.9, 73.5, 73.4, 73.2, 72.1, 71.6, 70.1, 68.0, 65.7, 53.8, 20.5; HRMS (ESI) *m/z* calcd for $C_{49}H_{47}FN_4O_{11}S$ $[M+Na]^+$, 941.2838; found, 941.2834.



10c

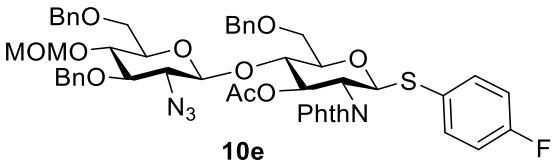
4-Fluorophenyl 4-*O*-acetyl-2-azido-di-*O*-benzyl-4-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benyl-2-deoxy-2phthalimido-1-thio-β-D-glucopyranoside (10c) TLC (Hexane/EtOAc 2:1) R_f = 0.46; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.25–7.39 (m, 15 H), 7.86–7.91 (m, 2 H), 7.74–7.76 (m, 2 H), 7.40–7.44 (m, 2 H), 6.12 (*pseudo*-t, *J* = 9.0 Hz, 2 H), 5.73 (*pseudo*-t, *J* = 10.2 Hz, 1 H), 5.63 (d, *J* = 10.2 Hz, 1 H), 4.75 (*pseudo*-t, *J* = 10.8 Hz, 1 H), 4.72 (d, *J* = 12.0 Hz, 1 H), 4.57 (d, *J* = 12.0 Hz, 1 H), 4.52 (d, *J* = 10.8 Hz, 1 H), 4.39 (d, *J* = 2.4 Hz, 2 H), 4.26 (dd, *J* = 4.2, 10.2 Hz, 1 H), 4.03 (*pseudo*-t, *J* = 9.6 Hz, 1 H), 3.97 (dd, *J* = 3.0, 11.4 Hz, 1 H), 3.87 (d, *J* = 11.4 Hz, 1 H), 3.82 (*pseudo*-t, *J* = 9.6 Hz, 1 H), 3.78 (*pseudo*-t, *J* = 10.8 Hz, 1 H), 3.49 (dd, *J* = 3.6, 10.2 Hz, 1 H), 3.41 (dd, *J* = 4.8, 10.2 Hz, 1 H), 3.31 (*pseudo*-t, *J* = 9.0 Hz, 1 H), 3.24 (*pseudo*-t, *J* = 9.0 Hz, 1 H), 2.05 (s, 3 H), 1.78 (s, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.4, 169.5, 168.1, 167.8, 164.2, 162.9, 138.1, 137.7, 137.5, 136.2, 136.14, 136.1, 134.5, 134.3, 128.6, 128.52, 128.48, 128.45, 128.41, 128.39, 128.1, 127.95, 127.93, 127.91, 127.86, 127.79, 127.68, 123.8, 123.6, 116.1, 115.9, 101.0, 83.0, 80.6, 78.9, 75.1, 75.0, 74.7, 73.6, 73.4, 73.3, 72.8, 71.4, 70.6, 69.1,

68.0, 65.9, 53.8, 20.8, 20.6, 20.4; HRMS (ESI) *m/z* calcd for $C_{69}H_{66}FN_5O_{20}S$ $[M+Na]^+$, 983.2944; found, 983.2938.



10d

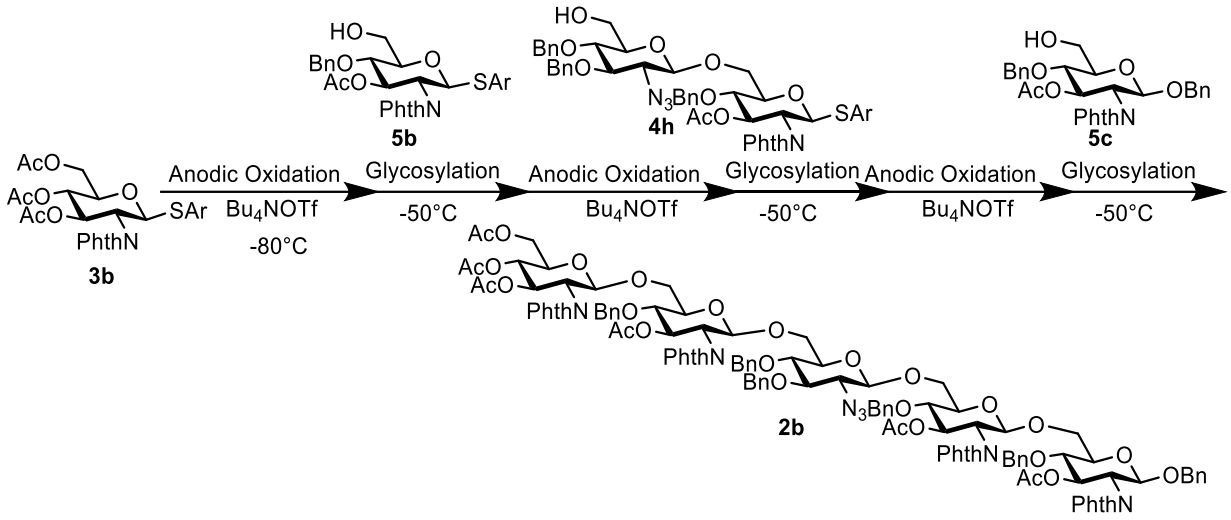
4-Fluorophenyl 4-allyl-2azido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (10d) $[\alpha]_D$ = 0.78 (*c* = 0.98, $CHCl_3$); TLC (Hexane/EtOAc 2:1) R_f = 0.397; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.87–7.83 (m, 2 H), 7.77–7.72 (m, 2 H), 7.44–7.41 (m, 2 H), 7.39–7.28(m, 21 H), 7.26–7.26 (m, 4 H), 6.92 (*pseudo*-t, *J* = 9.0 Hz, 2 H), 5.83–5.75 (m, 1 H), 5.72 (dd, *J* = 10.2, 9.0 Hz, 1 H), 5.62 (d, *J* = 10.2 Hz, 1 H), 5.17 (dd, *J* = 17.4, 1.8 Hz, 1 H), 5.13 (s, 1 H), 5.09 (dd, *J* = 10.8, 1.8 Hz, 1 H), 4.79 (d, *J* = 8.4 Hz, 1 H), 4.76 (d, *J* = 10.8 Hz, 1 H), 4.69 (d, *J* = 12.0 Hz, 1 H), 4.55 (d, *J* = 12.0 Hz, 1 H), 4.55 (s, 1 H), 4.45 (d, *J* = 11.4 Hz, 1 H), 4.41 (d, *J* = 12.0 Hz, 1 H), 4.21–4.16 (m, 3 H), 4.02–3.93 (m, 4 H), 3.87 (dd, *J* = 10.8, 1.2 Hz, 1 H), 3.82 (d, *J* = 4.2 Hz, 1 H), 3.79–3.76 (m, 2 H), 3.72 (d, *J* = 10.2 Hz, 1 H), 3.82–3.78 (m, 2 H), 3.73 (*pseudo*-t, *J* = 9.0 Hz, 2 H), 3.60 (td, *J* = 9.0, 1.2 Hz, 1 H), 3.50 (td, *J* = 9.6, 1.2 Hz, 1 H), 3.34 (td, *J* = 9.6, 3.6 Hz, 1 H), 1.76 (s, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.6, 167.8, 167.3, 163.1 (d, *J* = 247.2 Hz), 138.3, 138.1, 138.0, 137.8 137.6, 136.1 (d, *J* = 7.95 Hz), 134.62, 134.59, 134.2, 131.7, 131.3, 128.5, 128.44, 128.41, 128.2, 128.0, 127.88, 127.84, 127.80, 127.75, 127.7,127.5, 126.0 (d, *J* = 3.15 Hz), 123.80, 123.76, 116.0 (d, *J* = 21.75 Hz), 101.2, 99.2, 83.2, 82.9, 82.4, 79.9, 79.0, 78.7, 77.9, 75.3, 75.1, 74.5, 73.63, 73.55, 73.42, 73.39, 73.16, 71.52, 68.4, 68.0, 66.3, 63.5, 53.8, 20.4; HRMS (ESI) *m/z* calcd for $C_{52}H_{51}ClFN_4O_{11}S$ $[M+Na]^+$, 981.3151; found, 981.3189.



10e

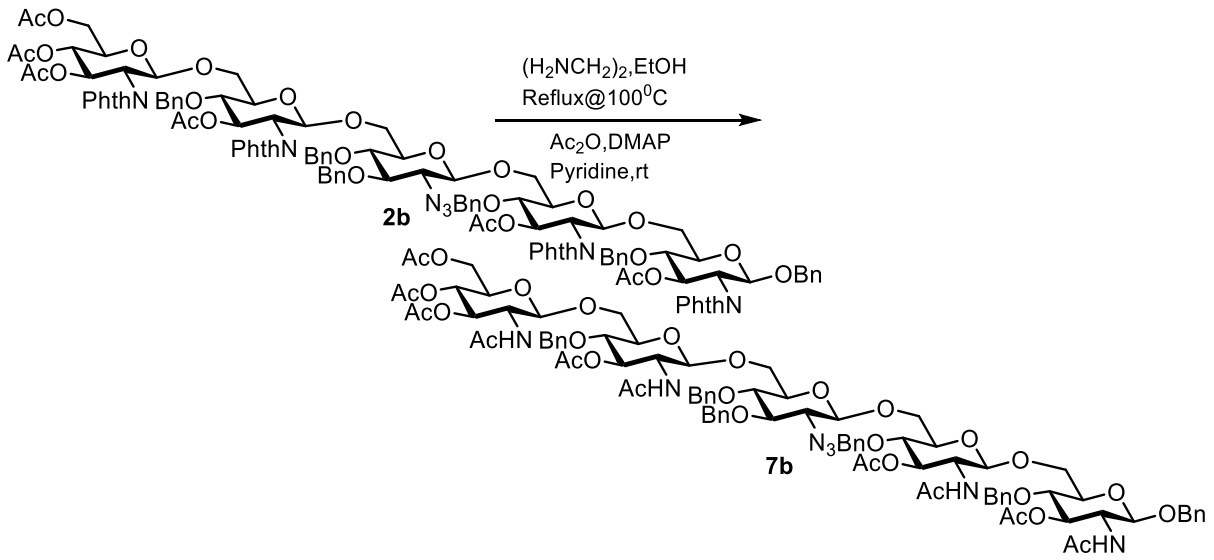
4-Fluorophenyl-2-deoxy-2-azido-3,6-di-*O*-benzyl-4-methoxymethyl-2-deoxy-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (10e) $[\alpha]_D$ = -3.238 (*c* = 1.05, $CHCl_3$); TLC (Hexane/EtOAc 2:1) R_f = 0.30; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.87–7.84 (m, 2 H), 7.75–7.72 (m, 2 H), 7.44–7.24 (m, 17 H), 6.92 (*pseudo*-t, *J* = 9.0 Hz, 2 H), 5.72 (dd, *J* = 10.2, 9.0 Hz, 1 H), 5.63 (d, *J* = 10.2 Hz, 1 H), 4.82 (d, *J* = 10.8 Hz, 1 H), 4.73 (d, *J* = 6.0 Hz, 1 H), 4.71 (d, *J* = 10.8 Hz, 1 H), 4.60 (d, *J* = 6.0 Hz, 1 H), 4.56 (d, *J* = 12.0 Hz, 1 H), 4.48 (d, *J* = 12.0 Hz, 1 H), 4.40 (d, *J* = 12.0 Hz, 1 H), 4.26 (*pseudo*-t, *J* = 10.2 Hz, 1 H), 4.23 (d, *J* = 8.4 Hz, 1 H), 4.02 (*pseudo*-t, *J* = 9.0 Hz, 1 H), 3.97 (dd, *J* = 10.8, 3.0 Hz, 1 H), 3.87 (d, *J* = 10.8 Hz, 1 H), 3.77 (ddd, *J* = 10.2, 3.0, 1.8 Hz, 1 H), 3.73 (dd, *J* = 10.8, 1.8 Hz, 1 H), 3.60 (dd, *J* = 10.8, 5.6 Hz, 1 H), 3.56 (*pseudo*-t, *J* = 9.0 Hz, 2 H), 3.28 - 3.21 (m, 4 H), 3.20 - 3.17 (m, 2 H), 1.78 (s, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.5, 167.8, 167.2, 163.0 (d, *J* = 247.2 Hz), 138.0, 137.8, 137.7, 136.0 (d, *J* = 8.1 Hz), 134.4, 134.2, 131.7, 131.2, 128.5, 128.41, 128.36, 127.84, 127.82, 127.7, 127.6, 126.0 (d, *J* = 3.3 Hz), 123.7, 123.5, 115.9 (d, *J* = 21.8 Hz), 101.0, 98.3, 83.0, 82.9, 78.9, 75.7, 75.2, 75.0, 74.3, 73.4, 73.1, 71.4, 68.7, 67.9, 66.2, 56.4, 53.8, 20.4; HRMS (ESI) *m/z* calcd for $C_{51}H_{51}FN_4O_{12}S$ $[M+Na]^+$, 985.3100; found, 985.3128.

Automated electrochemical assembly of 2b

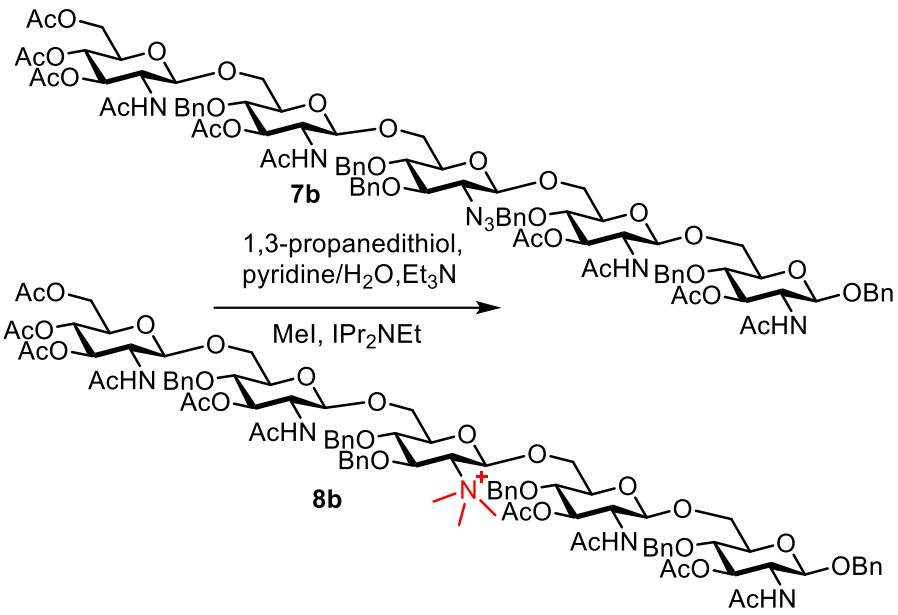


0.108 g (0.192 mmol) **3b** was taken into the cell anode of an electrolysis cell (H-type) and 0.587 g Electrolyte (Bu_4NOTf) was taken to both parts. The cell was vacuumed overnight and then taken under argon atmosphere. After vacuuming solvent dichloromethane (15 mL) was added to both anode and cathode and TfOH (52 μL) was added to cathode. Acceptor solution was made by this time **5c** (0.109 g in 1 mL of dichloromethane), **4h** (0.180 g in 1 mL of dichloromethane), **5b** (0.102 g in 1 mL of dichloromethane).

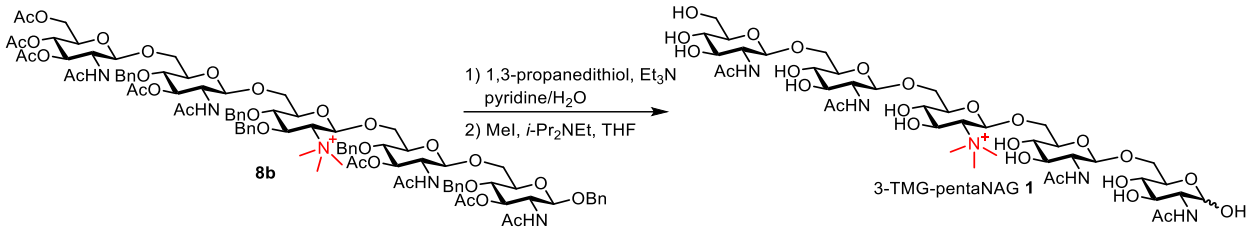
The synthesizer was started for three cycles with 8 mA and 1.05 F/mol. Acceptor solutions **5c, 4h** and **5b** were injected by the synthesizer respectively. After finishing by the synthesizer 1.0 mL triethylamine was added to both anode and cathode to quench the reaction. Completion of the reaction was confirmed by TLC. Taken the whole solution and then removed all the solvent by rotary evaporator and vacuumed line. The weight of the crude product was 2.16g. Column chromatography was done for purification. The weight of the pure product is 0.137g (0.063 mmol, 69% average in three cycles). **1-*O*- benzyl 3,4,6-tri-*O*-acetyl-2-phthalimido-β-D-glucopyranosyl-(1→6)-3-*O*-acetyl-4-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-*O*-benzyl-2-deoxy-2-azido-β-D-glucopyranosyl-(1→6)-3-*O*-acetyl-4-Obenzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3-*O*-acetyl-4-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (2b)** TLC (Hexane/EtOAc 2:1) R_f = 0.40; $[\alpha]_D$ = -4.9504 (*c* = 1.0, $CHCl_3$, 29 °C); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.76–7.54 (m, 16 H), 7.31–7.25 (m, 8 H), 7.24–7.20 (m, 8 H), 7.18–7.15 (m, 2 H), 7.07–7.02 (m, 4 H), 6.99–6.94 (m, 6 H), 5.83–5.77 (m, 2H), 5.65 (ddd, *J* = 12.6, 10.8, 9.0 Hz, 2 H), 5.58 (d, *J* = 8.4 Hz, 1 H), 5.47 (d, *J* = 8.4 Hz, 1 H), 5.37 (d, *J* = 8.4 Hz, 1 H), 5.24 (d, *J* = 8.4 Hz, 1 H), 5.16 (dd, *J* = 10.2, 9.3 Hz, 1 H), 4.73 (dd, *J* = 11.4, 3.5 Hz, 2 H), 4.65 (d, *J* = 11.7 Hz, 1 H), 4.62–4.57 (m, 2 H), 4.47 (d, *J* = 10.8 Hz, 1 H), 4.41 (dd, *J* = 10.8, 8.4 Hz, 1 H), 4.38–4.35 (m, 2 H), 4.34–4.27 (m, 5 H), 4.26 (d, *J* = 3.0 Hz, 1 H), 4.24–4.21 (m, 1 H), 4.18–4.12 (m, 3 H), 4.10–4.09 (m, 1 H), 4.06 (dd, *J* = 11.4, 1.5 Hz, 1 H), 3.87 (dd, *J* = 11.4, 1.5 Hz, 1 H), 3.83 (q, *J* = 5.7 Hz, 1 H), 3.80-3.75 (m, 5 H), 3.65–3.57 (m, 3 H), 3.53–3.47 (m, 2 H), 3.35–3.32 (m, 1 H), 3.26–3.22 (m, 2 H), 3.18–3.17 (m, 1 H), 2.09 (s, 3 H), 2.00 (s, 3 H), 1.85-1.83 (s, 3 H), 1.74 (s, 3 H), 1.67 (s, 3 H), 1.60 (s, 3 H);^{13}C NMR ($CDCl_3$, 150 MHz) δ170.7, 170.2, 170.1, 169.94, 169.91, 169.4, 167.9, 167.5, 138.0, 137.9, 137.54, 137.52, 136.9, 134.4, 131.6, 131.2, 128.5, 128.4, 128.3, 128.1, 128.0, 127.93, 127.92, 127.82, 127.80, 127.79, 127.76, 127.73, 127.71, 127.63, 127.57, 127.5, 127.4, 123.6, 123.5, 102.3, 98.4, 97.8, 97.6, 96.9, 82.8, 77.6, 75.2, 75.0, 74.8, 74.7, 74.6, 74.5, 74.4, 74.3, 74.2, 73.3, 72.9, 72.0, 70.8, 70.7, 68.9, 68.4, 68.3, 67.7. 67.2, 66.1, 20.8, 20.6, 20.52, 20.50, 20.4; HRMS (ESI) *m/z* calcd for $C_{116}H_{111}N_7O_{35}$ $[M+K]^+$, 2201.67862; found, 2201.6790.



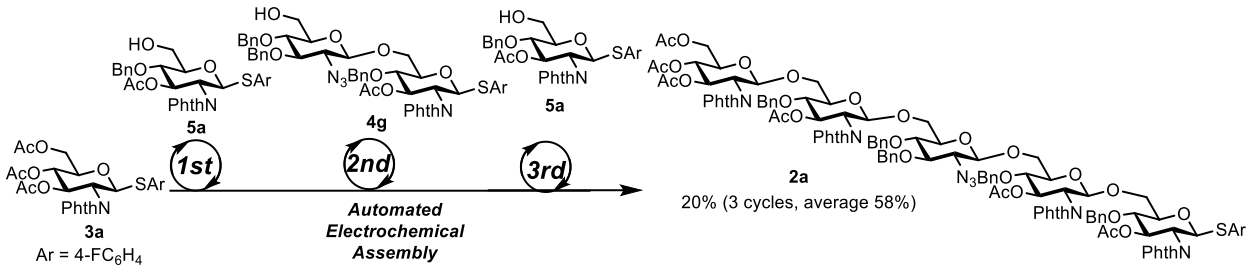
In a stirring solution of **2b** (0.046 mmol, 0.100 g) and ethanol (5 mL) ethylenediamine (6.83 mmol, 0.45 mL) was added to the reaction mixture and refluxed for 10 h. The solvent was removed under reduced pressure and dried under vacuum. The anhydrous product and DMAP (0.097 mmol, 12 mg) were dissolved in pyridine (5 mL) and finally acetic anhydride (7.93 mmol, 0.75 mL) was added at 0 °C and stirred at room temperature for 12 h. The reaction was quenched by addition of EtOAc, and the organic layer was washed with 1 M aqueous HCl solution, water, and brine, respectively. The organic layer was dried over Na_2SO_4, and the solvent was removed under reduced pressure. Thus-obtained crude product was purified with silica gel chromatography ($CHCl_3$/MeOH 8:1) and then by preparative-GPC with $CHCl_3$ as an eluent to obtain tetrasaccharide **7b** (0.038 mmol, 68 mg) as a white solid in 81.2% yield (2 steps); **1-*O*- benzyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-*N*-acetyl-β-D-glucopyranosyl-(1→6)-3-*O*-acetyl-4-*O*-benzyl-2-deoxy-2-*N*-acetyl-β-D-glucopyranosyl-(1→6)-3,4-*O*-benzyl-2-deoxy-2-azido-β-D-glucopyranosyl-(1→6)-3-*O*-acetyl-4-O-benzyl-2-deoxy-2-*N*-acetyl-β-D-glucopyranosyl-(1→6)-3-*O*-acetyl-4-*O*-benzyl-2-deoxy-2-*N*-acetyl-β-D-glucopyranoside (7b)** TLC (DCM/MeOH 8:1) R_f = 0.47; $[\alpha]_D$ = -39.865 (*c* = 1.5, $CHCl_3$, 26 °C); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.32–7.26 (m, 20 H), 7.23 (*pseudo*-t, *J* = 7.5 Hz, 8 H), 7.16 (d, *J* = 6.6 Hz, 2 H), 6.83 (d, *J* = 6.0 Hz, 1 H), 6.27 (d, *J* = 6.9 Hz, 1 H), 6.14 (d, *J* = 1.5 Hz, 1 H), 5.53 (*pseudo*-t, *J* = 9.9 Hz, 1 H), 5.47 (d, *J* = 9.3 Hz, 1 H), 5.11 (*pseudo*-t, *J* = 9.9 Hz, 1 H), 5.05–4.94 (m, 4 H), 4.84–4.78 (m, 3 H), 4.72 (d, *J* = 10.8 Hz, 1 H), 4.64–4.59 (m, 3 H), 4.57–4.47 (m, 6 H), 4.42–4.36 (m, 3 H), 4.31 (*pseudo*-t, *J* = 3.7 Hz, 1 H), 4.25 (*pseudo*-t, *J* = 9.4 Hz, 1 H), 4.21 (dd, *J* = 12.2, 5.2 Hz, 1 H), 4.16 (d, *J* = 9.6 Hz, 1 H), 4.11 (q, *J* = 9.4 Hz, 1 H), 4.06–3.94 (m, 3 H), 3.92–3.83 (m, 4 H), 3.81 (dd, *J* = 10.7, 6.0 Hz, 1 H), 3.76 (d, *J* = 10.4 Hz, 1 H), 3.68–3.63 (m, 3 H), 3.57 (dd, *J* = 11.4, 8.7 Hz, 4 H), 3.49 (dd, *J* = 15.5, 8.3 Hz, 2 H), 3.37–3.32 (m, 2 H), 2.01 (s, 3 H), 1.99 (s, 3 H), 1.99 (s, 3 H), 1.98 (s, 3 H), 1.92 (s, 3 H), 1.91 (s, 3 H), 1.88 (s, 3 H), 1.86 (s, 3 H), 1.81 (d, *J* = 3.3 Hz, 6 H);^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.4, 170.1, 169.9, 169.63, 169.55, 169.51, 169.3, 169.1, 168.5, 136.94, 136.87, 136.85, 136.81, 136.4, 136.3, 127.9, 127.61, 127.60, 127.5, 127.4, 127.3, 127.21, 127.20, 127.13, 126.98, 126.96, 126.94, 126.86, 126.82, 126.79, 126.77, 126.73, 126.4, 126.1, 102.1, 101.6, 99.61, 99.60, 98.7, 82.3, 77.1, 76.5, 75.7, 74.8, 74.3, 74.2, 74.1, 73.9, 73.8, 73.7, 73.6, 73.0, 72.9, 72.3, 71.1, 70.9, 70.1, 69.3, 68.5, 68.1, 66.9, 65.4, 61.2, 54.7, 53.5, 53.1, 53.0, 52.7, 22.2, 22.14, 22.11, 21.8, 19.9, 19.8, 19.71, 19.70; HRMS (ESI) *m/z* calcd for $C_{92}H_{111}NK_7O_{31}$ $[M+K]^+$, 1849.6989; found, 1849.6986.



Precursor **7b** (0.055 mmol, 100 mg) was dissolved in a mix-solvent of pyridine and water (3 mL/0.75 mL). Then Et_3N (0.22 mL) and 1.3-propanedithiol (2.26 mmol, 0.22 mL) were successively added to the reaction mixture and stirred at room temperature for 12 h. The completion of the reaction was confirmed by ESI-MS analysis and the solvent was removed under reduced pressure. The crude product was purified by silica gel chromatography (DCM/CH_3OH 20:1) and dried under vacuum. The dried compound was dissolved THF (4.2 mL) and iodomethane (45.86 mmol, 2.85 mL). Then *N,N*-di isopropyl amine (2.45 mmol, 0.43 mL) was added and stirred at room temperature for 10 h. The completion of the reaction was confirmed by ESI-MS analysis and the solvent was removed under reduced pressure. The crude product was purified by silica gel chromatography (DCM/MeOH 20:1) to obtain pentasaccharide (0.024 mmol, 43 mg) as a white solid in 44% yield (2 steps); **1-*O*-benzyl 3,4,6-tri-*O*-acetyl-2-phthalimido-β-D-glucopyranosyl-(1→6) -3-*O*-acetyl-4-*O*-benzyl-2-deoxy-2-*N*-acetyl-β-D-glucopyranosyl-(1→6)-3,4-O-benzyl-2-deoxy-2-trimethylammonium-β-D-glucopyranosyl-(1→6)-3-*O*-acetyl-4-*O*-benzyl-2-deoxy-2-*N*-acetyl-β-D-glucopyranosyl-(1→6)-3-*O*-acetyl-4-*O*-benzyl-2-deoxy-2-*N*-acetyl-β-D-glucopyranoside (8b)** TLC (DCM/MeOH 10:1) R_f = 0.33; $[\alpha]_D$ = -17.234 (*c* = 0.94, $CHCl_3$, 28 °C); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.33–7.31 (m, 7 H), 7.30–7.27 (m, 8 H), 7.25–7.21 (m, 9 H), 7.16 (s, 1 H), 7.01–7.00 (m, 1 H), 6.19 (*pseudo*-t, *J* = 8.7 Hz, 1 H), 5.34 (*pseudo*-t, *J* = 9.7 Hz, 1 H), 5.22 (*pseudo*-t, *J*= 9.7 Hz, 2 H), 5.03–4.97 (m, 3 H), 4.89 (*pseudo*-t, *J* = 10.0 Hz, 2 H), 4.84–4.81 (m, 3 H), 4.67 (d, *J* = 12.0 Hz, 1 H), 4.60 (d, *J* = 12.4 Hz, 1 H), 4.58–4.53 (m, 4 H), 4.45 (*pseudo*-t, *J* = 10.9 Hz, 3 H), 4.39 (d, *J* = 11.6 Hz, 1 H), 4.35 (d, *J* = 8.1 Hz, 1 H), 4.19–4.08 (m, 6 H), 3.99 (*pseudo*-t, *J* =7.9 Hz, 2 H), 3.91–3.84 (m, 4 H), 3.81–3.76 (m, 2 H), 3.70–3.60 (m, 6 H), 3.54 (*pseudo*-t, *J* = 9.3 Hz, 1 H), 3.45–3.39 (m, 2 H), 3.14 (s, 9 H), 2.04 (d, *J* = 11.7 Hz, 6 H), 1.98–1.95 (m, 15 H), 1.90-1.88 (dd, *J* = 7.8, 5.5 Hz, 12 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 171.6, 170.9, 170.8, 170.7, 170.6, 170.54, 170.51, 170.4, 169.5, 137.9, 137.73, 137.70, 137.63, 136.92, 136.90, 128.6, 128.53, 128.49, 128.45, 128.38, 128.35, 128.21,128.20, 128.13, 128.11, 127.92, 127.91, 127.7, 102.6, 101.7, 101.4, 99.3, 97.8, 78.1, 77.8, 77.6, 76.4, 76.0, 75.9, 75.1, 74.9, 74.7, 74.6, 74.2, 73.9, 73.3, 73.2, 72.9, 72.3, 71.9, 70.9, 70.7, 70.6, 70.3, 70.2, 69.0, 62.5, 55.5, 55.0, 54.6, 54.4, 54.3, 54.2, 42.8, 31.9, 31.6, 29.7, 29.4, 28.5, 25.7, 23.5, 23.4, 23.3, 23.1, 22.7, 22.6, 21.0, 20.94, 20.91, 20.90, 20.7, 18.7, 17.4, 14.1, 12.1; HRMS (ESI) *m/z* calcd for $C_{95}H_{120}N_5O_{31}$ $[M]^+$, 1827.79953, found, 1827.7999.



Pentasaccharide precursor **8b** (0.024 mmol, 43 mg) was dissolved in MeOH (3.3 mL) and water (1.7 mL). Then K_2CO_3 (33.2 mg, 0.24 mmol) was added to the reaction mixture at 0° C and stirred overnight at room temperature. The reaction was quenched by addition of Dowex® 50w*4 100-200 (cation exchange resin) and the resin was removed by filtration. The solvent was removed under reduced pressure. The residue was dissolved in a mixture of THF/water 4.6 mL (1:1) and to make it acidic 2 drops of hydrochloric acid were added. Then the reaction mixture was cooled by liquid N_2 and degassed under vacuum for 2 times. A catalyst $Pd(OH)_2/C$ (20%) (195 mg) was added to the cooled reaction mixture and filled with hydrogen gas. After stirring overnight, the catalyst was removed by filtration, and neutralized by Dowex™ 550A (Anion exchange resin). Thus-obtained reaction mixture was evaporated to remove the solvent and the crude product was purified by gel filtration chromatography to obtain pure 3-TMG pentaNAG (0.012 mmol, 12.4 mg) in 49.9% yield (2 steps). **β-D-Glucopyranose, *O*-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl-(1→6)-*O*-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl-(1→6)-*O*-2-deoxy-2-(trimethylammonio)-β-D-glucopyranosyl-(1→6)-*O*-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl-(1→6)-*O*-2-(acetylamino)-2-deoxy-β-D-glucopyranoside (3-TMG pentaNAG)** $[\alpha]_D = -13.504$ (c = 1.17, H_2O, 28 °C); ^{1}H-NMR (800 MHz, D_2O) δ 5.08–5.05 (m, 2 H), 4.51 (*pseudo*-t, J = 9.0 Hz, 1 H), 4.46 (dd, J = 8.7, 3.9 Hz, 2 H), 4.18 (*pseudo*-t, J = 9.9 Hz, 1 H), 4.10 (d, J = 11.4 Hz, 1 H), 4.04 (dd, J = 9.6, 6.0 Hz, 1 H), 3.96–3.94 (m, 2 H), 3.85–3.82 (m, 2 H), 3.71–3.59 (m, 11 H), 3.51–3.46 (m, 5H), 3.38–3.32 (m, 5 H), 3.24 (s, 10 H), 1.97–1.95 (m, 12 H); ^{13}C NMR (201 MHz, D_2O) δ 174.9, 174.84, 174.81, 174.7, 174.6, 174.5, 171.1, 162.8, 129.0, 101.7, 97.5, 97.4, 95.1, 91.0, 77.2, 77.1, 76.0, 75.13, 75.11, 74.7, 74.6, 74.3, 74.2, 74.0, 73.8, 73.74, 73.73, 70.7, 70.44, 70.40, 70.3, 70.1, 70.03, 70.0, 69.9, 69.7, 69.53, 69.51, 69.50, 69.4, 69.3, 69.2, 68.8, 68.6, 68.5, 68.3, 68.1, 60.8, 55.6, 55.53, 55.52, 54.3, 54.2, 54.1, 49.0, 22.44, 22.42, 22.3, 22.2, 22.1, 22.0, 20.1; HRMS (ESI) *m/z* calcd for $C_{41}H_{72}N_5O_{25}$, 1034.4511, found, 1034.4508.



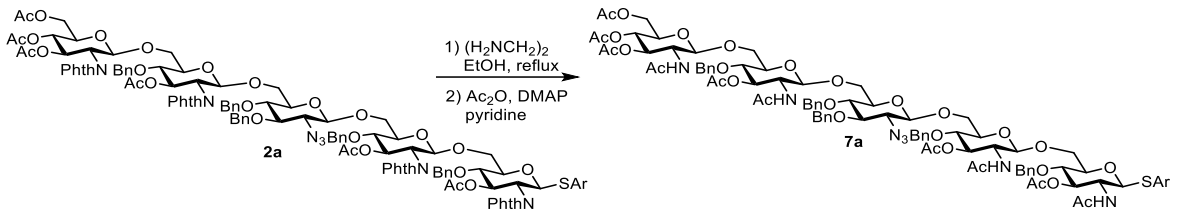
0.308 mmol **3a** was taken into the cell anode of an electrolysis cell (H-type) and 1.0 mmol Electrolyte (Bu_4NOTf) was taken to both parts. The cell was vacuumed overnight and then taken under argon atmosphere. After vacuuming solvent dichloromethane (10 mL) was added to both anode and cathode and TfOH (0.308 mmol) was added to cathode. Acceptor solution of **4g** and **5a** was made by this time.

The synthesizer was started for three cycles with 8 mA and 1.05 F/mol. Acceptor solutions **5a, 4g** and **5a** were injected by the synthesizer respectively. After finishing by the synthesizer 1.0 mL triethylamine was added to both anode and cathode to quench the reaction. Completion of the reaction was confirmed by TLC. Taken the whole solution and then removed all the solvent by rotary evaporator and vacuumed line. The

weight of the crude product was 2.34 g. Column chromatography was done for purification. We obtained target **2a** in 24% yield after three cycles of electrochemical reaction.

4-Fluorophenyl 3,4,6-tri-*O*-acetyl-2-phthalimido-β-D-glucopyranosyl-(1→6)-3-*O*-acetyl-4-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-*O*-benzyl-2-deoxy-2-azido-β-D-glucopyranosyl-(1→6)-3-*O*-acetyl-4-Obenzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3-*O*-acetyl-4-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (2a)

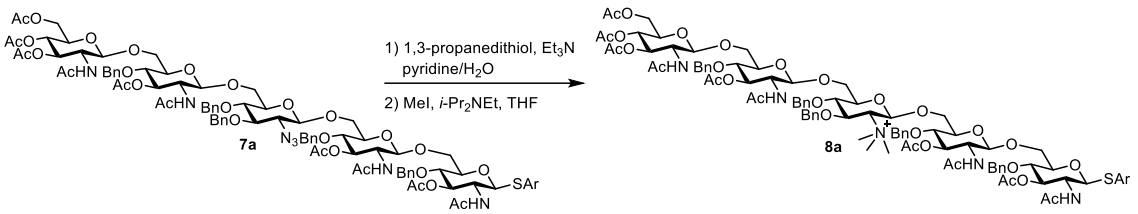
$[\alpha]_D$ = -3.4234 (*c* = 1.11, $CHCl_3$); TLC (Hexane/EtOAc 2:3) R_f 0.40; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.80–7.55 (m, 16 H), 7.34 (dd, *J* = 9.0, 5.4 Hz, 2 H), 7.32–7.21 (m, 16 H), 7.18–7.15 (m, 3 H), 7.10 (dd, *J* = 6.6, 1.2 Hz, 2 H), 7.05 (dd, *J* = 7.2, 1.2 Hz, 2 H), 7.03 (*pseudo*-t, *J* = 8.4 Hz, 2 H), 6.98 (dd, *J* = 7.2, 1.8 Hz, 1 H), 5.82 (dt, *J* = 8.4, 4.2 Hz, 1 H), 5.79 (dd, *J* = 10.8, 9.0 Hz, 1 H), 5.67 (*pseudo*-t, *J* = 9.0 Hz, 1 H), 5.54 (d, *J* = 8.4 Hz, 1 H), 5.51 (d, *J* = 10.2 Hz, 1 H), 5.46 (d, *J* = 8.4 Hz, 1 H), 5.38 (d, *J* = 8.4 Hz, 1 H), 5.17 (*pseudo*-t, *J* = 10.2 Hz, 1 H), 4.75 (dd, *J* = 10.8, 1.8 Hz, 2 H), 4.67 (d, *J* = 12.0 Hz, 1 H), 4.62 (d, *J* = 10.8 Hz, 1 H), 4.49 (d, *J* = 10.8 Hz, 1 H), 4.42 (dd, *J* = 10.8, 8.4 Hz, 1 H), 4.36 (*pseudo*-t, *J* = 9.6 Hz, 2 H), 4.34–4.29 (m, 5 H), 4.24 (dd, *J* = 10.8, 1.2 Hz, 1 H), 4.19 (d, *J* = 6.6 Hz, 1 H), 4.16 (dd, *J* = 10.8, 8.4 Hz, 2 H), 4.05 (d, *J* = 10.2 Hz, 1 H), 3.97 (*pseudo*-t, *J* = 10.2 Hz, 1 H), 3.88 (d, *J* = 10.8 Hz, 1 H), 3.79–3.74 (m, 5 H), 3.72 (dd, *J* = 7.2, 4.8 Hz, 1 H), 3.68 (ddd, *J* = 10.2, 2.4, 1.8 Hz, 1 H), 3.62–3.60 (m. 2 H), 3.52 (*pseudo*-t, *J* = 9.0 Hz, 1 H), 3.37 (*pseudo*-t, *J* = 9.6 Hz, 1 H), 3.34-3.31 (m, 2 H), 3.26 (dt, *J* = 14.4, 9.6 Hz, 2 H), 2.09 (s, 3 H), 2.02 (s, 3 H), 1.85 (s, 3 H), 1.77 (s, 3 H), 1.68 (s, 3 H), 1.56 (s, 3 H); ^{13}C NMR (CDCl3, 150 MHz) δ 170.8, 170.22, 170.19, 169.99, 169.92, 169.5, 168.2, 167.8, 167.2, 163.1 (d, *J* = 246.75 Hz), 138.1, 138.0, 137.7, 137.6, 137.5, 136.3 (d, *J* = 8.3 Hz), 134.4, 134.1, 131.7, 131.3, 128.5, 128.40, 128.38, 128.35, 127.95, 127.86, 127.83, 127.79, 127.76, 127.72, 127.65, 127.55, 127.4, 125.4 (d, *J* = 2.4 Hz), 123.63, 123.57, 123.5, 123.4, 116.0 (d, *J* = 8.3 Hz), 102.3, 98.5, 98.2, 97.6, 82.8, 81.8, 78.1, 77.61, 76.97, 75.2, 74.96, 74.8, 74.7, 74.6, 74.5, 74.3, 73.0, 72.0, 70.8, 69.0, 68.6, 68.5, 68.1, 67.2, 66.1, 62.0, 55.2, 55.0, 54.6, 53.9, 20.8, 20.7, 20.6, 20.5, 20.3; HRMS (ESI) *m/z* calcd for $C_{115}H_{108}FN_7O_{34}S$ [M+Na]+, 2204.6534; found, 2204.6519.



EtOH (3.1 mL) and $(H_2NCH_2)_2$ (9.5 mmol, 0.51 mL) were added to a flask containing compound **2a** (0.064 mmol, 140 mg) and stirred until homogeneous. The mixture was refluxed at 100 °C and stirred overnight. A TLC check (CH_2Cl_2/CH_3OH 8:1) was performed, followed by ESI-MS measurement. It was concentrated and dried in vacuo. 8.0ml pyridine and 1.04 mL acetic anhydride was added dropwise at 0 °C. A TLC check (CH_2Cl_2/CH_3OH 8:1) was performed, and ethyl acetate was added to separate the layers. After concentration and vacuum drying, the desired product **7a** was obtained with a yield of 0.0598 mmol, 109 mg, and a yield of 93%.

4- Fluorophenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-*N*-acetyl-β-D-glucopyranosyl-(1→6)-3-*O*-acetyl-4-*O*-benzyl-2-deoxy-2-*N*-acetyl-β-D-glucopyranosyl-(1→6)-3,4-*O*-benzyl-2-deoxy-2-azido-β-D-glucopyranosyl-(1→6)-3-*O*-acetyl-4-O-benzyl-2-deoxy-2-*N*-acetyl-β-D-glucopyranosyl-(1→6)-3-*O*-

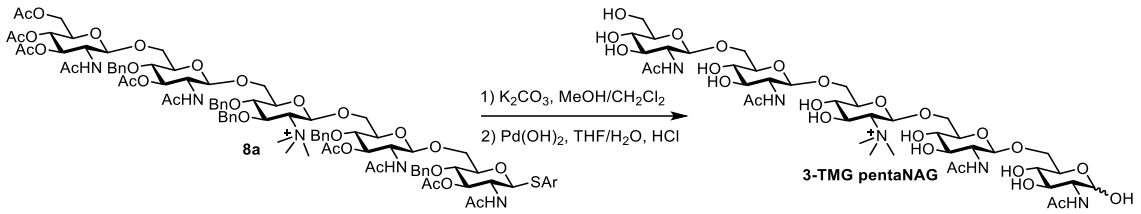
acetyl-4-*O*-benzyl-2-deoxy-2-*N*-acetyl-β-D-glucopyranoside (7a) $[\alpha]_D$ = -26.424 (*c* = 0.153, $CHCl_3$); TLC (CH_2Cl_2/MeOHc 8:1) R_f 0.42; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.52 (dd, *J* = 9.0, 3.6 Hz, 2 H), 7.37–7.23 (m, 23 H), 7.18–7.165 (m, 2 H), 7.06 (*pseudo*-t, *J* = 9.0 Hz, 2 H), 6.91 (s, 1 H), 6.20 (s, 1 H), 6.00 (s, 1 H), 5.70 (s, 1 H), 5.67 (*pseudo*-t, *J* = 9.6 Hz, 1 H), 5.53 (*pseudo*-t, *J* = 9.6 Hz, 1 H), 5.06 (*pseudo*-t, *J* = 9.0 Hz, 1 H), 5.00 (d, *J* = 10.2 Hz, 1H), 4.97 (d, *J* = 10.2 Hz, 1 H), 4.94 (dd *J* = 10.2, 3.0 Hz, 1 H), 4.85 (s, 1 H), 4.73 (d, *J* = 10.8 Hz, 1 H), 4.34 (d, *J* = 8.4 Hz, 1 H), 4.28–4.21 (m, 2 H), 4.16 (d, *J* = 9.6 Hz, 1 H), 4.13 (d, *J* = 8.4 Hz, 1 H), 4.05 (d, *J* = 10.2 Hz, 1 H), 4.02 (d, *J* = 9.0 Hz, 1 H), 3.95 (td, *J* = 12.0, 1.8 Hz, 2 H), 3.86–3.78 (m, 5 H), 3.71–3.71 (m, 2 H), 3.67 (ddd, *J* = 7.8, 3.6, 2.4 Hz, 1 H), 3.64–3.61 (m, 2 H), 3.58 (d, *J* = 9.6 Hz, 1 H), 3.55 (d, *J* = 9.6 Hz, 1 H), 3.52 (d, *J* = 9.0 Hz, 1 H), 3.47 (*pseudo*-t, *J* = 8.4 Hz, 1 H), 3.43 (*pseudo*-t, *J* = 9.6 Hz, 1 H), 3.37 (*pseudo*-t, *J* = 9.0 Hz, 1 H), 3.29 (*pseudo*-t, *J* = 9.0 Hz, 1 H), 2.05 (s, 3 H), 2.004 (s, 3 H), 2.00 (s, 3 H), 2.00 (s, 3 H), 1.95 (s, 3 H), 1.94 (s, 3 H), 1.93 (s, 3 H), 1.88 (s, 3 H), 1.839 (s, 3 H), 1.837 (s, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 171.6, 171.1, 171.00, 170.7, 170.6, 170.52, 170.48, 170.3, 170.1, 169.5, 162.7 (d, *J* = 246 Hz), 138.0, 137.9, 137.8, 137.5, 134.8 (d, *J* = 6.9 Hz), 128.6, 128.5, 128.4, 128.34, 128.27, 128.21, 128.02, 127.98, 127.92, 127.88, 127.85, 127.70, 125.1 (d, *J* = 3.8 Hz), 116.0 (d, *J* = 21.6 Hz), 102.9, 102.8, 100.7, 100.5, 86.9, 83.4, 78.24, 78.16, 77.6, 76.7, 76.4, 76.1, 75.3, 75.2, 75.1, 75.0, 74.9, 74.7, 74.6, 74.5, 73.9, 73.2, 72.0, 71.0, 70.5, 69.6, 69.0, 67.9, 62.2, 60.4, 55.7, 54.1, 53.7, 53.2, 23.3, 23.2, 21.1, 21.0, 20.9, 20.84, 20.75, 20.73, 20.66; HRMS (ESI) m/z calcd for $C_{91}H_{108}FN_7O_{30}S$ [M+Na]+, 1852.6743; found, 1852.6768.



An eggplant flask containing compound **7a** (0.059 mmol, 108.8 mg) was charged with pyridine/H_2O (3.2 mL/0.83 mL) and Et_3N (0.24 mL) was added. The mixture was stirred for 13 hours, subjected to ESI-MS measurement and TLC check (CH_2Cl_2/CH_3OH 8:1), purified by column chromatography (CH_2Cl_2/CH_3OH/Et_3N 80:2:1), and dried under vacuum. THF (4.5 mL) was added, DIPEA (2.63 mmol, 0.46 mL) and Iodomethane (49.2 mmol, 3.1 mL) were added dropwise, and the mixture was stirred for 17 hours. After ESI-MS measurement, solids were removed by Kiriyama filtration and concentrated. It was purified by chromatography (CH_2Cl_2/CH_3OH 10:1) to give the target compound **8a** in a yield of 0.031 mmol, 57.8 mg, and a yield of 53%.

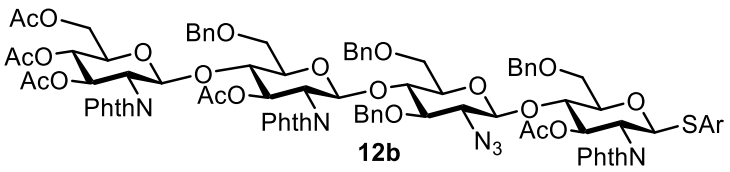
4-Fluorophenyl 3,4,6-tri-*O*-acetyl-2-phthalimido-β-D-glucopyranosyl-(1→6)-3-*O*-acetyl-4-*O*-benzyl-2-deoxy-2-*N*-acetyl-β-D-glucopyranosyl-(1→6)-3,4-O-benzyl-2-deoxy-2-trimethylammonium-β-D-glucopyranosyl-(1→6)-3-*O*-acetyl-4-*O*-benzyl-2-deoxy-2-*N*-acetyl-β-D-glucopyranosyl-(1→6)-3-*O*-acetyl-4-*O*-benzyl-2-deoxy-2-*N*-acetyl-β-D-glucopyranoside (8a) $[\alpha]_D$ = -7.8699 (c = 1.17, CH_2Cl_2); TLC (CH_2Cl_2/MeOHc 10:1) R_f 0.27; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.57 (dd, *J* = 8.4, 5.4 Hz, 2 H), 7.37–7.24 (m, 21 H), 7.21-7.18 (m, 4 H), 7.01 (*pseudo*-t, *J* = 8.4 Hz, 2 H), 6.88 (s, 1 H), 6.79 (s, 1 H), 6.53 (s, 1 H), 5.82 (s, 1 H), 5.37 (*pseudo*-t, *J* = 9.6 Hz, 1 H), 5.23 (*pseudo*-t, *J* = 9.6 Hz, 1 H), 5.21–5.19 (m, 1 H), 5.17 (d, *J* = 10.2 Hz, 1 H), 5.03 (d, *J* = 6.6 Hz, 1 H), 4.95 (*pseudo*-t, *J* = 9.0 Hz, 1 H), 4.89 (*pseudo*-t, *J* = 9.6 Hz, 1 H), 4.75 (d, *J* = 8.4 Hz, 1 H), 4.67 (d, *J* = 12.6 Hz, 1 H), 4.58 (d, *J* = 11.4 Hz, 1 H), 4.54 (d, *J* = 10.8 Hz, 1 H), 4.52 (dd, *J* = 10.2, 3.0 Hz, 1 H), 4.50 (d, *J* = 13.8 Hz, 1 H), 4.47 (d, *J* = 11.4 Hz, 1 H), 4.39 (d, *J* = 11.4 Hz, 1 H), 4.26 (d, *J* = 8.4 Hz, 1 H), 4.16 (d, *J* = 6.6 Hz, 1 H), 4.14 (td, *J* = 6.0 Hz, 2 H), 4.08–4.00 (m, 2 H), 3.98 (d, *J* = 10.2 Hz, 1 H), 3.92–3.87 (m, 4 H), 3.85 (d, *J* = 7.2 Hz, 1 H), 3.84–3.81 (m, 1 H),

3.77–3.74 (m, 3 H), 3.55 (*pseudo*-t, *J* = 9.0 Hz, 1 H), 3.36 (*pseudo*-t, *J* = 9.6 Hz, 1 H), 3.33 (*pseudo*-t, *J* = 9.0 Hz, 1 H), 3.20 (s, 9 H), 2.06 (s, 3 H), 2.00 (s, 3 H), 1.98 (s, 3 H), 1.98 (s, 3 H), 1.97 (s, 3 H), 1.97 (s, 3 H), 1.96 (s, 3 H), 1.92 (s, 3 H), 1.89. (s, 3 H), 1.87. (s, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 171.7, 170.8, 170.7, 170.6, 170.5, 170.4, 170.2, 169.4, 162.6 (d, *J* = 262.95 Hz), 137.9, 137.6, 137.5, 134.9 (d, *J* = 9.64 Hz), 134.8, 134.7, 128.72, 128.65, 128.57, 128.53, 128.50, 128.47, 128.41,128.3, 128.23, 128.16, 127.95, 127.91, 127.7, 125.5 (d, *J* = 7.05 Hz), 115.9 (d, *J* = 21.6 Hz), 102.5, 101.6, 101.4, 98.5, 97.8, 85.5, 78.1, 77.8, 76.2, 75.2, 75.1, 74.8, 74.6, 74.5, 73.6, 73.4, 72.5, 71.9, 68.9, 67.4, 62.4, 55.2, 54.3, 54.2, 23.52, 23.45, 23.41, 23.36, 23.2, 21.07, 21.05, 20.98, 20.91, 20.88, 20.7; HRMS (ESI) *m/z* calcd for $C_{94}H_{117}FN_5O_{30}S^+$ $[M+Na]^+$, 1846.7483; found, 1847.7466.



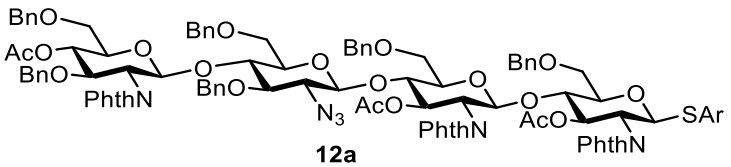
CH_3OH/CH_2Cl_2 (4.3 mL/2.1 mL) and K_2CO_3 (0.31 mmol, 42.8 mg) were added to an eggplant flask containing compound **8a** (0.031 mmol, 57.8 mg). After 6 hours, the reaction was completed by ESI-MS. After confirming this, Dowex-50wx4-20 (cation exchange resin H^+) washed with CH_3OH was added to stop the reaction. The resin was removed by Kiriyama filtration, concentrated, and dried in vacuo. H_2O/THF (3.0 mL/3.0 mL) was added, and 2 drops of concentrated hydrochloric acid were added with $Pd(OH)_2$/C. After completion of the reaction $Pd(OH)_2$/C was filtered and solvent were concentrated. In the concentrated solution, Amberlite HO-exchange resin was added to stop the reaction. Amberlite OH-exchange resin was filtered by Kiriyama filtration. The Amberlite Cl^- exchange resin was filtered by Kiriyama filtration and solvent was removed under reduced pressure. Then, 3-TMG pentaNAG was purified by gel filtration chromatography.

Synthesis of 2-TMG chitotriomycin and 3-TMG chitotriomycin

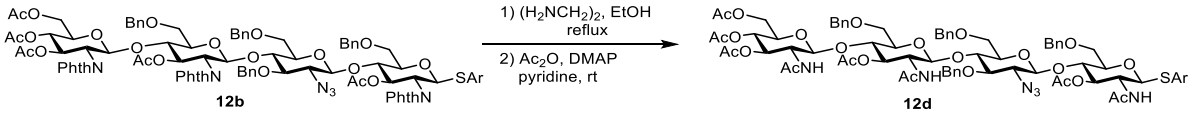


4- Fluorophenyl 3,4,6-tri-O-acetyl-2-phthalimido-β-D-glucopyranosyl-(1→4)-3-O-acetyl-6-Obenzyl-2-deoxy-2- phthalimido-β-D-glucopyranosyl-(1→4)-3-O-acetyl-6-O-benzyl-2-deoxy-2-azido-β-D-glucopyranosyl-(1→4)-3-O-acetyl-6-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (12b)
TLC (Hexane/EtOAc 1:1) R_f 0.20; $[\alpha]_D$ = -7.8699 (c = 1.17, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.91–7.88 (m, 2 H), 7.84–7.81 (m, 2 H), 7.79–7.77 (m, 4 H), 7.74–7.70 (m, 2 H), 7.38 (dd, *J* = 8.4, 5.4 Hz, 2 H), 7.35 (d, *J* = 7.2 Hz, 2 H), 7.31–7.23 (m, 3 H), 7.22 (d, *J* = 7.2 Hz, 2 H), 7.19–7.16 (m, 4 H), 7.14–7.11 (m, 3 H), 7.08 (*pseudo*-t, *J* = 7.8 Hz, 2 H), 6.94 (*pseudo*-t, *J* = 7.2 Hz, 1 H), 6.92–6.87 (m, 4 H), 6.66 (*pseudo*-t, *J* = 7.2 Hz, 1 H), 5.64 (*pseudo*-t, *J* = 9.0 Hz, 2 H), 5.57 (dd, *J* = 10.8, 9.0 Hz, 1 H), 5.56 (d, *J* = 10.8 z, 1 H), 5.39 (d, *J* = 8.4 Hz, 1 H), 5.19 (d, *J* = 8.4 Hz, 1 H), 5.08 (*pseudo*-t, *J* = 9.0 Hz, 1 H), 4.93 (d, *J* = 11.4 Hz, 1 H), 4.64 (d, *J* = 11.4 Hz, 1 H), 4.60 (d, *J* = 12.0 Hz, 1 H), 4.43 (d, *J* = 12.0 Hz, 1 H), 4.39 (d, *J* = 12.0

Hz, 1 H), 4.38–4.35 (m, 2 H), 4.32 (d, *J* = 12.0 Hz, 2 H), 4.28 (d, *J* = 10.8 Hz, 1 H), 4.16 (*pseudo*-t, *J* = 10.2 Hz, 1 H), 4.155 (dd, *J* = 10.8, 8.4 Hz, 1 H), 4.147 (*pseudo*-t, *J* = 9.0 Hz, 1 H), 4.12 (*pseudo*-t, *J* = 9.0 Hz, 1 H), 4.01 (*pseudo*-t, *J* = 9.6 Hz, 1 H), 3.91-3.89 (m, 2 H), 3.86 (dd, *J* = 10.8, 3.0 Hz, 1 H), 3.83 (*pseudo*-t, *J* = 9.0 Hz, 1 H), 3.79 (d, *J* = 10.2 Hz, 1 H), 3.67 (d, *J* = 10.2 Hz, 1 H), 3.47 (d, *J* = 10.2 Hz, 1 H), 3.40 (dt, *J* = 10.2, 3.0 Hz, 1 H), 3.27 (dd, *J* = 10.8, 3.0 Hz, 1 H), 3.25 (d, *J* = 11.4 Hz, 1 H), 3.08 (dd, *J* = 9.0, 8.4 Hz, 1 H), 3.03 (dd, *J* = 11.4, 3.0 Hz, 1 H), 3.01 (*pseudo*-t, *J* = 9.6 Hz, 1 H), 2.69 (dd, *J* = 9.6, 1.8 Hz, 1 H), 2.61 (dd, *J* = 9.6, 1.8 Hz, 1 H), 2.01 (s, 3 H), 1.97 (s, 3 H), 1.92 (s, 3 H), 1.81 (s, 3 H), 1.69 (s, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.44, 170.37, 170.1, 169.9, 169.3, 168.0, 167.7, 167.5, 167.2, 162.9 (d, *J* = 248.7 Hz), 138.4, 138.1, 137.9, 137.7, 135.9 (d, *J* = 8.3 Hz), 134.5, 134.4, 134.3, 134.1, 131.51, 131.45, 131.3, 131.2, 131.1, 128.2, 128.14, 128.05, 127.9, 127.7, 127.5, 127.34, 127.29, 127.1, 127.04, 126.99, 125.9 (d, *J* = 3.3 Hz), 123.7, 123.6, 123.50, 123.46, 115.9 (d, *J* = 21.8 Hz), 100.6, 96.7, 95.8, 82.9, 80.5, 78.7, 74.7, 74.6, 74.0, 73.7, 73.5, 73.1, 73.0, 72.2, 71.9, 71.4, 71.2, 70.9, 70.6, 68.2, 68.0, 67.8, 66.8, 65.6, 61.3, 55.6, 54.8, 53.6, 20.6, 20.5, 20.4, 20.3, 20.2; HRMS (ESI) *m/z* calcd for $C_{92}H_{87}FN_6O_{27}S$ $[M+Na]^+$, 1781.5216; found, 1781.5240.

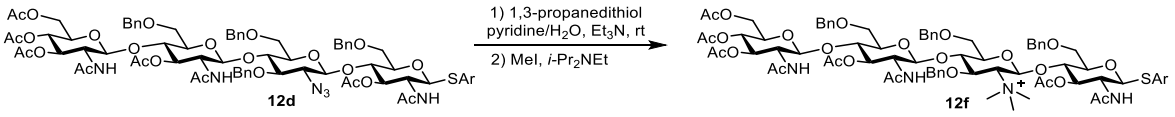


4-Fluorophenyl 4-*O*-acetyl-3,6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-*O*-benzyl-2-deoxy-2-azido-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (12a) TLC (Hexane/EtOAc 1:1) R_f 0.50; $[\alpha]_D$ = -4.3557 (*c* = 1.10, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.74–7.72 (m, 2 H), 7.82–7.76 (m, 4 H), 7.73–7.72 (m, 2 H), 7.67 (s, 2 H), 7.43 (d, *J* = 7.2 Hz, 2 H), 7.36–7.13 (m, 6 H), 7.30–7.27 (m, 3 H), 7.24–7.18 (m, 11 H), 7.08 (d, *J* = 6.6 Hz, 2 H), 6.98 (*pseudo*-t, *J* = 7.8 Hz, 4 H), 6.95–6.88 (m, 3 H), 6.83 (*pseudo*-t, *J* = 8.4 Hz, 2 H), 6.95–6.88 (m, 3 H), 6.83 (*pseudo*-t, *J* = 8.4 Hz, 2 H), 6.79 (*pseudo*-t, *J* = 7.2 Hz, 2 H), 5.65 (dd, *J* = 10.2, 9.0 Hz, 1 H), 5.55 (dd, *J* = 10.8, 9.0 Hz, 1 H), 5.48 (d, *J* = 8.4 Hz, 1 H), 5.36 (d, *J* = 8.4 Hz, 1 H), 5.13 (*pseudo*-t, *J* = 9.0 Hz, 1 H), 4.90 (d, *J* = 10.8 Hz, 1 H), 4.69 (*pseudo*-t, *J* = 10.8 Hz, 1 H), 4.59 (d, *J* = 12.0 Hz, 1 H), 4.49 (dd, *J* = 16.2, 10.8 Hz, 1 H), 4.42–4.37 (m, 3 H), 4.34 (d, *J* = 12.0 Hz, 1 H), 4.28 (*pseudo*-t, *J* = 12.0 Hz, 2 H), 4.23 (d, *J* = 10.8 Hz, 1 H), 4.20 (*pseudo*-t, *J* = 12.0 Hz, 1 H), 4.16 (*pseudo*-t, *J* = 10.8 Hz, 1 H), 4.04–4.00 (m, 2 H), 3.97 (*pseudo*-t, *J* = 10.8 Hz, 1 H), 3.83 (*pseudo*-t, *J* = 9.6 Hz, 1 H), 3.79 (d, *J* = 7.8 Hz, 1 H), 3.76 (dd, *J* = 10.2, 2.4 Hz, 1 H), 3.67 (d, *J* = 10.2 Hz, 1 H), 3.52–3.48 (m, 2 H), 3.43–3.40 (m, 2 H), 3.37–3.35 (m, 3 H), 3.27 (dd, *J* = 10.8, 6.0 Hz, 1 H), 3.14 (dd, *J* = 10.2, 3.0 Hz, 1 H), 2.94 (d, *J* = 10.2 Hz, 1 H), 2.91 (d, *J* = 10.2 Hz, 1 H), 3.15–3.12 (m, 2 H), 1.89 (s, 3 H), 1.85 (s, 3 H), 1.58 (s, 3 H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.5, 170.2, 169.7, 167.8, 167.3, 163.1 (d, *J* = 247.2 Hz), 138.32, 138.29, 138.19, 137.9, 137.8, 137.7, 136.1 (d, *J* = 8.4 Hz), 134.4, 134.2, 131.3, 128.5, 128.34, 128.30, 128.27, 128.2, 128.13, 128.05, 127.94, 127.8, 127.70, 127.61, 127.56, 127.51, 127.50, 127.48, 127.45, 127.1, 125.8 (d, *J* = 4.5 Hz), 123.7, 123.5, 115.9 (d, *J* = 21.75 Hz), 100.3, 97.2, 96.5, 82.7, 80.7, 78.5, 76.8, 74.9, 74.5, 74.3, 74.1, 73.9, 73.7, 73.6, 73.5, 72.94, 72.88, 72.76, 72.3, 72.2, 70.2, 69.6, 68.0, 67.9, 67.6, 66.0, 56.3, 55.1, 53.8, 20.9, 20.5, 20.2; HRMS (ESI) *m/z* calcd for $C_{102}H_{95}FN_6O_{25}S$ $[M+Na]^+$,1877.5944; found, 1877.5988.



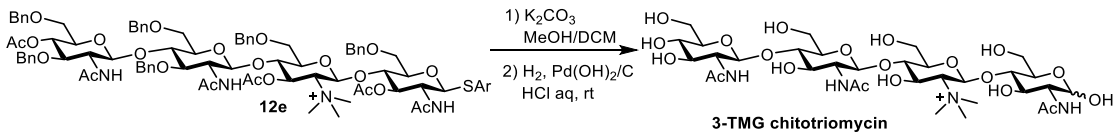
EtOH (6.1 mL) and $(H_2NCH_2)_2$ were added to a flask containing compound **12b** (0.125 mmol, 0.221 g) and stirred until uniform. The mixture was refluxed at 100°C and stirred overnight. TLC check (CH_2Cl_2/ CH_3OH 4:1) was carried out, and ESI-MS measurement was carried out. It was concentrated and dried in vacuo. A TLC check (CH_2Cl_2/CH_3OH 8:1) was performed, and CH_2Cl_2 was added to separate the layers (1N-HCl, $NaHCO_3$, H_2O). Column chromatography (CH_2Cl_2/CH_3OH 40:1) was performed. After concentration and vacuum drying, the target product **12d** was obtained in a yield of 0.078 mmol, 117 mg, and a yield of 62%.

4-Fluorophenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-*N*-acetyl-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-*N*-acetyl-β-D-glucopyranosyl-(1→4)-3,6-*O*-benzyl-2-deoxy-2-*N*-azido-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (12d)
TLC (CH_2Cl_2/MeOH 40:1) R_f 0.20; $[\alpha]_D$ = -56.909 (*c* = 1.13, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.52 (dd, *J* = 9.0, 5.4 Hz, 2 H), 7.49–7.22 (m, 20 H), 6.95 (*pseudo*-t, *J* = 8.4 Hz, 2 H), 5.48 (d, *J* = 9.6 Hz, 1 H), 4.87 (dd, *J* = 10.2, 9.6 Hz, 1 H), 4.70 (d, *J* = 12.0 Hz, 1 H), 4.66–4.59 (m, 5 H), 4.50 (dd, *J* = 12.0, 9.6 Hz, 2 H), 4.47 (d, *J* = 12.0 Hz, 1 H), 4.34 (dd, *J* = 12.6, 4.2 Hz, 1 H), 4.31 (d, *J* = 8.4 Hz, 1 H), 4.23 (d, *J* = 12.0 Hz, 1 H), 4.18 (d, *J* = 8.4 Hz, 1 H), 4.10–4.08 (m, 2 H), 4.04 (d, *J* = 12.0 Hz, 1 H), 3.97 (dd, *J* = 12.0, 1.8 Hz, 1 H), 3.93 (d, *J* = 8.4 Hz, 1 H), 3.90–3.77 (m, 7 H), 3.51 (ddd, *J* = 10.8, 7.8, 1.2 Hz, 1 H), 3.49 (*pseudo*-t, *J* = 2.4 Hz, 2 H), 3.45 (ddd, *J* = 10.2, 4.2, 2.4 Hz, 1 H), 3.41 (d, *J* = 3.6 Hz, 2 H), 3.19–3.16 (m, 3 H), 2.97 (d, *J* = 9.6 Hz, 1 H), 2.06 (s, 3 H), 2.058 (s, 3 H), 2.05 (s, 3 H), 2.02 (s, 3 H), 2.00 (s, 3 H) 1.984 (s, 3 H), 1.980, (s, 3 H), 1.69 (s, 3 H) 1.66 (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 171.6, 170.7, 170.6, 169.9, 169.7, 169.4, 160.1 (d, *J* = 595.95 Hz), 138.9, 137.9, 137.7, 136.9, 135.7 (d, *J* = 8.25 Hz), 129.3, 129.2, 129.04, 128.98, 128.87, 128.77, 128.5, 128.2, 127.94, 127.89, 127.6. 127.3, 127.0 (d, *J* = 2.85 Hz), 115.9 (d, *J* = 21.75 Hz), 100.9, 100.7, 100.3, 86.9, 81.3, 79.0, 76.1, 74.9, 74.4, 74.3, 74.0, 73.9, 73.6, 73.5, 73.3, 73.2, 72.7, 71.5, 68.2, 67.8, 67.3, 66.0, 61.8, 54.2, 54.0, 52.7, 23.4, 23.09, 23.07, 20.69, 20.65, 20.61, 20.5; HRMS (ESI) *m/z* calcd for $C_{74}H_{87}FN_6O_{24}S$ $[M+Na]^+$, 1517.5368; found, 1517.5337.



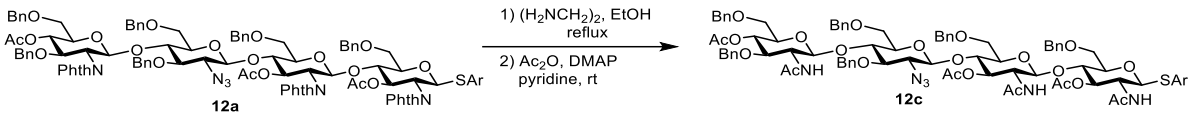
Pyridine/H_2O (6.3 mL/1.63 mL) was added to an eggplant flask containing compound **12d** (0.115 mmol, 172 mg), Et_3N (0.48 mL) was added, 1,3-propanedithiol (0.47 mL) was added, and the mixture was stirred overnight. ESI-MS measurement and TLC check (CH_2Cl_2/CH_3OH 8:1) were performed. The product was purified by column chromatography (CH_2Cl_2/CH_3OH/Et_3N 80:2:1) and dried in vacuo. THF (9.3 ml) was added, DIPEA (5.4 mmol, 0.94 mL) and Iodomethane (101.1 mmol, 6.3 mL) were added dropwise, and the mixture was stirred overnight. After ESI-MS measurement, solids were removed by Kiriyama filtration and concentrated. Purification with (CH_2Cl_2/CH_3OH 10:1) yielded 0.044 mmol, 67 mg, 70% yield of target compound **12f**.

4-Fluorophenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-*N*-acetyl-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-*N*-acetyl-β-D-glucopyranosyl-(1→4)-3,6-*O*-benzyl-2-deoxy-2-trimethylammonium-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (12f) TLC (CH_2Cl_2/MeOH 10:1) R_f 0.15; $[\alpha]_D$ = -15.672 (c = 0.99, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.40–7.30 (m, 18 H), 6.94 (*pseudo*-t, *J* = 8.4 Hz, 2 H), 5.29 (*pseudo*-t, *J* = 9.0 Hz, 1 H), 5.21 (d, *J* = 9.0 Hz, 1 H), 5.17 (d, *J* = 6.2 Hz, 2 H), 5.06 (*pseudo*-t, *J* = 9.6 Hz, 1 H), 4.98 (*pseudo*-t, *J* = 9.0 Hz, 1 H), 4.82 (d, *J* = 10.2 Hz, 1 H), 4.71 (d, *J* = 12.0 Hz, 2 H), 4.60 (*pseudo*-t, *J* = 11.4 Hz, 3 H), 4.522 (d, *J* = 12.0 Hz, 1 H), 4.517 (d, *J* = 11.4 Hz, 1 H), 4.48 (d, *J* = 11.40 Hz, 1 H), 4.46 (d, *J* = 10.8, 2.4 Hz, 1 H), 4.41 (d, *J* = 11.4 Hz, 1 H), 4.36 (dd, *J* = 12.6 4.2 Hz, 1 H), 4.33 (s, 1 H), 4.16 (s, 1 H), 4.07 (d, *J* = 4.2 Hz, 1 H), 4.05 4.01 (m, 3 H), 3.98 (*pseudo*-t, *J* = 9.0 Hz, 1 H), 4.07 (dd, *J* = 10.8, 4.2 Hz, 1 H), 3.80-3.72 (m, 5 H), 3.85 (d, *J* = 9.0 Hz, 1 H), 3.61 (dd, *J* = 9.0, 4.8 Hz, 3 H), 3.541 (d, *J* = 9.0 Hz, 2 H), 3.50 (*pseudo*-t, *J* = 9.0 Hz, 1 H), 3.28 (d, *J* = 7.2 Hz, 1 H), 3.17 (dd, *J* = 7.8, 4.8 Hz, 2 H), 3.04 (s, 9 H), 2.02 (s, 3 H), 2.008 (s, 3 H), 2.003 (s, 3 H), 1.98 (s, 3 H) 1.94 (s, 3 H), 1.91, (s, 3 H), 1.86 (s, 3 H) 1.81 (s, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 171.7, 171.2, 170.7, 170.64, 170.56, 170.0, 169.8, 169.7, 169.4, 162.9 (d, *J* = 246.45 Hz), 139.4, 138.0, 137.7, 137.1, 135.5 (d, *J* = 8.25 Hz), 129.2, 129.1, 129.0, 128.9, 128.8, 128.7, 128.4, 128.3, 127.3, 127.1 (d, *J* = 3.0 Hz), 115.9 (d, *J* = 21.45 Hz), 103.3, 100.6, 100.3, 86.7, 83.0, 79.2, 76.4, 74.9, 74.7, 74.5, 74.2, 74.0, 73.9, 73.48, 73.45, 73.3, 73.2, 72.7, 71.5, 68.2, 68.0, 67.72, 67.69, 61.7, 60.4, 56.8, 54.2, 54.1, 52.5, 23.3, 23.09, 23.06, 21.04, 20.77, 20.67, 20.60, 20.5; HRMS (ESI) *m/z* calcd for $C_{74}H_{87}FN_6O_{24}S$ $[M]^+$, 1511.6113; found, 1511.6177.



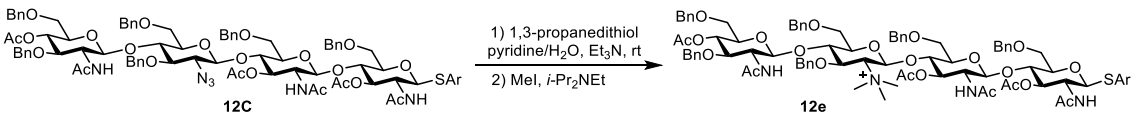
CH_3OH/CH_2Cl_2 (6.0 mL/3 mL) and K_2CO_3 (0.5 mmol, 70.4 mg) were added to an eggplant flask containing compound **12e** (0.041 mmol, 66 mg) for overnight. Completion of the reaction was confirmed by ESI-MS. Dowex-50wx4-20 (cation exchange resin H^+) washed with CH_3OH was added to stop the reaction. After concentration, liquid separation was carried out $Pd(OH)_2/C$ (240 mg) was added in the recovered clean target product and the mixture was replaced with hydrogen, then the mixture was stirred at the maximum stirring speed for one day. After the completion of the reaction exchange resin was added to stop the reaction. The amberlite OH-exchange resin was filtered by Kiriyama filtration. Amberlite Cl-exchange resin was added to change the anion to Cl^-.The Amberlite Cl^- exchange resin was filtered by Kiriyama filtration. The target product was confirmed by ESI-MS. Gel filtration was performed to isolate the pure product from the crude product. The obtained pure product is 12.6 mg (0.015 mmol, 37%)

β-D-Glucopyranose, *O*-2-deoxy-2-(acetylamino)-β-D-glucopyranosyl-(1 → 4)-2-deoxy-β-D-glucopyranosyl-(1 → 4)-*O*-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl-(1 → 4)-*O*-2-deoxy-2-(trimethylammonio-2-(acetylamino)-2-deoxy-β-D-glucopyranoside (3-TMG chitotriomycin) ^{1}H-NMR (600 MHz, D_2O) δ 5.26 (s, 1 H), 4.41 (d, *J* = 7.7 Hz, 2 H), 4.06–4.13 (1 H), 3.81–3.99 (m, 2 H), 3.75 (d, *J* = 11.9 Hz, 2H), 3.64–3.61 (m, 2 H), 3.59–3.54 (m, 4 H), 3.49 (s, 2H), 3.39 (d, *J* = 9.6 Hz, 2H), 3.33–3.30 (m, 1H), 3.14 (s, 9H), 1.89 (d, *J* = 8.8 Hz, 9H);^{13}C-NMR (151 MHz, D_2O) δ 175.2, 175.1, 102.01, 102.0, 101.94, 101.91, 96.4, 89.5, 79.8, 79.7, 79.2, 78.6, 77.94, 77.86, 76.4, 76.02, 76.0, 75.04, 75.01, 73.9, 72.53, 72.51, 70.6, 70.4, 70.2, 68.6, 68.4, 61.2, 61.04, 61.02, 60.7, 60.5, 56.1, 55.5, 54.93, 54.89, 54.22, 54.19, 54.03, 53.99, 53.96, 22.7, 22.6, 22.4; HRMS (ESI) *m/z* calcd for $C_{33}H_{59}FN_4O_{20}^+$ $[M+Na]^+$,831.3717; found, 831.3740.



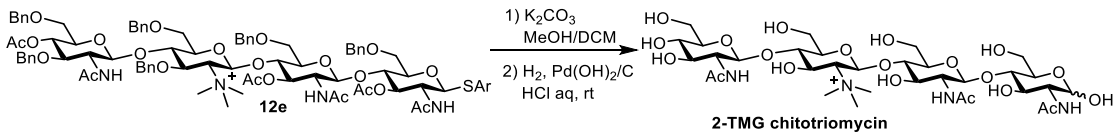
EtOH (5.7 mL) and $(H_2NCH_2)_2$ (17.6 mmol, 1.2 mL) were added to a flask containing compound 12a (0.12 mmol, 223 mg) and stirred until homogeneous. The mixture was refluxed at 100 °C and stirred overnight. A TLC check (CH_2Cl_2/CH_3OH 8:1) was performed, followed by ESI-MS measurement. 7.9ml pyridine and 1.94 ml acetic anhydride was added dropwise at 0 °C. A TLC check (CH_2Cl_2/CH_3OH 8:1) was performed, and ethyl acetate was added to separate the layers. The organic layer was collected, and Na_2SO_4 was added to dehydrate. Pure product was obtained by column chromatography (CH_2Cl_2/CH_3OH 10:1) as eluent. By concentrating and vacuum drying, the desired product **12c** was obtained in a yield of 0.104 mmol, 166 mg, and a yield of 86%.

4-Fluorophenyl 4-*O*-acetyl-3,6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-*O*-benzyl-2-deoxy-2-azido-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (12c)
TLC (CH_2Cl_2/MeOH 20:1) R_f =0.29; $[\alpha]_D$ = -45.599 (*c* =1.02, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.74–7.72 (m, 2 H), 7.82–7.76 (m, 4 H), 7.73–7.72 (m, 2 H), 7.67 (s, 2 H), 7.43 (d, *J* = 7.2 Hz, 2 H), 7.36–7.13 (m, 6 H), 7.30–7.27 (m, 3 H), 7.24–7.18 (m, 11 H), 7.08 (d, *J* = 6.6 Hz, 2 H), 6.98 (*pseudo*-t, *J* = 7.8 Hz, 4 H), 6.95–6.88 (m, 3 H), 6.83 (*pseudo*-t, *J* = 8.4 Hz, 2 H), 6.95-6.88 (m, 3 H), 6.83 (*pseudo*-t, *J* = 8.4 Hz, 2 H), 6.79 (*pseudo*-t, *J* = 7.2 Hz, 2 H), 5.65 (dd, *J* = 10.2, 9.0 Hz, 1 H), 5.55 (dd, *J* = 10.8, 9.0 Hz, 1 H), 5.48 (d, *J* = 8.4 Hz, 1 H), 5.36 (d, *J* = 8.4 Hz, 1 H), 5.13 (*pseudo*-t, *J* = 9.0 Hz, 1 H), 4.90 (d, *J* = 10.8 Hz, 1 H), 4.69 (*pseudo*-t, *J* = 10.8 Hz, 1 H), 4.59 (d, *J* = 12.0 Hz, 1 H), 4.49 (dd, *J* = 16.2, 10.8 Hz, 1 H), 4.42–4.37 (m, 3 H), 4.34 (d, *J* = 12.0 Hz, 1 H), 4.28 (*pseudo*-t, *J* = 12.0 Hz, 2 H), 4.23 (d, *J* = 10.8 Hz, 1 H), 4.20 (*pseudo*-t, *J* = 12.0 Hz, 1 H), 4.16 (*pseudo*-t, *J* = 10.8 Hz, 1 H), 4.04–4.00 (m, 2 H), 3.97 (*pseudo*-t, *J* = 10.8 Hz, 1 H), 3.83 (*pseudo*-t, *J* = 9.6 Hz, 1 H), 3.79 (d, *J* = 7.8 Hz, 1 H), 3.76 (dd, *J* = 10.2, 2.4 Hz, 1 H), 3.67 (d, *J* = 10.2 Hz, 1 H), 3.52–3.48 (m, 2 H), 3.43–3.40 (m, 2 H), 3.37–3.35 (m, 3 H), 3.27 (dd, *J* = 10.8, 6.0 Hz, 1 H), 3.14 (dd, *J* = 10.2, 3.0 Hz, 1 H), 2.94 (d, *J* = 10.2 Hz, 1 H), 2.91 (d, *J* = 10.2 Hz, 1 H), 3.15–3.12 (m, 2 H), 1.89 (s, 3 H), 1.85 (s, 3 H), 1.58 (s, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.5, 170.2, 169.7, 167.8, 167.3, 163.1 (d, *J* = 247.2 Hz), 138.32, 138.29, 138.19, 137.9, 137.8, 137.7, 136.1 (d, *J* = 8.4 Hz), 134.4, 134.2, 131.3, 128.5, 128.34, 128.30, 128.27, 128.23, 128.13, 128.05, 127.9, 127.8, 127.7, 127.61, 127.56, 127.51, 127.50, 127.48, 127.45, 127.1, 125.8 (d, *J* = 4.5 Hz), 123.7, 123.5, 115.9 (d, *J* = 21.75 Hz), 100.3, 97.2, 96.5, 82.7, 80.7, 78.5, 76.8, 74.9, 74.5, 74.3, 74.1, 73.9, 73.7, 73.6, 73.5, 72.94, 72.88, 72.76, 72.3, 72.2, 70.2, 69.6, 68.0, 67.9, 67.6, 66.0, 56.3, 55.2, 53.8, 20.9, 20.5, 20.2; HRMS (ESI) *m/z* calcd for $C_{102}H_{95}FN_6O_{25}S$ $[M+Na]^+$,1877.5944; found, 1877.5982.



Pyridine/H_2O (5.7 mL/1.4 mL) was added to an eggplant flask containing **12c** (0.104 mmol, 166 mg), Et_3N (0.43 mL) was added, 1,3-propanedithiol (0.85 mL) was added, and the mixture was stirred for 13 hours. ESI-MS measurement and TLC check (CH_2Cl_2/CH_3OH 8:1) were performed. The product was purified by column chromatography ($CH_2Cl_2/CH_3OH/Et_3N$ 80:2:1) and dried in vacuo. THF (8.4 mL) was added, DIPEA (4.92 mmol, 0.85 mL) and Iodomethane (91.6 mmol, 5.7 mL) were added dropwise, and the mixture

was stirred for 17 hours. TLC (CH_2Cl_2/CH_3OH 10:1) was performed folled by ESI-MS. Pure **12e** was isolated as white solid. **4-Fluorophenyl 4-*O*-acetyl-3,6-*O*-benzyl-2-deoxy-2-*N*-acetyl-β-D-glucopyranosyl-(1→4)-3,6-*O*-benzyl-2-deoxy-2-trimethylammonium-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-*N*-acetyl-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-*N*-acetyl-β-D-glucopyranoside (12e)** TLC (CH_2Cl_2/MeOH/Et_3N 40:1:0.5) R_f 0.46; $[\alpha]_D$ = -97.999 (c =-0.010, $CHCl_3$); TLC (CH_2Cl_2/MeOH 8:1) Rf 0.46; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.49 (dd, *J* = 9.0, 5.4 Hz, 2 H), 7.34–7.30 (m, 18 H), 7.26–7.21 (m, 10 H), 6.87 (*pseudo*-t, *J* = 9.0 Hz, 2 H), 6.47 (s, 1 H), 6.14 (s, 1 H), 5.28 (*pseudo*-t, *J* = 9.6 Hz, 1 H), 4.94 (*pseudo*-t, *J* = 7.2 Hz, 2 H), 5.07 (*pseudo*-t, *J* = 9.0 Hz, 1 H), 4.93 (*pseudo*-t, *J* = 10.2 Hz, 1 H), 4.73 (d, *J* = 8.4 Hz, 1 H), 4.69 (d, *J* = 12.0 Hz, 1 H), 4.66–4.62 (m, 3 H), 4.60 (d, *J* = 6.6 Hz, 1 H), 4.57 (d, *J* = 4.8 Hz, 1 H), 4.55 (*pseudo*-t, *J* = 6.0 Hz, 2 H), 4.47 (dd, *J* = 12.0, 6.0 Hz, 2 H), 4.39 (s, 4 H), 4.31 (d, *J* = 12.0 Hz, 1 H), 4.16 (*pseudo*-t, *J* = 9.6 Hz, 1 H), 4.13 (*pseudo*-t, *J* = 7.2 Hz, 1 H), 4.09 (*pseudo*-t, *J* = 10.2 Hz, 1 H), 4.05 (s, 1 H), 3.87 (dd, *J* = 18, 9.6 Hz, 1 H), 3.82 (*pseudo*-t, *J* = 8.4 Hz, 1 H), 3.77–3.57 (m, 8 H), 3.49 (*pseudo*-t, *J* = 9.0 Hz, 1 H), 3.42 (d, *J* = 4.2 Hz, 2 H), 3.01 (d, *J* = 6.6 Hz, 1 H), 1.97 (s, 3 H), 1.93 (s, 3 H), 1.910 (s, 3 H), 1.907 (s, 3 H), 1.88 (s, 3 H), 1.82 (s, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 171.2, 171.1, 170.9, 170.2, 169.8, 162.7 (d, *J* = 8246.6 Hz), 138.3, 137.8 (d, *J* = 4.5 Hz), 137.6, 136.1, 135.1 (d, *J* = 7.95 Hz), 128.73, 128.66, 128.62, 128.5, 128.43, 128.40, 128.00, 127.95, 127.91, 127.8, 127.7, 115.8 (d, *J* = 21.6 Hz), 100.1, 99.6, 92.6, 86.3, 80.1, 79.3, 78.7, 75.1, 74.7, 74.4, 74.3, 73.5, 73.4, 73.3, 73.2, 73.0, 72.4, 71.6, 71.3, 70.9, 70.1, 69.5, 56.0, 54.5, 53.9, 52.7, 29.7, 23.6, 23.3, 23.2, 21.2, 20.9, 20.8; HRMS (ESI) *m/z* calcd for $C_{87}H_{104}FN_4O_{22}S$ $[M+Na]^+$,1607.6841; found, 1607.6803.



CH_3OH/CH_2Cl_2 (7.0 mL/3.5 mL) and K_2CO_3 (0.51 mmol, 70.4 mg) were added to an eggplant flask containing compound 12e (0.051 mmol, 82.9 mg). After 6 hours, the reaction was completed by ESI-MS. Dowex-50wx4-20 (cation exchange resin H^+) washed with CH_3OH was added to stop the reaction. After concentration, liquid separation was carried out. The recovered clean target product (0.033 mmol, 50 mg) was benzylated. $Pd(OH)_2$/C (240 mg) was added, the mixture was replaced with hydrogen, and the mixture was stirred at the maximum stirring speed for one day. The exchange resin was added to stop the reaction. The Amberlite OH-exchange resin was filtered by Kiriyama filtration. Amberlite Cl-exchange resin was added to change the anion to Cl^-. The Amberlite Cl^- exchange resin was filtered by Kiriyama filtration. The target product was confirmed by ESI-MS, but the by-product was also confirmed. Gel filtration was performed, but it could not be completely removed. The by-product dissolved in CH_2Cl_2, and the target product did not dissolve in CH_2Cl_2. After that, it was washed with CH_2Cl_2. After that, it was dissolved in a small amount of MeOH and recrystallized by adding CH_2Cl_2. **β-D-Glucopyranose, *O*-2-deoxy-2-(acetylamino)-β-D-glucopyranosyl-(1 → 4)-*O*-2-deoxy-2-(trimethylammonio)-2-deoxy-β-D-glucopyranosyl-(1 → 4)-*O*-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl-(1 → 4)-2-(acetylamino)-2-deoxy-β-D-glucopyranoside (2-TMG chitotriomycin)** $[\alpha]_D$ = -0.3999 (*c* = 1.06, CH_3OH); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 5.37 (d, *J* = 3.6 Hz, 1 H), 4.53 (d, *J* = 8.4 Hz, 1 H), 4.22 (dd, *J* = 13.2, 6.0 Hz, 1 H), 4.00–3.95 (m, 1 H), 3.89–3.80 (m, 4 H), 3.77–3.65 (m, 10 H), 3.63 (d, *J* = 3.6 Hz, 1 H), 3.61–3.58 (m, 2 H), 3.52 (*pseudo*-t, *J* = 9.6 Hz, 1 H), 3.47–3.44 (m, 1 H), 4.42 (*pseudo*-t, *J* = 10.8 Hz, 1 H), 3.42 (s, 9 H), 2.02 (s, 3 H), 2.01 (s, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 101.6, 101.3, 95.1, 90.5, 78.4, 78.1, 76.7, 75.9, 75.4, 74.5, 73.3, 71.0, 70.9, 69.8, 68.2, 60.8, 60.6, 60.4, 56.1, 55.6, 53.9, 53.8, 48.9, 22.2, 21.9; HRMS (ESI) *m/z* calcd for $C_{33}H_{59}FN_4O_{20}^+$ $[M+Na]^+$, 831.3717; found, 831.3703.

References

(1) Nokami, T.; Sasaki, N.; Isoda, Y.; Itoh, T. *ChemElectroChem* **2016**, *3*, 2012–2016.

(2) Manmode, S.; Sato, T.; Sasaki, N.; Notsu, I.; Hayase, S.; Nokami, T.; Itoh, T. *Carbohydr. Res.* **2017**, *450*, 44–48.

Chapter 2.

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

Abstract

Both Chitin and its derivative chitosan are biopolymers with excellent bioactive properties, such as biodegradability, non-toxicity, biocompatibility, hemostatic activity, and antimicrobial activity. But the synthesis of longer chitin molecule remains challenging. In this study I described the synthesis of protected precursor of chitin oligosaccharides by electrochemical polyglycosylation of thioglycoside as a monomer. I synthesized up to hexasaccharide under optimized condition of reaction which was elongated till octasaccharide by the modification of polyglycosylation reaction. The reaction mechanism including optimization of several parameters are described in this study.

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 2-1).[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.

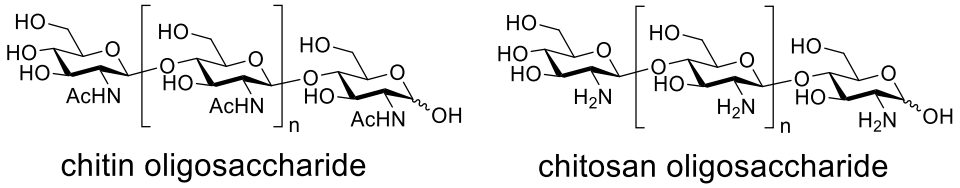


Figure 2-1. Structures of chitin and chitosan oligosaccharides.

Results and discussion

I initiated my 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 2-2).[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_6H_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_6H_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] In this case, lower conversion of the building block **1c** and lower yields of oligosaccharides **2c**-**4c** indicated that thus-generated oligosaccharides **2c**-**4c** with lower oxidation potentials also consumed electricity and converted to the corresponding hydroxy sugars as observed by MS analysis. Thioglycoside **1d** (Ar = 2,4-$F_2C_6H_3$, 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%).

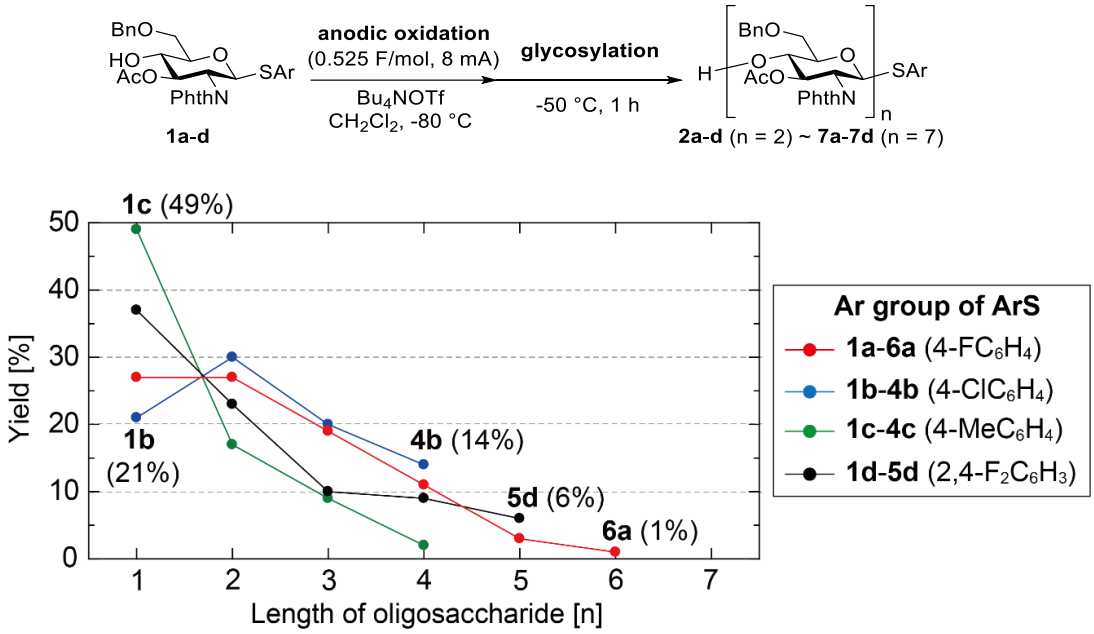


Figure 2-2. Effect of the anomeric leaving group on yields of oligosaccharides.

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 (**T**[2]), and it was revealed that glycosylation proceeded even at -80 °C (pink) (Figure 2-3). Although higher conversions of thioglycoside **1a** were observed at higher temperatures, we did not test glycosylation temperature above -30°C because of low stability of glycosylation intermediate at elevated temperature .[9] 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.

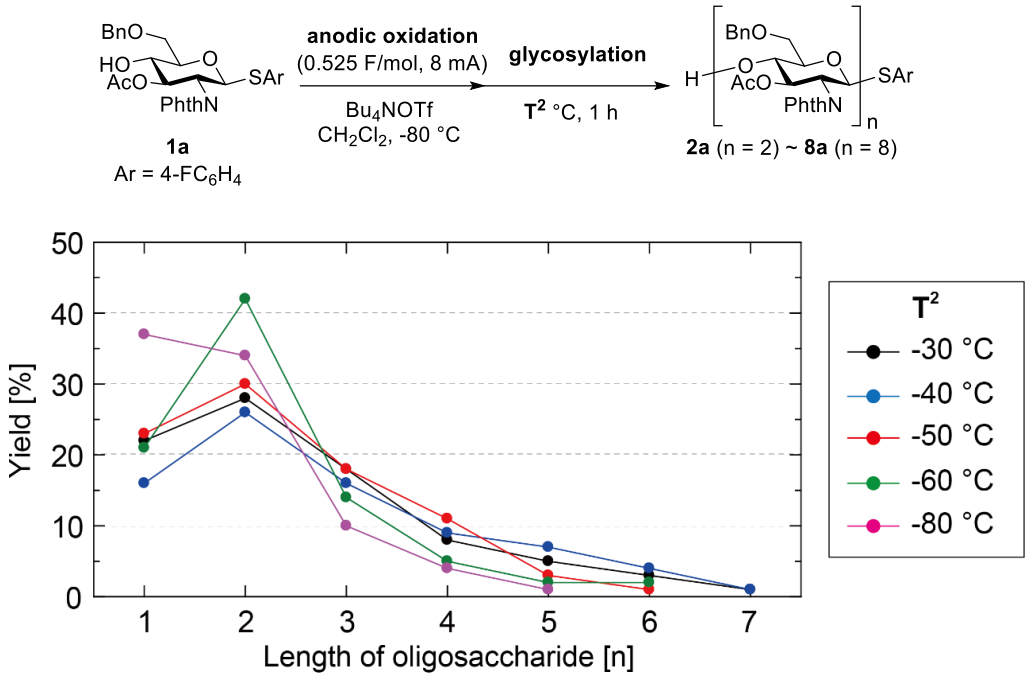
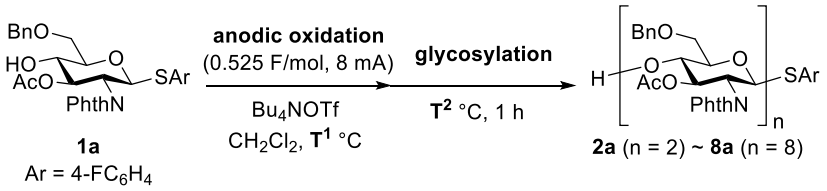


Figure 2-3. Influence of the glycosylation temperature on yields of oligosaccharides.

The temperature of anodic oxidation (**T**^{1}) was also investigated together with glycosylation temperature (**T**^{2}) because glycosylation must occur during the anodic oxidation at elevated temperature (Figure 2-4). 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 **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 **T**^{1}/**T**^{2} = -30 °C /-30 °C (Figure 2-5). 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 2-6. These by-products 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.



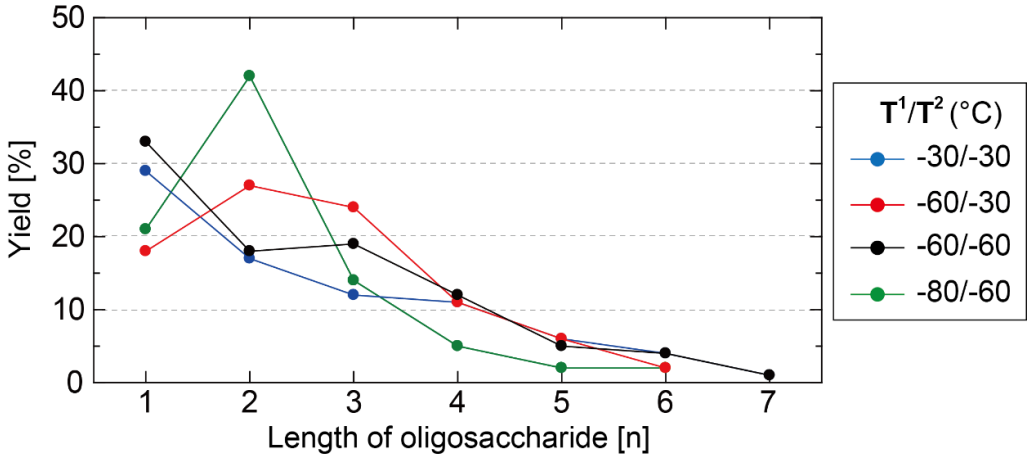


Figure 2-4. Influence of temperatures of anodic oxidation and glycosylation.

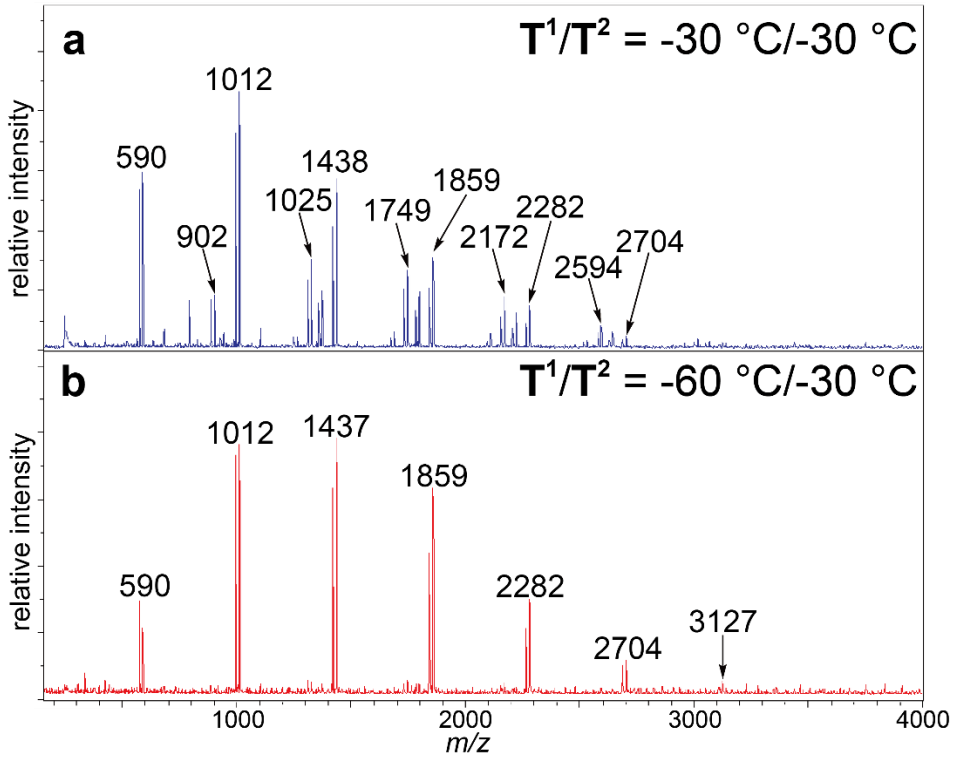


Figure 2-5. Spectra of MALDI-TOF MS of oligosaccharides.

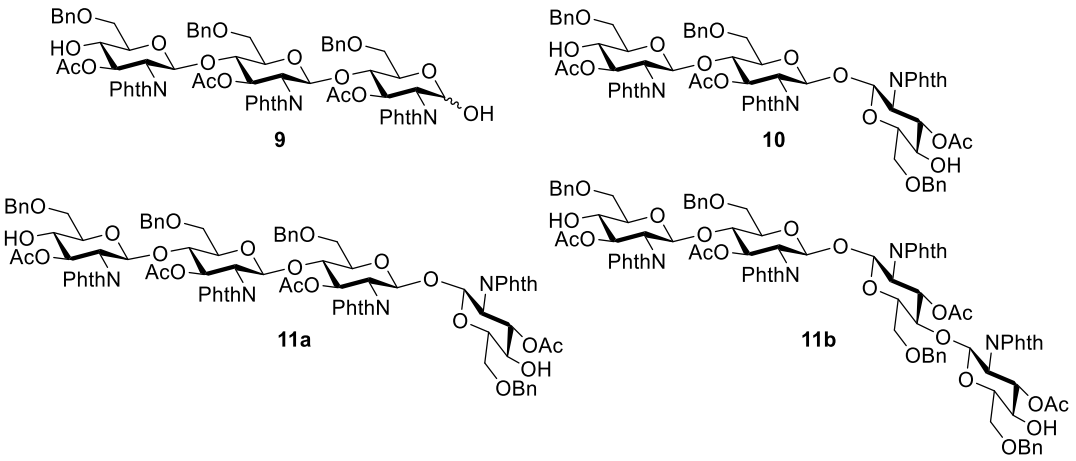


Figure 2-6. Proposed structures of by-products of electrochemical polyglycosylation.

There are two possible pathways for chain elongation in electrochemical polyglycosylation (Figure 2-7). In the path a, monosaccharide **1a** is converted to the corresponding glycosyl triflate **12** and 4-OH of oligosaccharide **2a-6a** reacts with **12**.[9] 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%.[10] 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 2-8). 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 2-1). These results strongly suggested that path a of Figure 7 is the most probable mechanism of the reaction.

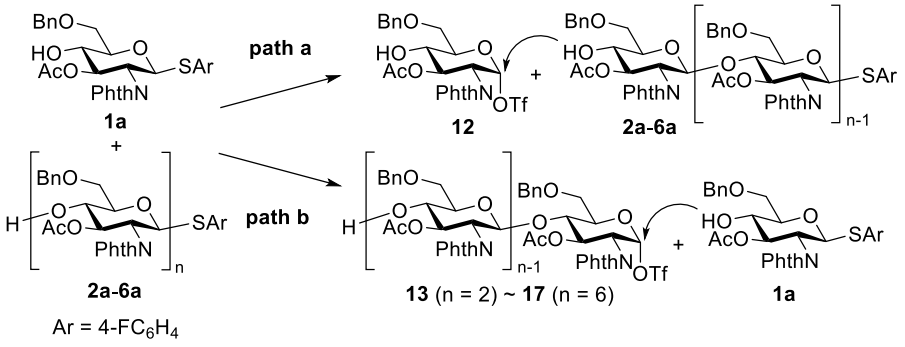


Figure 2-7. Proposed mechanisms of electrochemical polyglycosylation.

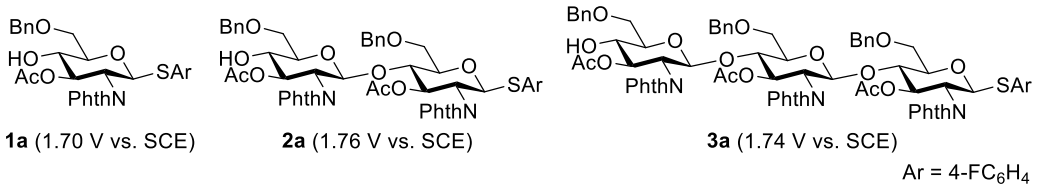
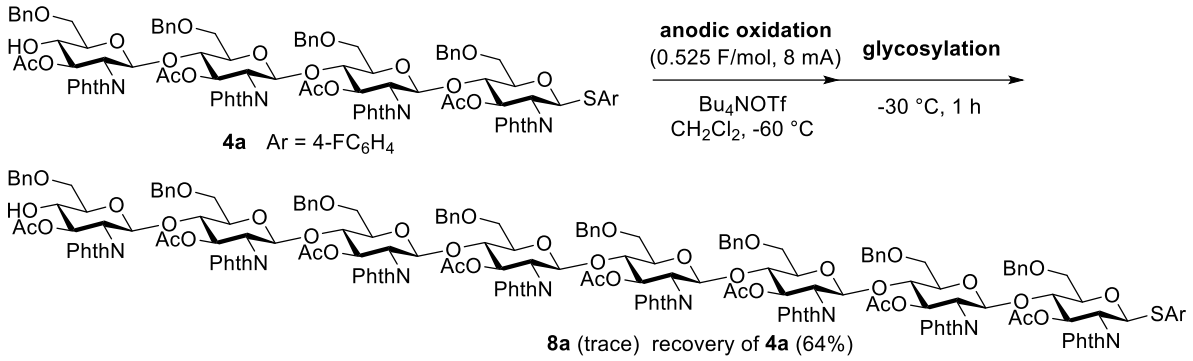
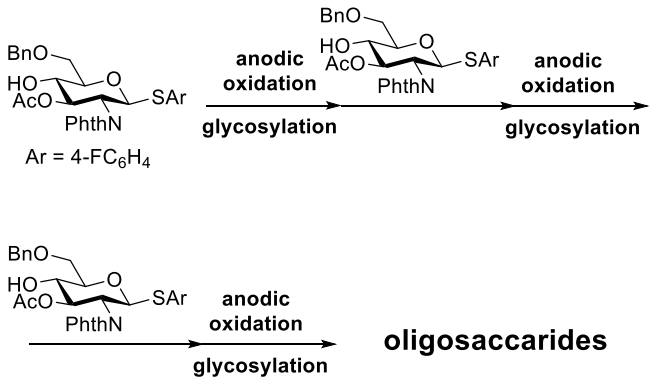


Figure 2-8. Oxidative potential of monosaccharide, disaccharide, and trisaccharide.



Scheme 2-1. Electrochemical dimerization of tetrasaccharide

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.[11] Higher reactivity of monosaccharide **1a** than oligosaccharides encouraged us to modify the protocol of electrochemical polyglycosylation (Figure 2-9). 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** (0.20 mmol, 109 mg) was added before the second anodic oxidation. After the second cycle we could isolate heptasaccharide **7a** (0.0015 mmol, 4.7 mg) together with the increasing amount of hexasaccharide **6a** (0.0031 mmol, 8.2 mg). We run the process up to the third cycle and isolated octasaccharide **8a** (0.0022 mmol, 7.6 mg) 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.



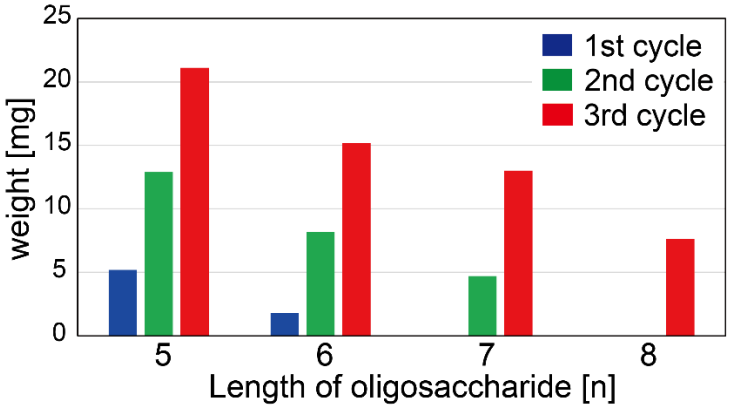


Figure 2-9. Weights of longer oligosaccharides **5a** (n = 5) ~ **8a** (n = 8) after cycles. Electrolysis conditions: Constant current (8.0 mA, 0.52 F/mol), Temperature (-60 °C for anodic oxidation, -30 °C glycosylation) Building block (**1a** (0.20 mmol, 109 mg) per one cycle as 0.2 M solution in dry CH_2Cl_2).

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.

References

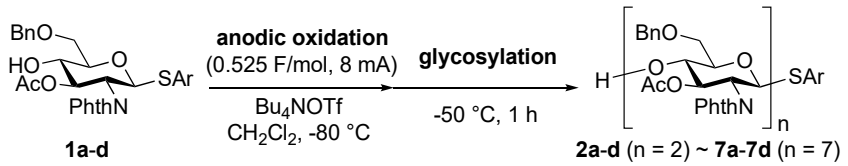
(1) Fittolani, G.; Tyrikos-Ergas, T.; Vargová, D.; Chaube, M. A.; Delbianco, M. *Beilstein J. Org. Chem.* **2021**, *17*, 1981–2025.
(2) Yang, Y.; Biao, Y.; *Tetrahedron* **2014**, *70,* 1023–1046.
(3) Nokami, T.; Hayashi, R.; Saigusa, Y.; Shimizu, A.; Liu, C.-Y.; Mong, K.-K. T.; *Org. Lett.* **2013**, *15*, 4520–4523.
(4) Isoda, Y.; Sasaki, N.; Kitamura, K.; Takahashi, S.; Manmode, S.; Takeda-Okuda, N.; Tamura, J.; Nokami, T.; Itoh, T. *Beilstein J. Org. Chem.* **2017**, *13*, 919–924.
(5) Hashimoto, H.; Abe, Y.; Yoshimura, J. *J. Carbohydr. Chem.* **1989**, *8,* 307–311.
(6) Kadokawa, J.; Watanabe, Y.; Karasu, M.; Tagaya, H.; Chiba, K. *Macromol. Rapid Commun.* **1996**, *17,* 367–372.
(7) Endo, H.; Ochi, M.; Rahman, M. A.; Hamada, T.; Kawano, T.; Nokami, T. *Chem. Commun.* **2022**, *58*, 7948-7951.
(8) Nokami, T.; Isoda, Y.; Sasaki, N.; Takaiso, A.; Hayase, S.; Itoh, T.; Hayashi, R.; Shimizu, A.; Yoshida, J. *Org. Lett.* **2015**, *17*, 1525–1528.
(9) Nokami, T.; Shibuya, A.; Tsuyama, H.; Suga, S.; Bowers, A. A.; Crich, D.; Yoshida, J. *J. Am. Chem. Soc.* **2007**, *129*, 10922–10928.
(10) The theoretical amount of electricity necessary for 100% conversion of the monosaccharide building block into disaccharide is 0.5 F/mol. This theoretical value was based on the hypothesis of selective activation of the monosaccharide.
(11) Li, K.; Xing, R.; Liu, S. *J. Agric. Food Chem.* **2020**, *68*, 12203–12211.

Experimental Section

General

All reactions were carried out under argon atmosphere except notice. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AVANCE II 600 (600 MHz for ^{1}H and 150 MHz for ^{13}C) and JEOL JNM-ECZ600 (600 MHz for ^{1}H and 150 MHz for ^{13}C). ESI-MS spectra were recorded on Thermo Scientific Exactive spectrometer. MALDI-TOF MS spectra were recorded on Bruker Ultraflextreme spectrometer. Optical rotation data was recorded on JASCO DIP-370 digital polarimeter. Merck TLC (silica gel 60 F_{254}) was employed for TLC analysis. Gel permeation chromatography (GPC) was used with JAI Labo Ace LC-5060 recycling preparative HPLC (eluent: $CHCl_3$). Kanto silica gel (spherical, neutral, 63-210 μm) and Sephadex LH-20 were used for Silica gel chromatography and gel filtration chromatography, respectively. Rotating-disk electrode voltammetry was carried out using BAS 700c analyzer and RRDE-3 rotating ring disk electrode. Measurements of oxidation potential of substrates (conc. 4.0 mM) were carried out in 0.1 M Bu_4NOTf/CH_2Cl_2 using a glassy carbon disk working electrode, a platinum wire counter electrode, and a saturated calomel electrode (SCE) as a reference electrode with sweep rate of 10 mV/s at 2000 r.p.m.. Compounds **1a,**[1] **1b,**[2] **1c,**[1] and **1d**[1] were synthesized according to the reported procedures. Unless otherwise mentioned, all reagents were obtained from commercial suppliers and used without extra purification.

1. Synthesis of oligosaccharides by electrochemical poly-glycosylation



The electrochemical polymerization synthesis of linear oligosaccharides (**2a**~**7a**) was carried out an H-type divided cell (4G glass filter). The cell had a carbon felt anode (Nippon Carbon JF-20-P7) and platinum square plate (20 mm×20 mm). Building block **1a** (0.39 mmol, 218 mg), Bu_4NOTf (1.00 mmol, 393 mg), and CH_2Cl_2 (20 mL) were added to the anodic chamber. Trifluoromethanesulfonic acid (0.4 mmol, 35 μL), Bu_4NOTf (1.00 mmol, 393 mg), and CH_2Cl_2 (20 mL) were added to the cathodic chamber. The constant current (8 mA (current density: 2.0 mA/cm^2), 45 V (electrode distance: 4.5 cm)) was employed at –80 °C with magnetic stirring until 0.52 F/mol of the electricity was consumed. After the electrolysis, the reaction was kept stirring at –50 °C for 1 h. After that, triethylamine (0.3 mL) was added to both chambers. The solution in both chambers was collected in eggplant flask, and the solvent was removed under reduced pressure. The mixture was dissolved in EtOAc and washed with water (3 times) and brine, respectively. The solution was dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified with preparative-GPC to afford linear oligosaccharides **2a** (n = 2, 0.053 mmol, 52.0 mg, 27%), **3a** (n = 3, 0.0248 mmol, 34.7 mg, 19%), **4a** (n = 4, 0.0106 mmol, 19.3 mg, 11%), **5a** (n = 5, 2.22 μmol, 5.0 mg, 3%), **6a** (n = 6, 0.090 μmol, 2.4 mg, 1%), and **7a** (n = 7, trace) as white solids. Recovered yield of buliding block **1a** was 27% (58.2 mg, 0.1055 mmol).

4-Fluorophenyl (3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**2a**) TLC (Hexane/EtOAc 1:2) R_f 0.57; $[\alpha]_D$ = –7.88 (c = 1.0, $CHCl_3$, 26 °C); E_{ox} = 1.76 V vs. SCE; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.86–7.77 (m, 4 H), 7.76–7.72 (m, 2 H), 7.71–7.67 (m, 2 H), 7.35–7.32 (m, 6 H), 7.31–7.26 (m, 4 H), 7.22 (d, J

= 7.0 Hz, 2 H), 6.82 (*pseudo*-t, *J* = 8.6 Hz, 2 H), 5.67 (dd, *J* = 9.9, 8.9 Hz, 1 H), 5.57 (dd, *J* = 10.6, 8.9 Hz, 1 H), 5.50 (d, *J* = 10.5 Hz, 1 H), 5.45 (d, *J* = 8.3 Hz, 1 H), 4.54 (d, *J* = 11.8 Hz, 1 H), 4.49 (d, *J* = 11.8 Hz, 1 H), 4.37 (d, *J* = 11.8 Hz, 1 H), 4.31 (d, *J* = 11.9 Hz, 1 H), 4.15 (*pseudo*-t, *J* = 10.3 Hz, 1 H), 4.11 (dd, *J* = 10.7, 8.3 Hz, 1 H), 4.03 (*pseudo*-t, *J* = 9.2 Hz, 1 H), 3.81 (td, *J* = 9.2, 3.2 Hz, 1 H), 3.75 (dd, *J* = 10.0, 4.0 Hz, 1 H), 3.66 (dd, *J* = 10.0, 4.9 Hz, 1 H), 3.52 (dd, *J* = 9.8, 2.3 Hz, 2 H), 3.49–3.43 (m, 2 H), 2.96 (d, *J* = 2.8 Hz, 1 H), 1.88 (s, 3 H), 1.82 (s, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 171.0, 170.0, 167.8, 167.3, 163.0 (d, *J* = 247.5 Hz), 138.2, 137.4, 136.1 (d, *J* = 9.0 Hz), 134.4, 134.3, 133.2, 131.7,131.4, 131.2, 128.5, 128.3, 128.0, 127.7, 127.5, 127.4, 125.8 (d, *J* = 3.0 Hz), 123.7, 123.5, 115.9 (d, *J* = 22.5 Hz), 97.2, 82.6, 78.5, 74.1, 73.6, 73.4, 73.2, 72.7, 72.4, 71.4, 70.0, 67.8, 54.9, 53.8, 20.63, 20.61; HRMS (ESI) *m/z* calculated for $C_{52}H_{47}FKN_2O_{14}S$ $[M+K]^+$, 1013.2364; found, 1013.2322.

4-Fluorophenyl (3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-(3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**3a**) TLC (Hexane/EtOAc 1:2) R_f 0.50; $[\alpha]_D$ = –15.8 (*c* = 1.0, $CHCl_3$, 26 °C). E_{ox} = 1.74 V vs. SCE; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.88–7.77 (m, 6 H), 7.76–7.67 (m, 6 H), 7.35–7.31 (m, 4 H), 7.30–7.26 (m, 5 H), 7.25–7.20 (m, 5 H), 7.14 (*pseudo*-t, *J* = 7.8 Hz, 2 H), 6.82 (*pseudo*-t, *J* = 8.6 Hz, 2 H), 5.58 (*pseudo*-t, *J* = 9.4 Hz, 1 H), 5.54 (td, *J* = 10.6, 1.6 Hz, 1 H), 5.51 (td, *J* = 10.6, 1.6 Hz, 1 H), 5.46 (d, *J* = 10.5 Hz, 1 H), 5.38 (d, *J* = 8.3 Hz, 1 H), 5.27 (d, *J* = 8.4 Hz, 1 H), 4.52 (d, *J* = 11.7 Hz, 1 H), 4.47 (d, *J* = 11.8 Hz, 1 H), 4.43 (d, *J* = 11.8 Hz, 1 H), 4.42 (d, *J* = 11.6 Hz, 1 H), 4.38 (d, *J* = 11.8 Hz, 1 H), 4.31 (d, *J* = 11.6 Hz, 1 H), 4.14 (dd, *J* = 9.4, 5.5 Hz, 1 H), 4.12 (dd, *J* = 9.4, 4.4 Hz, 1 H), 4.07 (dd, *J* = 10.7, 8.3 Hz, 1 H), 4.02 (dd, *J* = 10.4, 8.2 Hz, 1 H), 3.99 (*pseudo*-t, *J* = 9.4 Hz, 1 H), 3.79 (td, *J* = 9.2, 3.2 Hz, 1 H), 3.72 (dd, *J* = 9.9, 4.0 Hz, 1 H), 3.63 (dd, *J* = 9.9, 4.9 Hz, 1 H), 3.54 (d, *J* = 10.4 Hz, 1 H), 3.46 (dd, *J* = 10.7, 3.7 Hz, 1 H), 3.42 (d, *J* = 10.9 Hz, 2 H), 3.30 (dd, *J* = 11.2, 3.5 Hz, 1 H), 3.27 (dd, *J* = 9.2, 4.4 Hz, 1 H), 3.10 (d, *J* = 8.8 Hz, 1 H), 2.88 (d, *J* = 3.3 Hz, 1 H), 1.80 (s, 3 H), 1.71 (s, 3 H), 1.63 (s, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 171.0, 170.2, 170.1, 168.1, 167.8, 167.2 163.0 (d, *J* = 247.5 Hz), 138.2, 138.1, 137.4, 136.0 (d, *J* = 9.0 Hz), 134.4, 134.3, 134.1, 131.7, 131.2, 128.5, 128.2, 128.1, 127.9, 127.6, 127.4, 127.36, 127.26, 127.1, 125.9 (d, *J* = 3.3 Hz), 123.6, 123.5, 115.9 (d, *J* = 22.5 Hz), 96.6, 96.5, 82.6, 78.5, 74.0, 73.6, 72.6, 72.3, 71.7, 71.4, 71.2, 70.0, 67.9, 67.3 55.3, 54.9, 53.8, 20.61, 20.57, 20.46; HRMS (ESI) *m/z* calculated for $C_{75}H_{68}FKN_3O_{21}S$ $[M+K]^+$, 1436.3682; found, 1436.3613.

4-Fluorophenyl (3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-(3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-(3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**4a**) TLC (Hexane/EtOAc 1:2) R_f 0.37; $[\alpha]_D$ = –22.9 (*c* = 1.1, $CHCl_3$, 24 °C); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.89–7.65 (m, 16 H), 7.35–7.26 (m, 9 H), 7.25–7.17 (m, 7 H), 7.10 (*pseudo*-t, *J* = 7.8 Hz, 2 H), 6.99 (*pseudo*-t, *J* = 7.8 Hz, 2 H), 6.94 (*pseudo*-t, *J* = 7.2 Hz, 1 H), 6.82 (*pseudo*-t, *J* = 8.4 Hz, 2 H), 6.69 (*pseudo*-t, *J* = 7.2 Hz, 1 H), 5.57 (dd, *J* = 10.2, 9.0 Hz, 1 H), 5.49 (dd, *J* = 10.8, 9.0 Hz, 1 H), 5.48–5.44 (m, 3 H), 5.34 (d, *J* = 8.4 Hz, 1 H), 5.21 (d, *J* = 8.4 Hz, 1 H), 5.18 (d, *J* = 8.4 Hz, 1 H), 4.52 (d, *J* = 11.4 Hz, 1 H), 4.47 (d, *J* = 11.4 Hz, 1 H), 4.45–4.33 (m, 5 H), 4.32 (d, *J* = 11.4 Hz, 1 H), 4.14 (d, *J* = 10.8 Hz, 1 H), 4.10 (d, *J* = 9.0 Hz, 1 H), 4.09–3.95 (m, 5 H), 3.77 (*pseudo*-t, *J* = 9.6 Hz, 1 H), 3.71 (dd, *J* = 10.2, 4.2 Hz, 1 H), 3.62 (dd, *J* = 9.6, 4.8 Hz, 1 H), 3.53 (d, *J* = 9.6 Hz, 1 H), 3.47–3.43 (m, 2 H), 3.41–3.37 (m, 2 H), 3.26–3.20 (m, 3 H), 3.01 (dd, *J* = 9.6, 1.8 Hz, 1 H), 2.86 (s, 1 H), 2.79 (d, *J* = 9.0 Hz, 1 H), 1.87 (s, 3 H), 1.79 (s, 3 H), 1.73 (s, 3 H), 1.67 (s, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 171.0, 170.4, 170.3, 170.2, 168.2, 167.8, 167.3, 163.0 (d, *J* = 247.2 Hz), 138.3, 138.19, 138.17, 137.5, 136.0 (d, *J* = 8.7 Hz), 134.5, 134.4, 134.3, 134.2, 131.7, 131.5, 131.2, 128.6, 128.3, 128.1, 128.03, 127.97, 127.7, 127.5, 127.3, 127.2, 127.0, 126.0 (d, *J* = 3.3 Hz), 123.7, 123.63, 123.58, 123.45, 123.39, 115.9 (d, *J* = 20.9 Hz), 96.60, 96.56, 96.0, 82.7, 78.5, 73.9, 73.8, 73.6, 73.4, 73.3, 73.1, 73.0, 72.6, 72.33, 72.30, 72.1, 71.8, 71.3, 70.8,

69.9, 67.9, 67.5, 55.4, 55.2, 55.0, 53.8, 20.67, 20.65, 20.53; HRMS (ESI) *m/z* calculated for $C_{98}H_{89}FN_4NaO_{28}S$ $[M+Na]^+$, 1843.5260; found, 1843.5217.

4-Fluorophenyl (3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-(3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-(3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-(3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**5a**) TLC (Hexane/EtOAc 1:2) R_f 0.28; $[\alpha]_D$ = –27.0 (*c* = 1.2, $CHCl_3$, 25 °C); ^1H NMR ($CDCl_3$, 600 MHz) δ 7.89–7.64 (m, 20 H), 7.34–7.25 (m, 10 H), 7.23–7.14 (m, 9 H), 7.07 (*pseudo*-t, *J* = 7.8 Hz, 2 H), 6.92 (*pseudo*-t, *J* = 7.8 Hz, 2 H), 6.90 (*pseudo*-t, *J* = 7.8 Hz, 2 H), 6.81 (*pseudo*-t, *J* = 9.0 Hz, 2 H), 6.61 (*pseudo*-t, *J* = 7.2 Hz, 1 H), 6.55 (*pseudo*-t, *J* = 7.2 Hz, 1 H), 5.55 (dd, *J* = 10.2, 9.6 Hz, 1 H), 5.50–5.40 (m, 4 H), 5.36 (dd, *J* = 10.2, 9.0 Hz, 1 H), 5.31 (d, *J* = 8.4 Hz, 1 H), 5.18 (d, *J* = 8.4 Hz, 1 H), 5.12 (d, *J* = 8.4 Hz, 1 H), 5.10 (d, *J* = 8.4 Hz, 1 H), 4.50 (d, *J* = 11.4 Hz, 1 H), 4.46 (d, *J* = 11.4 Hz, 1 H), 4.44–4.28 (m, 8 H), 4.13–3.90 (m, 10 H), 3.75 (td, *J* = 9.6, 3.6 Hz, 1 H), 3.69 (dd, *J* = 9.6, 3.6 Hz, 1 H), 3.61 (dd, *J* = 9.6, 4.8 Hz, 1 H), 3.51 (d, *J* = 9.6 Hz, 1 H), 3.46–3.33 (m, 4 H), 3.23–3.12 (m, 4 H), 2.97 (d, *J* = 9.0 Hz, 1 H), 2.89 (d, *J* = 3.6 Hz, 1 H), 2.71 (d, *J* = 9.0 Hz, 1 H), 2.65 (d, *J* = 8.4 Hz, 1 H), 1.85 (s, 3 H), 1.77 (s, 3 H), 1.72 (s, 3 H), 1.69 (s, 3 H), 1.62 (s, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.9, 170.33, 170.30, 170.2, 170.1, 168.0, 167.7, 167.20, 167.16, 162.9 (d, *J* = 247.2 Hz), 138.2, 138.13, 138.12, 138.08, 137.4, 135.9 (d, *J* = 7.7 Hz), 134.4, 134.3, 134.23, 134.16, 134.1, 131.6, 131.5, 131.3, 131.1, 128.5, 128.3, 128.2, 128.1, 128.03, 127.89, 127.87, 127.6, 127.42, 127.38, 127.36, 127.19, 127.15, 127.06, 126.90, 126.85, 125.9 (d, *J* = 3.3 Hz), 123.63, 123.54, 123.49, 123.4, 123.3, 115.8 (d, *J* = 21.9 Hz), 96.5, 96.4, 95.9, 95.8, 82.7, 73.8, 73.6, 73.3, 73.2, 73.0, 72.9, 72.5, 72.2, 72.0, 71.9, 71.7, 71.3, 71.2, 70.7, 70.6, 69.9, 67.8, 67.5, 67.4, 55.3, 55.2, 55.1, 54.8, 53.7, 20.58, 20.55, 20.44; HRMS (ESI) *m/z* calculated for $C_{121}H_{110}FN_5NaO_{35}S$ $[M+Na]^+$, 2266.6578; found, 2266.6513.

4-Fluorophenyl (3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-(3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-(3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-(3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-(3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**6a**) TLC (Hexane/EtOAc 1:2) R_f 0.20; $[\alpha]_D$ = –28.9 (*c* = 0.9, $CHCl_3$, 28 °C); ^1H NMR ($CDCl_3$, 600 MHz) δ 7.90–7.64 (m, 24 H), 7.34–7.26 (m, 9 H), 7.23–7.15 (m, 12 H), 7.08 (*pseudo*-t, *J* = 7.8 Hz, 2 H), 6.94 (*pseudo*-t, *J* = 7.8 Hz, 2 H), 6.93–6.85 (m, 4 H), 6.82 (*pseudo*-t, *J* = 8.4 Hz, 2 H), 6.61 (*pseudo*-t, *J* = 7.2 Hz, 1 H), 6.53 (*pseudo*-t, *J* = 7.8 Hz, 1 H), 6.49 (*pseudo*-t, *J* = 7.2 Hz, 1 H), 5.56 (dd, *J* = 10.2, 9.0 Hz, 1 H), 5.48 (dd, *J* = 10.8, 9.0 Hz, 1 H), 5.46–5.41 (m, 3 H), 5.38–5.31 (m, 3 H), 5.18 (d, *J* = 8.4 Hz, 1 H), 5.12 (d, *J* = 8.4 Hz, 1 H), 5.10 (d, *J* = 8.4 Hz, 1 H), 5.06 (d, *J* = 8.4 Hz, 1 H), 4.51 (d, *J* = 11.4 Hz, 1 H), 4.46 (d, *J* = 11.4 Hz, 1 H), 4.44–4.29 (m, 10 H), 4.14–3.89 (m, 12 H), 3.76 (td, *J* = 9.6, 3.6 Hz, 1 H), 3.70 (dd, *J* = 9.6, 3.6 Hz, 1 H), 3.62 (dd, *J* = 9.6, 4.8 Hz, 1 H), 3.52 (d, *J* = 10.2 Hz, 1 H), 3.45–3.35 (m, 5 H), 3.23–3.16 (m, 3 H), 3.14–3.09 (m, 2 H), 2.98 (d, *J* = 9.0 Hz, 1 H), 2.89 (d, *J* = 3.6 Hz, 1 H), 2.70 (d, *J* = 9.6 Hz, 1 H), 2.64 (d, *J* = 9.0 Hz, 1 H), 2.59 (d, *J* = 9.6 Hz, 1 H), 1.86 (s, 3 H), 1.78 (s, 3 H), 1.73 (s, 3 H), 1.71 (s, 3 H), 1.69 (s, 3 H), 1.65 (s, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 171.2, 170.34, 170.30, 170.2, 170.1, 168.01, 167.97, 167.7, 167.2, 167.1 162.9 (d, *J* = 247.2 Hz), 138.2, 138.15, 138.11, 138.08, 137.3, 135.9 (d, *J* = 7.7 Hz), 134.3, 134.1, 131.60, 131.56, 131.4, 131.1, 128.5, 128.2, 128.0, 127.90, 127.85, 127.80, 127.6, 127.42, 127.35, 127.26, 127.18, 127.14, 127.05, 126.82, 126.79, 125.9 (d, *J* = 3.3 Hz), 123.66, 123.54, 123.49, 123.38, 115.8 (d, *J* = 21.9 Hz), 96.49, 96.41, 95.89, 95.74, 82.7, 78.4, 73.8, 73.6, 73.5, 73.3, 73.1, 73.0, 72.5, 72.2, 71.9, 71.7, 71.3, 71.2, 70.73, 70.70, 70.6, 69.9, 67.8, 67.5, 67.4, 55.27, 55.14, 55.11, 55.08, 54.8,

53.7, 20.58, 20.55, 20.44; HRMS (ESI) *m/z* calculated for $C_{144}H_{131}FN_6NaO_{42}S$ $[M+Na]^+$, 2689.7896; found, 2689.7849.

4-Fluorophenyl (3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-(3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-(3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-(3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-(3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-(3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**7a**) TLC (Hexane/EtOAc 1:2) R_f 0.17; $[\alpha]_D$ = –28.9 (*c* = 0.64, $CHCl_3$, 28 °C); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.89–7.66 (m, 28 H), 7.35–7.27 (m, 10 H), 7.24–7.13 (m, 13 H), 7.08 (*pseudo*-t, *J* = 7.8 Hz, 2 H), 6.94 (*pseudo*-t, *J* = 7.8 Hz, 2 H), 6.92–6.84 (m, 6 H), 6.82 (*pseudo*-t, *J* = 7.8 Hz, 2 H), 6.61 (*pseudo*-t, *J* = 7.8 Hz, 1 H), 6.52 (*pseudo*-t, *J* = 7.2 Hz, 1 H), 6.48 (*pseudo*-t, *J* = 7.8 Hz, 1 H), 6.46 (*pseudo*-t, *J* = 7.8 Hz, 1 H), 5.56 (dd, *J* = 10.2, 9.0 Hz, 1 H), 5.48 (dd, *J* = 10.8, 9.0 Hz, 1 H), 5.46–5.40 (m, 3 H), 5.37–5.30 (m, 4 H), 5.18 (d, *J* = 8.4 Hz, 1 H), 5.11 (d, *J* = 9.0 Hz, 1 H), 5.09 (d, *J* = 8.4 Hz, 1 H), 5.048 (d, *J* = 8.4 Hz, 1 H), 5.046 (d, *J* = 8.4 Hz, 1 H), 4.51 (d, *J* = 11.4 Hz, 1 H), 4.46 (d, *J* = 11.4 Hz, 1 H), 4.44–4.37 (m, 6 H), 4.36–4.28 (m, 6 H), 4.14–3.88 (m, 16 H), 3.79–3.74 (m, 1 H), 3.70 (dd, *J* = 9.6, 3.6 Hz, 1 H), 3.61 (dd, *J* = 10.2, 4.8 Hz, 1 H), 3.52 (d, *J* = 10.2 Hz, 1 H), 3.45–3.35 (m, 6 H), 3.23–3.16 (m, 3 H), 3.13–3.07 (m, 2 H), 2.97 (d, *J* = 9.6 Hz, 1 H), 2.87 (s, 1 H), 2.70 (d, *J* = 10.2 Hz, 1 H), 2.63 (d, *J* = 9.0 Hz, 1 H), 2.58–2.55 (m, 1 H), 1.86 (s, 3 H), 1.77 (s, 3 H), 1.73 (s, 3 H), 1.71 (s, 3 H), 1.70 (s, 3 H), 1.68 (s, 3 H), 1.64 (s, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 171.0, 170.43, 170.41, 170.38, 170.30, 170.2, 168.05, 167.99, 167.8, 167.3, 167.23, 167.18, 167.17, 163.0 (d, *J* = 247.5 Hz), 138.2, 138.11, 138.08, 138.04, 138.0, 137.4, 136.0 (d, *J* = 8.3 Hz), 134.39, 134.32, 134.25, 134.17, 131.6, 131.5, 131.4, 131.3, 131.1, 128.5, 128.2, 128.0, 127.91, 127.88, 127.87, 127.81, 127.6, 127.40, 127.37, 127.23, 127.21, 127.18, 127.15, 127.0, 126.9, 126.84, 126.80, 125.9 (d, *J* = 2.9 Hz), 123.69, 123.66, 123.58, 123.52, 123.4, 115.8 (d, *J* = 21.9 Hz), 96.5, 96.4, 95.9, 95.7, 82.7, 78.4, 77.3, 77.0, 76.8, 73.8, 73.57, 73.53, 73.47, 73.32, 73.27, 73.0, 72.8, 72.5, 72.2, 71.9, 71.7, 71.2, 71.1, 70.72, 70.69, 70.61, 69.8, 67.8, 67.5, 67.4, 55.3, 55.12, 55.08, 54.9, 53.7, 20.59, 20.56, 20.45, 20.43; HRMS (ESI) *m/z* calculated for $C_{167}H_{152}FKN_7O_{49}S$ $[M+K]^+$, 3128.8954; found, 3128.8948.

Buliding block **1b** (0.40 mmol, 220 mg) afforded oligosaccharides **2b** (*n* = 2, 0.060 mmol, 60 mg, 30%), **3b** (*n* = 3, 0.027 mmol, 40 mg, 20%), and **4b** (*n* = 4, 0.014 mmol, 26 mg, 14%) as white solids. Recovered yield of buliding block **1b** was 21% (47 mg, 0.083 mmol).

4-Chlorophenyl (3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**2b**); TLC (Hexane/EtOAc 1:2) R_f 0.63; $[\alpha]_D$ = –8.62 (*c* = 1.3, $CHCl_3$, 27 °C); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.86–7.78 (m, 4 H), 7.76–7.68 (m, 4 H), 7.35–7.26 (m, 10 H), 7.23–7.21 (m, 2 H), 7.10–7.07 (m, 2 H), 5.68 (dd, *J* = 10.2, 9.0 Hz, 1 H), 5.57 (dd, *J* = 10.8, 9.0 Hz, 1 H), 5.54 (d, *J* = 10.2 Hz, 1 H), 5.49 (d, *J* = 8.4 Hz, 1 H), 4.55 (d, *J* = 12.0 Hz, 1 H), 4.49 (d, *J* = 12.0 Hz, 1 H), 4.37 (d, *J* = 12.0 Hz, 1 H), 4.31 (d, *J* = 11.4 Hz, 1 H), 4.18 (*pseudo*-t, *J* = 10.2 Hz, 1 H), 4.11 (dd, *J* = 10.8, 8.4 Hz, 1 H), 4.04 (*pseudo*-t, *J* = 8.4 Hz, 1 H), 3.84–3.79 (m, 1 H), 3.76 (dd, *J* = 10.2, 4.2 Hz, 1 H), 3.66 (dd, *J* = 9.6, 4.8 Hz, 1 H), 3.56–3.51 (m, 2 H), 3.50–3.43 (m, 2 H), 2.95 (d, *J* = 3.6 Hz, 1 H), 1.89 (s, 3 H), 1.82 (s, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 171.0, 170.0, 167.8, 167.2, 138.1, 137.3, 134.7, 134.6, 134.4, 134.3, 134.2, 131.7, 131.42, 131.39, 131.2, 129.4, 129.0, 128.5, 128.3, 127.9, 127.7, 127.5, 127.3, 123.7, 123.5, 97.2, 82.4, 78.5, 74.1, 73.6, 73.5, 73.2, 72.8, 72.3, 71.3,

69.9, 67.8, 54.9, 53.9, 20.61, 20.58; HRMS (ESI) *m/z* calculated for $C_{52}H_{47}ClN_2NaO_{14}S$ $[M+Na]^+$, 1013.2329; found, 1013.2300.

4-Chlorophenyl (3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-(3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**3b**) TLC (Hexane/EtOAc 1:2) R_f 0.57; $[\alpha]_D$ = –17.5 (*c* = 1.3, $CHCl_3$, 27 °C); ^1H NMR ($CDCl_3$, 600 MHz) δ 7.88–7.79 (m, 6 H), 7.76–7.67 (m, 6 H), 7.36–7.32 (m, 2 H), 7.31–7.26 (m, 7 H), 7.25–7.20 (m, 5 H), 7.15 (*pseudo*-t, *J* = 7.8 Hz, 2 H), 7.10–7.08 (m, 2 H), 7.01 (*pseudo*-t, *J* = 7.2 Hz, 1 H), 5.60 (dd, *J* = 10.2, 9.0 Hz, 1 H), 5.55 (dd, *J* = 10.2, 8.4 Hz, 1 H), 5.52 (dd, *J* = 10.8, 9.0 Hz, 1 H), 5.50 (d, *J* = 10.8 Hz, 1 H), 5.38 (d, *J* = 8.4 Hz, 1 H), 5.27 (d, *J* = 8.4 Hz, 1 H), 4.53 (d, *J* = 11.4 Hz, 1 H), 4.48 (d, *J* = 12.0 Hz, 1 H), 4.43 (d, *J* = 11.4 Hz, 1 H), 4.42 (d, *J* = 11.4 Hz, 1 H), 4.38 (d, *J* = 11.4 Hz, 1 H), 4.32 (d, *J* = 11.4 Hz, 1 H), 4.17 (*pseudo*-t, *J* = 10.2 Hz, 1 H), 4.13 (*pseudo*-t, *J* = 9.0 Hz, 1 H), 4.07 (dd, *J* = 10.8, 8.4 Hz, 1 H), 4.03 (dd, *J* = 10.8, 8.4 Hz, 1 H), 4.00 (*pseudo*-t, *J* = 9.0 Hz, 1 H), 3.79 (td, *J* = 9.6, 3.0 Hz, 1 H), 3.72 (dd, *J* = 10.2, 4.2 Hz, 1 H), 3.63 (dd, *J* = 10.2, 4.8 Hz, 1 H), 3.55 (d, *J* = 9.6 Hz, 1 H), 3.48 (ddd, *J* = 9.6, 3.6, 1.2 Hz, 1 H), 3.45–3.41 (m, 2 H), 3.31–3.25 (m, 2 H), 3.11 (dd, *J* = 10.2, 1.2 Hz, 1 H), 2.89 (d, *J* = 3.6 Hz, 1 H), 1.88 (s, 3 H), 1.80 (s, 3 H), 1.71 (s, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.9, 170.2, 170.1, 168.1, 167.7, 167.3, 167.2, 138.2, 138.0, 137.4, 134.6, 134.5, 134.4, 134.3, 134.1, 131.6, 131.5, 131.44, 131.38, 131.1, 129.5, 128.9, 128.5, 128.2, 128.1, 127.9, 127.6, 127.42, 127.39, 127.3, 127.1, 123.7, 123.5, 123.4, 96.6, 96.5, 82.4, 78.5, 74.0, 73.6, 73.26, 73.23, 73.1, 73.0, 72.6, 72.3, 71.1, 71.4, 71.2, 69.9, 67.9, 67.4, 55.3, 54.9, 53.8, 20.61, 20.57, 20.46; HRMS (ESI) *m/z* calculated for $C_{75}H_{68}ClN_3NaO_{21}S$ $[M+Na]^+$, 1436.3647; found, 1436.3621.

4-Chlorophenyl (3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-(3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-(3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**4b**) TLC (Hexane/EtOAc 1:2) R_f 0.50; $[\alpha]_D$ = –32.9 (*c* = 0.7, $CHCl_3$, 27 °C); ^1H NMR ($CDCl_3$, 600 MHz) δ 7.90–7.65 (m, 16 H), 7.36–7.32 (m, 2 H), 7.30–7.26 (m, 6 H), 7.25–7.18 (m, 8 H), 7.12–7.07 (m, 4 H), 6.99 (*pseudo*-t, *J* = 7.8 Hz, 2 H), 6.94 (*pseudo*-t, *J* = 7.8 Hz, 1 H), 6.70 (*pseudo*-t, *J* = 7.2 Hz, 1 H), 5.58 (dd, *J* = 10.2, 9.0 Hz, 1 H), 5.51–5.44 (m, 4 H), 5.34 (d, *J* = 8.4 Hz, 1 H), 5.21 (d, *J* = 8.4 Hz, 1 H), 5.18 (d, *J* = 8.4 Hz, 1 H), 4.52 (d, *J* = 12.0 Hz, 1 H), 4.47 (d, *J* = 11.4 Hz, 1 H), 4.45–4.37 (m, 4 H), 4.35 (d, *J* = 11.4 Hz, 1 H), 4.32 (d, *J* = 11.4 Hz, 1 H), 4.15 (*pseudo*-t, *J* = 10.2 Hz, 1 H), 4.09 (*pseudo*-t, *J* = 9.6 Hz, 1 H), 4.07–3.95 (m, 5 H), 3.77 (td, *J* = 9.0, 3.0 Hz, 1 H), 3.71 (dd, *J* = 10.2, 4.2 Hz, 1 H), 3.62 (dd, *J* = 10.2, 5.4 Hz, 1 H), 3.54 (d, *J* = 10.2 Hz, 1 H), 3.48–3.45 (m, 2 H), 3.41–3.38 (m, 2 H), 3.26–3.21 (m, 3 H), 3.01 (dd, *J* = 10.2, 1.8 Hz, 1 H), 2.86 (d, *J* = 3.0 Hz, 1 H), 2.79 (dd, *J* = 10.2, 1.2 Hz, 1 H), 1.87 (s, 3 H), 1.79 (s, 3 H), 1.73 (s, 3 H), 1.67 (s, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 171.9, 170.3, 170.2, 170.1, 168.1, 167.7, 167.23, 167.18, 138.2, 138.11, 138.09, 137.4, 134.6, 134.5, 134.4, 134.3, 134.2, 134.1, 131.6, 131.5, 131.4, 131.1, 129.6, 128.9, 128.5, 128.2, 128.1, 127.95, 127.92, 127.6, 127.4, 127.21, 127.18, 127.1, 127.0, 123.7, 123.54, 123.51, 123.4, 123.3, 96.52, 96.46, 95.9, 82.4, 78.5, 73.9, 73.7, 73.6, 73.3, 73.13, 73.07, 72.9, 72.5, 72.3, 72.2, 72.0, 71.6, 71.4, 71.2, 70.7, 69.9, 67.8, 67.4, 55.3, 55.2, 54.8, 53.7, 20.60, 20.57, 20.45; HRMS (ESI) *m/z* calculated for $C_{98}H_{89}ClN_4NaO_{28}S$ $[M+Na]^+$, 1859.4965; found, 1859.4932.

Buliding block **1c** (0.20 mmol, 110 mg) afforded oligosaccharides **2c** (*n* = 2, 0.017 mmol, 16 mg, 17%), **3c** (*n* = 3, 0.0060 mmol, 8.3 mg, 9%), and **4c** (*n* = 4, 0.99 μmol, 1.8 mg, 2%) as white solids. Recovered yield of buliding block **1c** was 49% (53.3 mg, 0.0973 mmol).

4-Methylphenyl (3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**2c**) TLC (Hexane/EtOAc 1:2) R_f 0.50; $[\alpha]_D$ = –6.37 (*c* = 1.6, $CHCl_3$, 27 °C); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.87–7.71 (m, 4 H), 7.75–7.71 (m, 2 H), 7.71–7.66 (m, 2 H), 7.35–7.27 (m, 8 H), 7.23 (d, *J* = 7.8 Hz, 2 H), 7.21 (d, *J* = 8.4 Hz, 2 H), 6.94 (d, *J* = 7.8 Hz, 2 H), 5.68 (dd, *J* = 10.2, 9.0 Hz, 1 H), 5.57 (dd, *J* = 10.8, 9.0 Hz, 1 H), 5.52 (d, *J* = 10.8 Hz, 1 H), 5.45 (d, *J* = 8.4 Hz, 1 H), 4.54 (d, *J* = 12.0 Hz, 1 H), 4.49 (d, *J* = 12.0 Hz, 1 H), 4.37 (d, *J* = 12.0 Hz, 1 H), 4.32 (d, *J* = 12.0 Hz, 1 H), 4.18 (*pseudo*-t, *J* = 10.2 Hz, 1 H), 4.11 (dd, *J* = 10.8, 8.4 Hz, 1 H), 4.04 (*pseudo*-t, *J* = 9.0 Hz, 1 H), 3.84–3.78 (m, 1 H), 3.75 (dd, *J* = 9.6, 3.6 Hz, 1 H), 3.65 (dd. *J* = 10.2, 5.4 Hz, 1 H), 3.54–3.50 (m, 2 H), 3.48–3.43 (m, 2 H), 2.96 (d, *J* = 3.0 Hz, 1 H), 2.25 (s, 3 H), 1.88 (s, 3 H), 1.82 (s, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.9, 170.0, 167.8, 167.3, 138.4, 138.2, 137.4, 134.3, 134.2, 134.1, 133.8, 131.7, 131.4, 131.2, 129.5, 128.5, 128.2, 127.9, 127.7, 127.4, 127.1, 123.6, 123.5, 97.2, 82.7, 78.6, 74.1, 73.6, 73.5, 73.2, 72.7, 72.5, 71.3, 70.0, 67.8, 54.9, 54.0, 21.1, 20.6; HRMS (ESI) *m/z* calculated for $C_{53}H_{50}N_2NaO_{14}S$ $[M+Na]^+$, 993.2875; found, 993.2875.

4-Methylphenyl (3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-(3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3-*O*-acetyl-6-*O*-benzy-l-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**3c**) TLC (Hexane/EtOAc 1:2) R_f 0.57; $[\alpha]_D$ = –17.9 (*c* = 1.7, $CHCl_3$, 27 °C); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.89–7.77 (m, 6 H), 7.76–7.66 (m, 6 H), 7.36–7.32 (m, 2 H), 7.31–7.27 (m, 5 H), 7.24–7.20 (m, 7 H), 7.13 (*pseudo*-t, *J* = 7.8 Hz, 2 H), 6.99 (*pseudo*-t, *J* = 7.2 Hz, 1 H), 6.94 (d, *J* = 7.8 Hz, 2 H), 5.60 (dd, *J* = 10.2, 9.0 Hz, 1 H), 5.55 (dd, *J* = 10.8, 9.0 Hz, 1 H), 5.51 (dd, *J* = 10.2, 8.4 Hz, 1 H), 5.48 (d, *J* = 10.8 Hz, 1 H), 5.38 (d, *J* = 8.4 Hz, 1 H), 5.27 (d, *J* = 7.8 Hz, 1 H), 4.53 (d, *J* = 11.4 Hz, 1 H), 4.48 (d, *J* = 12.0 Hz, 1 H), 4.44–4.38 (m, 3 H), 4.32 (d, *J* = 11.4 Hz, 1 H), 4.17 (*pseudo*-t, *J* = 10.8 Hz, 1 H), 4.12 (*pseudo*-t, *J* = 9.0 Hz, 1 H), 4.07 (dd, *J* = 10.8, 8.4 Hz, 1 H), 4.03–3.99 (m, 2 H), 3.79 (td, *J* = 9.0, 3.0 Hz, 1 H), 3.72 (dd, *J* = 10.2, 4.2 Hz, 1 H), 3.63 (dd, *J* = 9.6, 4.8 Hz, 1 H), 3.55 (d, *J* = 10.2 Hz, 1 H), 3.46 (dd, *J* = 10.2, 3.6 Hz, 1 H), 3.44–3.39 (m, 2 H), 3.31–3.25 (m, 2 H), 3.09 (dd, *J* = 10.2, 1.2 Hz, 1 H), 2.88 (d, *J* = 3.6 Hz, 1 H), 2.24 (s, 3 H), 1.88 (s, 3 H), 1.80 (s, 3 H), 1.70 (s, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 171.0, 170.2, 170.1, 168.1, 167.7, 167.32, 167.26, 138.3, 138.1, 137.4, 134.3, 134.09, 134.05, 133.7, 131.7, 131.54, 131.45, 131.3, 129.5, 128.5, 128.2, 128.0, 127.9, 127.6, 127.41, 127.39, 127.3, 127.1, 123.60, 123.55, 123.47, 123.3, 96.6, 96.5, 82.9, 78.6, 74.0, 73.6, 73.26, 73.21, 73.16, 73.09, 72.6, 72.3, 71.9, 71.4, 71.3, 69.9, 67.9, 67.3, 55.3, 54.9, 53.9, 21.1, 20.61, 20.58, 20.49; HRMS (ESI) *m/z* calculated for $C_{76}H_{71}N_3NaO_{21}S$ $[M+Na]^+$, 1416.4193; found, 1416.4163.

4-Methylphenyl (3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-(3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-(3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**4c**) TLC (Hexane/EtOAc 1:2) R_f 0.48; $[\alpha]_D$ = –24.3 (*c* = 0.6, $CHCl_3$, 27 °C); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.90–7.84 (m, 4 H), 7.83–7.74 (m, 8 H), 7.73–7.65 (m, 4 H), 7.37–7.26 (m, 8 H), 7.25–7.18 (m, 8 H), 7.09 (d, *J* = 6.0 Hz, 1 H), 7.08 (d, *J* = 7.8 Hz, 1 H), 6.99 (*pseudo*-t, *J* = 7.8 Hz, 2 H), 6.95–6.89 (m, 3 H), 6.69 (*pseudo*-t, *J* = 7.2 Hz, 1 H), 5.58 (*pseudo*-t, *J* = 9.6 Hz, 1 H), 5.52–5.48 (m, 2 H), 5.45 (d, *J* = 7.8 Hz, 2 H), 5.34 (d, *J* = 8.4 Hz, 1 H), 5.21 (d, *J* = 8.4 Hz, 1 H), 5.18 (d, *J* = 8.4 Hz, 1 H), 4.52 (d, *J* = 12.0 Hz, 1 H), 4.47 (d, *J* = 11.4 Hz, 1 H), 4.43 (d, *J* = 11.4 Hz, 1 H), 4.42 (d, *J* = 11.4 Hz, 1 H), 4.41–4.38 (m, 2 H), 4.35 (d, *J* = 11.4 Hz, 1 H), 4.32 (d, *J* = 11.4 Hz, 1 H), 4.16 (*pseudo*-t, *J* = 10.2 Hz, 1 H), 4.12–4.02 (m, 3 H), 4.01–3.95 (m, 3 H), 3.77 (*pseudo*-t, *J* = 9.0 Hz, 1 H), 3.75–3.69 (m, 1 H), 3.62 (dd, *J* = 9.6, 4.8 Hz, 1 H), 3.54 (d, *J* = 10.8 Hz, 1 H), 3.46–3.42 (m, 2 H), 3.41–3.37 (m, 2 H), 3.26–3.19 (m, 3

H), 2.99 (d, J = 10.2 Hz, 1 H), 2.87 (s, 1 H), 2.78 (d, J = 9.6 Hz, 1 H), 2.24 (s, 3 H), 1.87 (s, 3 H), 1.79 (s, 3 H), 1.73 (s, 3 H), 1.67 (s, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 171.0, 170.3, 170.2, 170.1, 168.1, 167.7, 167.3, 138.4, 138.3, 138.2, 138.1, 137.4, 134.29, 134.27, 134.23, 134.19, 134.17, 134.0, 133.7, 131.7, 131.6, 131.5, 131.49, 131.46, 131.42, 131.39, 131.27, 129.5, 128.5, 128.2, 127.99, 127.96, 127.92, 127.6, 127.41, 127.38, 127.37, 127.2, 127.10, 127.08, 126.94, 123.67, 123.63, 123.60, 123.57, 123.51, 123.48, 123.39, 123.30, 96.51, 96.48, 95.9, 82.9, 78.6, 73.9, 73.7, 73.6, 73.3, 73.14, 73.13, 72.9, 72.5, 72.26, 72.24, 72.0, 71.8, 71.4, 70.7, 70.0, 67.9, 67.5, 55.3, 55.2, 54.9, 53.9, 21.1, 20.60, 20.57, 20.48, 20.45; HRMS (ESI) *m/z* calculated for $C_{99}H_{92}KN_4O_{28}S$ $[M+K]^+$, 1855.5250; found, 1855.5226.

Buliding block **1d** (0.2 mmol, 114 mg) afforded oligosaccharides **2d** (n = 2, 0.023 mmol, 22.8 mg, 23%), **3d** (n = 3, 0.0067 mmol, 9.5 mg, 10%), **4d** (n = 4, 0.0043 mmol, 8.0 mg, 9%), and **5d** (n = 5, 0.0026 mmol, 5.8 mg, 6%) as white solids. Recovered yield of buliding block **1d** was 37% (42 mg, 0.074 mmol).

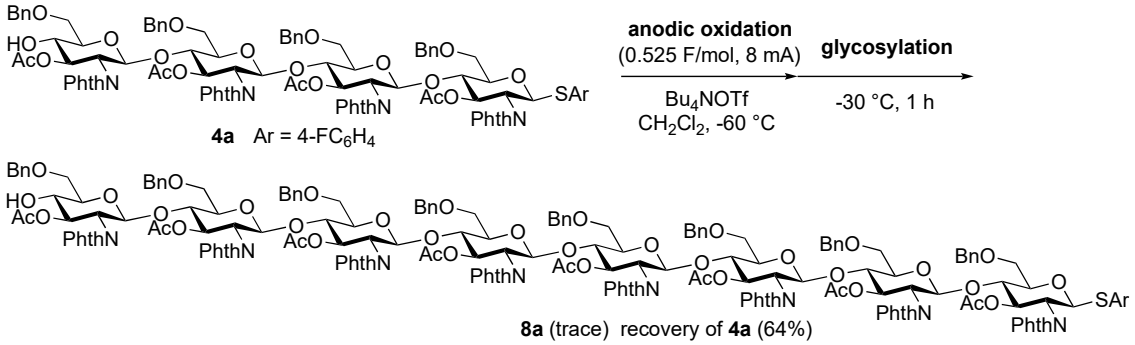
2,4-Difluorophenyl (3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**2d**) TLC (Hexane/EtOAc 1:2) R_f 0.60; $[\alpha]_D$ = –4.06 (c = 1.4, $CHCl_3$, 27 °C); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.86–7.67 (m, 8 H), 7.46 (td, J = 8.4, 6.0 Hz, 1 H), 7.36–7.28 (m, 8 H), 7.20 (d, J = 8.4 Hz, 2 H), 6.70 (td, J = 8.4, 2.4 Hz, 1 H), 6.64 (td, J = 8.4, 2.4 Hz, 1 H), 5.66 (*pseudo*-t, J = 9.6 Hz, 1 H), 5.57 (dd, J = 10.8, 9.6 Hz, 1 H), 5.52 (d, J = 10.2 Hz, 1 H), 5.45 (d, J = 8.4 Hz, 1 H), 4.54 (d, J = 12.0 Hz, 1 H), 4.49 (d, J = 11.4 Hz, 1 H), 4.35 (d, J = 12.0 Hz, 1 H), 4.32 (d, J = 11.4 Hz, 1 H), 4.14−4.08 (m, 2 H), 4.03 (*pseudo*-t, J = 9.0 Hz, 1 H), 3.82 (td, J = 10.8, 3.6 Hz, 1 H), 3.75 (dd, J = 9.6, 3.6 Hz, 1 H), 3.66 (dd, J = 10.2, 5.4 Hz, 1 H), 3.53–3.50 (m, 2 H), 3.48 (dd, J = 9.6, 4.8 Hz, 1 H), 3.45 (dd, J = 11.4, 3.6 Hz, 1 H), 2.96 (d, J = 3.0 Hz, 1 H), 1.88 (s, 3 H), 1.82 (s, 3 H).; ^{13}C NMR ($CDCl_3$, 150 MHz) δ 171.0, 170.0, 167.8, 167.3, 163.6 (dd, J = 250.7, 11.4 Hz), 162.8 (dd, J = 248.6, 12.3 Hz), 138.2, 137.8 (d, J = 9.3 Hz), 137.4, 134.4, 134.3, 134.2, 131.6, 131.4, 131.2, 128.5, 128.2, 127.9, 127.7, 127.5, 127.3, 123.7, 123.5, 112.9 (dd, J = 18.5, 4.1 Hz), 111.9 (dd, J = 21.3, 3.6 Hz), 104.4 (t, J = 26.3 Hz), 97.3, 82.0, 78.6, 74.0, 73.6, 73.5, 73.2, 72.8, 72.3, 71.2, 69.9, 67.8, 54.9, 53.8, 20.60, 20.58; HRMS (ESI) *m/z* calculated for $C_{52}H_{46}F_2KN_2O_{14}S$ $[M+K]^+$, 1031.2269; found, 1031.2269.

2,4-Difluorophenyl (3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-(3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**3d**) TLC (Hexane/EtOAc 1:2) R_f 0.55; $[\alpha]_D$ = –18.1 (c = 1.7, $CHCl_3$, 27 °C); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.88–7.67 (m, 12 H), 7.46 (td, J = 8.4, 6.6 Hz, 1 H), 7.37–7.19 (m, 12 H), 7.15 (*pseudo*-t, J = 7.8 Hz, 2 H), 7.02 (*pseudo*-t, J = 7.2 Hz, 1 H), 6.71 (td, J = 8.4, 2.4 Hz, 1 H), 6.65 (td, J = 8.4, 2.4 Hz, 1 H), 5.58 (dd, J = 9.6, 9.0 Hz, 1 H), 5.55 (dd, J = 10.2, 8.4 Hz, 1 H), 5.51 (dd, J = 10.8, 9.0 Hz, 1 H), 5.47 (d, J = 10.2 Hz, 1 H), 5.38 (d, J = 8.4 Hz, 1 H), 5.27 (d, J = 8.4 Hz, 1 H), 4.53 (d, J = 12.0 Hz, 1 H), 4.48 (d, J = 11.4 Hz, 1 H), 4.45–4.40 (m, 2 H), 4.38 (d, J = 11.4 Hz, 1 H), 4.31 (d, J = 12.0 Hz, 1 H), 4.13 (*pseudo*-t, J = 9.6 Hz, 1 H), 4.11–4.05 (m, 2 H), 4.02 (dd, J = 10.8, 8.4 Hz, 1 H), 4.00 (*pseudo*-t, J = 9.6 Hz, 1 H), 3.79 (td, J = 9.0, 3.0 Hz, 1 H), 3.72 (dd, J = 10.2, 4.2 Hz, 1 H), 3.63 (dd, J = 10.2, 4.8 Hz, 1 H), 3.54 (d, J = 10.8 Hz, 1 H), 3.48–3.40 (m, 3 H), 3.33–3.25 (m, 2 H), 3.11 (dd, J = 9.6, 1.2 Hz, 1 H), 2.88 (d, J = 3.6 Hz, 1 H), 1.88 (s, 3 H), 1.80 (s, 3 H), 1.71 (s, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.9, 170.2, 170.1, 168.1, 167.7, 167.3, 167.2, 163.5 (dd, J = 250.5, 11.0 Hz), 162.6 (dd, J = 248.3, 12.2 Hz), 138.2, 138.0, 137.7 (d, J = 9.9 Hz), 137.4, 134.34, 134.27, 134.1, 131.6, 131.5, 131.44, 131.38, 131.2, 128.5, 128.2, 128.1, 127.9, 127.6, 127.4, 127.31, 127.25, 127.1, 123.6, 123.54, 123.46, 123.4, 113.1 (dd, J = 17.6, 3.3 Hz), 111.9 (dd, J = 21.8, 3.3 Hz), 104.4 (t, J = 26.3 Hz), 96.6, 96.5, 82.1, 78.6, 74.0, 73.6, 73.2, 73.0, 72.6, 72.3, 71.7, 71.3, 71.2, 69.9, 67.8, 67.3, 55.3, 54.9, 53.8, 20.60, 20.57, 20.45; HRMS (ESI) *m/z* calculated for $C_{75}H_{67}F_2KN_3O_{21}S$ $[M+K]^+$, 1454.3587; found, 1454.3563.

2,4-Difluorophenyl (3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-(3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-(3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**4d**) TLC (Hexane/EtOAc 1:2) R_f 0.47; $[\alpha]_D$ = –5.00 (*c* = 1.4, $CHCl_3$, 27 °C); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.89–7.65 (m, 16 H), 7.46 (td, *J* = 7.8, 6.6 Hz, 1 H), 7.38–7.18 (m, 15 H), 7.10 (*pseudo*-t, *J* = 7.8 Hz, 1 H), 6.99 (*pseudo*-t, *J* = 7.8 Hz, 2 H), 6.95 (*pseudo*-t, *J* = 7.2 Hz, 1 H), 6.72–6.68 (m, 2 H), 6.65 (td, *J* = 8.4, 2.4 Hz, 1 H), 5.57 (*pseudo*-t, *J* = 10.2 Hz, 1 H), 5.51–5.43 (m, 4 H), 5.34 (d, *J* = 7.8 Hz, 1 H), 5.21 (d, *J* = 8.4 Hz, 1 H), 5.18 (d, *J* = 8.4 Hz, 1 H), 4.52 (d, *J* = 12.0 Hz, 1 H), 4.47 (d, *J* = 11.4 Hz, 1 H), 4.44–4.34 (m, 5 H), 4.32 (d, *J* = 11.4 Hz, 1 H), 4.13–3.95 (m, 7 H), 3.77 (td, *J* = 9.6, 3.0 Hz, 1 H), 3.71 (dd, *J* = 9.6, 3.6 Hz, 1 H), 3.62 (dd, *J* = 10.2, 4.8 Hz, 1 H), 3.52 (d, *J* = 10.2 Hz, 1 H), 3.49–3.35 (m, 4 H), 3.29–3.19 (m, 3 H), 3.02 (d, *J* = 10.2 Hz, 1 H), 2.87 (d, *J* = 3.0 Hz, 1 H), 2.79 (d, *J* = 10.2 Hz, 1 H), 1.87 (s, 3 H), 1.79 (s, 3 H), 1.73 (s, 3 H), 1.67 (s, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.9, 170.3, 170.2, 168.1, 167.7, 167.2, 163.5 (dd, *J* = 249.3, 9.8 Hz), 162.6 (dd, *J* = 248.4, 12.2 Hz), 138.2, 138.11, 138.09, 137.6 (d, *J* = 8.7 Hz), 137.4, 134.4, 134.3, 134.2, 134.1, 131.6, 131.44, 131.35, 131.2, 128.5, 128.2, 128.03, 127.96, 127.91, 127.6, 127.4, 127.3, 127.2, 127.1, 127.0, 123.61, 123.57, 123.45, 123.36, 113.1 (dd, *J* = 18.6, 4.4 Hz), 111.9 (dd, *J* = 21.9, 4.4 Hz), 104.4 (t, *J* = 26.3 Hz), 96.54, 96.46, 95.9, 82.1, 78.6, 73.9, 73.7, 73.6, 73.3, 73.2, 73.0, 72.9, 72.5, 72.2, 72.0, 71.7, 71.4, 71.2, 70.7, 69.9, 67.8, 67.5, 67.4, 55.3, 55.1, 54.9, 53.7, 20.60, 20.56, 20.44; HRMS (ESI) *m/z* calculated for $C_{98}H_{88}F_2N_4NaO_{28}S$ $[M+Na]^+$, 1861.5166; found, 1861.5137.

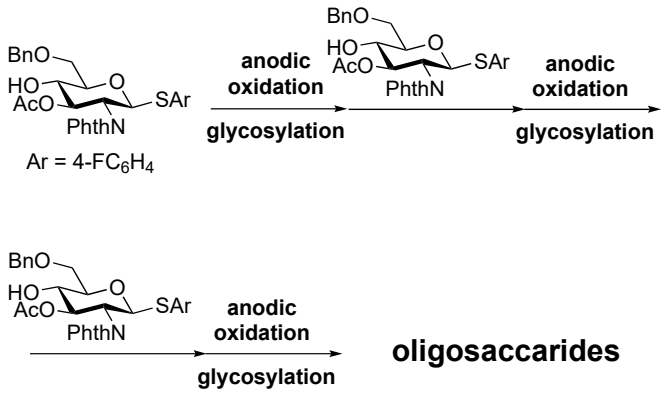
2,4-Difluorophenyl (3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-(3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-(3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-(3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**5d**) TLC (Hexane/EtOAc 1:2) R_f 0.40; $[\alpha]_D$ = –27.1 (*c* = 1.0, $CHCl_3$, 30 °C); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.90–7.64 (m, 20 H), 7.45 (td, *J* = 7.8, 6.0 Hz, 1 H), 7.36–7.26 (m, 8 H), 7.25–7.15 (m, 9 H), 7.09 (*pseudo*-t, *J* = 7.2 Hz, 2 H), 6.97–6.90 (m, 4 H), 6.70 (td, *J* = 8.4, 2.4 Hz, 1 H), 6.66–6.61 (m, 2 H), 6.57 (*pseudo*-t, *J* = 7.8 Hz, 1 H), 5.57 (*pseudo*-t, *J* = 9.6 Hz, 1 H) 5.50–5.41 (m, 4 H), 5.37 (dd, *J* = 10.8, 9.6 Hz, 1 H), 5.32 (d, *J* = 8.4 Hz, 1 H), 5.19 (d, *J* = 8.4 Hz, 1 H), 5.12 (*pseudo*-t, *J* = 8.4 Hz, 2 H), 4.51 (d, *J* = 11.4 Hz, 1 H), 4.47 (d, *J* = 12.0 Hz, 1 H), 4.45–4.30 (m, 8 H), 4.10–3.91 (m, 10 H), 3.77 (td, *J* = 9.6, 3.6 Hz, 1 H), 3.71 (dd, *J* = 9.6, 3.6 Hz, 1 H), 3.62 (dd, *J* = 10.2, 4.8 Hz, 1 H), 3.51 (d, *J* = 10.8 Hz, 1 H), 3.46–3.36 (m, 4 H), 3.23–3.13 (m, 4 H), 3.00 (d, *J* = 10.2 Hz, 1 H), 2.85 (d, *J* = 2.4 Hz, 1 H), 2.72 (d, *J* = 9.0 Hz, 1 H), 2.61 (d, *J* = 9.0 Hz, 1 H), 1.88 (s, 3 H), 1.78 (s, 3 H), 1.73 (s, 3 H), 1.70 (s, 3 H), 1.65 (s, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.9, 170.4, 170.3, 170.18, 170.15, 168.0, 167.7, 167.22, 167.16, 163.5 (dd, *J* = 250.7, 11.3 Hz), 162.6 (dd, *J* = 249.1, 11.9 Hz), 138.2, 138.10, 138.08, 138.05, 137.6 (d, *J*= 9.0 Hz), 137.4, 134.3, 134.24, 134.22, 134.15, 134.09, 131.55, 131.48, 131.4, 131.3, 131.1, 128.46, 128.18, 128.00, 127.87, 127.86, 127.6, 127.4, 127.3, 127.19, 127.14, 127.04, 126.89, 126.84, 123.7, 123.6, 123.5, 123.4, 123.3, 113.1 (dd, *J* = 18.3, 3.6 Hz), 111.9 (dd, *J* = 22.4, 3.3 Hz), 104.3 (t, *J* = 25.5 Hz), 96.5, 96.4, 95.9, 95.7, 82.1, 78.5, 73.8, 73.55, 73.52, 73.2, 73.0, 72.8, 72.5, 72.2, 71.97, 71.95, 71.6, 71.2, 70.7, 70.6, 69.8, 67.7, 67.5, 67.4, 55.2, 55.0, 54.8, 53.7, 20.55, 20.52, 20.4; HRMS (ESI) *m/z* calculated for $C_{121}H_{109}F_2KN_5O_{35}S$ $[M+K]^+$, 2300.6223; found, 2300.6287.

2. Electrochemical dimerization of tetrasaccharide



The electrochemical dimerization of tetrasaccharide **4a** was carried out on an H-type divided cell (4G glass filter). The cell had a carbon felt anode (Nippon Carbon JF-20-P7) and platinum square plate (20 mm×20 mm). Tetrasaccharide **4a** (0.1 mmol, 182 mg), Bu_4NOTf (0.5 mmol, 196 mg), and CH_2Cl_2 (5 mL) were added to the anodic chamber. Trifluoromethanesulfonic acid (0.1 mmol, 9 μL), Bu_4NOTf (0.5 mmol, 196 mg), and CH_2Cl_2 (5 mL) were added to the cathodic chamber. The constant current (6 mA (current density: 2.0 mA/cm^2), 29 V (electrode distance: 4.5 cm)) was employed at –60 ℃ with magnetic stirring until 0.52 F/mol of the electricity was consumed. After the electrolysis, the reaction was kept stirring at –30 ℃ for 1 h. After that, triethylamine (0.2 mL) was added to both chambers. The solution in both chambers was collected in eggplant flask, and the solvent was removed under reduced pressure. The mixture was dissolved in EtOAc and washed with water (3 times) and brine, respectively. The solution was dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified with preparative-GPC to afford octasaccharides **8a** (n = 8, trace), and recovered yield of tetrasaccharides **4a** (n = 4, 0.6422 mmol, 117 mg, 64%) as white solids.

3. Protocol modification of electrochemical poly-glycosylation



The electrochemical polymerization synthesis of linear oligosaccharides (**2a**~**8a**) was carried out an H-type divided cell (4G glass filter). The cell had a carbon felt anode (Nippon Carbon JF-20-P7) and platinum square plate (20 mm×20 mm). Building block **1a** (0.200 mmol, 109 mg), Bu$_4$NOTf (1.00 mmol, 393 mg), and CH$_2$Cl$_2$ (10 mL) were added to the anodic chamber. Trifluoromethanesulfonic acid (0.200 mmol, 18 μL), Bu$_4$NOTf (1.00 mmol, 393 mg), and CH$_2$Cl$_2$ (10 mL) were added to the cathodic chamber. The constant current (8 mA (current density: 2.0 mA/cm^2), 53 V (electrode distance: 4.5 cm)) was employed at –60 °C with magnetic stirring until 0.52 F/mol of the electricity was consumed. After the electrolysis, the reaction was kept stirring at –30 °C for 1 h. After that, building block **1a** (0.400 mmol, 218 mg) dissolved in CH$_2$Cl$_2$ (2.0 mL) was subsequently added by the syringe (1.0 mL (0.200 mmol) for one cycle) at –30 °C. The reaction temperature was cooled down to –60 °C and the next cycle started. After the 2nd cycle, triethylamine (0.3 mL) was added to both chambers. The solution in both chambers was collected in eggplant flask, and the solvent was removed under reduced pressure. The reaction mixture was dissolved in EtOAc and washed with water (3 times) and brine, respectively. The solution was dried over Na$_2$SO$_4$ and removed under reduced pressure. The crude product was purified with preparative-GPC to afford linear oligosaccharides **2a** (*n* = 2, 0.065 mmol, 63 mg, 22%), **3a** (*n* = 3, 0.034 mmol, 47 mg, 17%), **4a** (*n* = 4, 0.017 mmol, 31 mg, 11%), **5a** (*n* = 5, 0.0094 mmol, 21 mg, 8%), **6a** (*n* = 6, 0.0056 mmol, 15 mg, 6%), **7a** (*n* = 7, 0.0042 mmol, 13 mg, 5%), and **8a** (*n* = 8, 0.0023 mmol, 7.6 mg, 3%) as white solids.

4-Fluorophenyl (3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-(3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-(3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-(3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-(3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-(3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-(3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**8a**) TLC (Hexane/EtOAc 1:2) R$_f$ 0.13; [α]$_D$ = –25.8 (*c* = 0.9, CHCl$_3$, 32 °C); ^{1}H NMR (CDCl$_3$, 600 MHz) δ 7.90–7.60 (m, 32 H), 7.36–7.25 (m, 10 H), 7.23–7.11 (m, 16 H), 7.08 (*pseudo*-t, *J* = 7.8 Hz, 2 H), 6.96–6.84 (m, 10 H), 6.81 (*pseudo*-t, *J* = 7.8 Hz, 2 H), 6.61 (*pseudo*-t, *J* = 7.2 Hz, 1 H), 6.52 (*pseudo*-t, *J* = 7.2 Hz, 1 H), 6.50–6.43 (m, 2 H), 5.56 (*pseudo*-t, *J* = 10.2 Hz, 1 H), 5.48 (dd, *J* = 10.2, 9.0 Hz, 1 H), 5.46–5.41 (m, 4 H), 5.38–5.30 (m, 5 H), 5.18 (d, *J* = 7.8 Hz, 1 H), 5.11 (d, *J* = 8.4 Hz, 1 H), 5.09 (d, *J* = 8.4 Hz, 1 H), 5.06–5.01 (m, 2 H), 4.51 (d, *J* = 11.4 Hz, 1 H), 4.47 (d, *J* = 11.4 Hz, 1 H), 4.44–4.27 (m, 14 H), 4.14–3.87 (m, 16 H), 3.76 (*pseudo*-t, *J* = 9.0 Hz, 1 H), 3.70 (dd, *J* = 9.6, 3.6 Hz, 1 H), 3.62 (dd, *J* = 9.6, 4.2 Hz, 1 H), 3.52 (d, *J* = 10.2 Hz, 1 H), 3.45–3.35 (m, 8 H), 3.24–3.16 (m, 4 H), 3.14–3.06 (m, 4 H), 2.97 (d, *J* = 9.6 Hz, 1 H), 2.70 (d, *J* = 10.2 Hz, 1 H), 2.63 (d, *J* = 9.6 Hz, 1 H),

2.60–2.54 (m, 1 H), 1.86 (s, 3 H), 1.77 (s, 3 H), 1.73 (s, 3 H), 1.703 (s, 3 H), 1.698 (s, 3 H), 1.695 (s, 3 H), 1.68 (s, 3 H), 1.64 (s, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.9, 170.4, 170.3, 170.2, 170.1, 168.0, 167.9, 167.2, 167.1, 163.0 (d, *J* = 247.4 Hz), 138.2, 138.12, 138.09, 138.07, 137.4, 135.9 (d, *J* = 8.3 Hz), 134.3, 134.23, 134.20, 134.1, 131.6, 131.55, 131.48, 131.40, 128.81, 128.78, 128.5, 128.2, 128.0, 127.89, 127.86, 127.84, 127.82, 127.78, 127.6, 127.35, 127.19, 127.17, 127.14, 127.05, 126.88, 126.82, 126.77, 126.76, 125.9, 123.66, 123.65, 123.64, 123.58, 123.54, 123.49, 123.48, 123.44, 123.36 115.8 (d, *J* = 21.9 Hz), 96.5, 96.4, 95.9, 95.7, 82.66, 82.65, 78.4, 73.8, 73.6, 73.5, 73.25, 73.20, 73.0, 72.8, 72.5, 72.2, 71.94, 71.91, 71.7, 71.25, 71.19, 70.7, 70.6, 69.8, 67.7, 67.45, 67.41, 66.2, 65.9, 55.25, 55.12, 55.07, 54.8, 20.56, 20.52, 20.4; HRMS (ESI) *m/z* calculated for $C_{190}H_{173}FKN_8O_{56}S$ $[M+K]^+$, 3552.0272; found, 3552.0203.

References of experimental section

(1) T. Nokami, Y. Isoda, N. Sasaki, A. Takaiso, S. Hayase, T. Itoh, R. Hayashi, A. Shimizu, and J. Yoshida, *Org. Lett.*, **2015**, *17*, 1525-1528.
(2) K. Yano, T. Itoh and T. Nokami, *Carbohydr. Res.*, **2020**, *492*, 108018.

Chapter 3
Synthesis of Cyclic Oligosaccharides of PNAG by Electrochemical Polyglycosylation

Abstract
Synthesis of chitin oligosaccharide precursor is already reported by electrochemical polyglycosylation. In this chapter I showed the synthesis of cyclic and linear PNAG oligosaccharide by electrochemical polyglycosylation method along with the structure of cyclic hetero oligosaccharide and the possible reaction mechanism.

Introduction

Electrochemical polyglycosylation has been proved as a useful alternative to prepare oligosaccharides.[1] Thus, we envisioned that the method must be useful to prepare PNAG oligosaccharide precursors which have been prepared by automated electrochemical assembly (Figure 3-1).[2] One of the things we must consider is that the oligosaccharide intermediates might afford cyclic products as well. Indeed, we have already reported electrochemical conversion of linear PNAG oligosaccharides into the corresponding cyclic oligosaccharides in high yields.[3] Therefore, we investigated the influence of substituents, protecting groups of hydroxy groups, and reaction conditions to obtain linear or cyclic oligosaccharides selectively. By the chemical polyglycosylation glucoside and galactoside monomers afforded cyclic oligosaccharide; however, polyglycosylation of the glucosamine monomer has never been reported.[4]

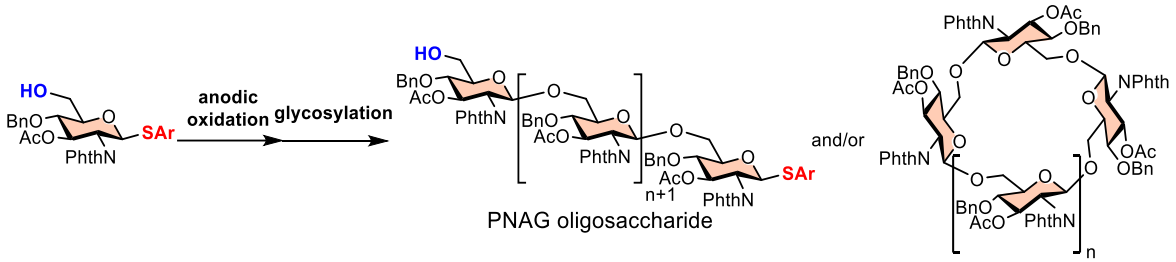
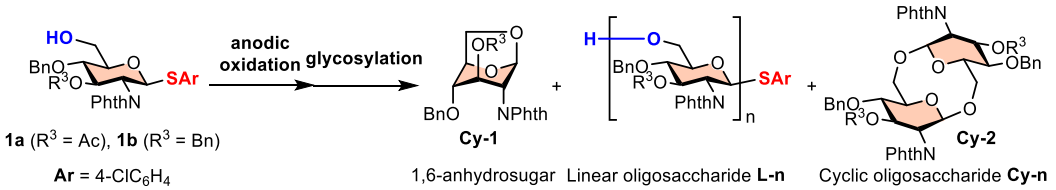


Figure 3-1. Electrochemical polyglycosylation of PNAG monosaccharide.

Results and discussion

I initiated this research from the electrochemical polyglycosylation of PNAG monosaccharide with 2-deoxy-2-phtalolyl (2-PhthN) group (Table 3-1). The monosaccharide was completely consumed with the slight excess amount of electricity (1.05 F/mol); however, 1,6-anhydrosugar **Cy-1** was formed as a major product together with cyclic disaccharide **Cy-2** and trace amounts of larger cyclic oligosaccharides **Cy-3** and **Cy-4** (entry 1). Then we reduced the amount of electricity from 1.05 to 0.52 F/mol because no linear oligosaccharide **L-n** was obtained (entry 2). Linear disaccharides **L-2** and **L-3** were obtained in 13% and 6% yields, respectively. The protecting group (R^3) of 3-OH was changed from acetyl (Ac) group to benzyl (Bn) group; however, yields of linear oligosaccharides **L-2** and **L-3** were decreased and yields of **Cy-1** and **Cy-2** were increased (entry 3). I assume that electron donating property of Bn group may accelerate intramolecular glycosylation to form **Cy-1** and **Cy-2**. In all cases the major product was **Cy-1** which was the product of intramolecular glycosylation of the PNAG monosaccharide. Therefore, we supposed that the 2-PhthN protecting group might accelerate the intramolecular glycosylation to form **Cy-1** and cyclic oligosaccharide **Cy-n**.

Table 3-1. Electrochemical polyglycosylation of PNAG monosaccharide with 2-Phth group.

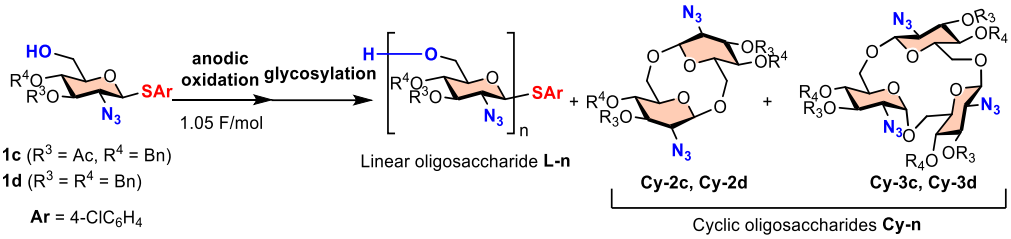


entry	R^3	amount of electricity	conv.	yield of **Cy-1**	yields of oligosaccharides		
					L-2	**L-3**	**Cy-2**
1[a]	Ac	1.05 F/mol	>99%	28% **Cy-1a**	-	-	6% **Cy-2a**
2	Ac	0.52 F/mol	67%	25% **Cy-1a**	13% **L-2a**	6% **L-3a**	7% **Cy-2a**
3	Bn	0.52 F/mol	59%	35% **Cy-1b**	4% **L-2b**	2% **L-3b**	25% **Cy-2b**

[a]Trace amounts of cyclic oligosaccharides **Cy-3a** and **Cy-4a** were obtained.

Based on the results of Table 3-1, we changed the substituent of C-2 position from phthaloyl (PhthN) group to azido (N_3) group which has no neighboring group effect. Although glycosyl donors with N_3 group at C-2 position have been used for α-selective glycosylation, we have already found that β-selective glycosylation proceeded using a glycosyl donor with N_3 group under the electrochemical conditions.[5] The results of electrochemical polyglycosylation using the thioglycoside with N_3 group are summarized in Table 3-2. Larger cyclic oligosaccharides up to trisaccharide **Cy-3c** were obtained together with the trace amount of linear tetrasaccharide **L-4c** by the introduction of N_3 group (entry 1). Protecting groups (R^3 and R^4) at 3-OH and 4-OH also affected the distribution of products (entries 2 and 3). Linear trisaccharide **L-3d** and cyclic disaccharide **Cy-2d** were produced with monosaccharide with 3,4-di-*O*-benzyl group (entry 2). In all cases formation of **Cy-1** was not observed.

Table 3-2. Electrochemical polyglycosylation of PNAG monosaccharide with 2-azido group.



entry	R^3	R^4	conv.	yields of oligosaccharides[a]					
				L-2	**L-3**	**L-4**	**Cy-2**	**Cy-3**	**Cy-4**
1	Ac	Bn	>99%	-	-	trace **L-4c**	49% **Cy-2c**	17% **Cy-3c**	trace **Cy-4c**
2	Bn	Bn	73%	-	13% **L-3d**	-	14% **Cy-2d**	-	-

[a]NMR yields.

The successful synthesis of cyclic disaccharide and trisaccharide with a good amount of yield we choose the monosaccharide of entry 1 in Table 3-2 as our suitable building block for the next experiments. Another

reason for choosing this building block is its satisfactory result in overcoming the formation of 1,6-anhydrosugar.

We were interested in the atomic structure of cyclic disaccharide and trisaccharide. Cyclic disaccharide is homogeneous cyclic oligosaccharide by their structure. The noticeable part is its coupling constant is 1.8 Hz only. That notices the formation of α-cyclic oligosaccharide which is not real structurally. So, we proposed a structure of cyclic disaccharides according to a previous report.[6] Moreover the cyclic disaccharides we synthesized are the precursors of reported products.

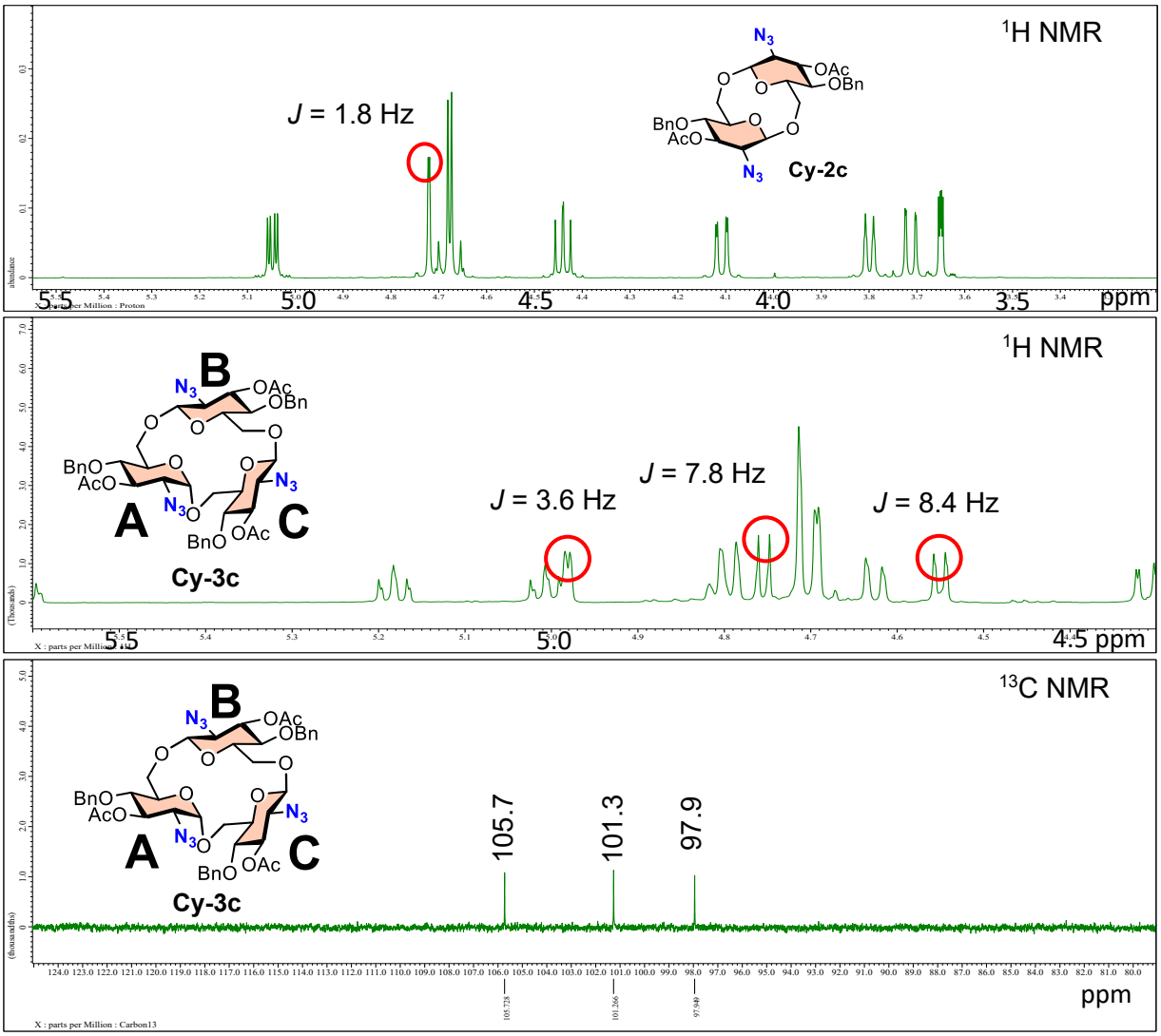


Figure 3-2. NMR spectrum and proposed structure of cyclic disaccharide and cyclic trisaccharide.

According to the NMR we propose a structure of cyclic trisaccharide **Cy-3c** with mixed stereochemistry. At first, we measured the NMR using chloroform d solvent. But we could not measure the coupling constant of all anomeric protons because of the overlapping of an anomeric proton. From the other two protons we were assured that they are mixed by α and β type. For the remaining proton we changed the solvent for NMR to acetone-d_6, toluene-d_8 but obtained the NMR with overlapped anomeric proton. The fully isolated anomeric protons were observed using benzene-d_6 solvent with acetone-d_6. We obtained three

different coupling constants for three anomeric protons of three pyranose rings, (A; J = 3.36 Hz at 4.79 ppm at ^{1}H NMR and 98 ppm at ^{13}C NMR, B; J = 8.04 Hz at 4.20 ppm at ^{1}H NMR and 106 ppm at ^{13}C NMR, C; J = 7.68 Hz at 4.50 ppm at ^{1}H NMR and 101 ppm at ^{13}C NMR). It clearly indicates a formation of α, β, β-type cyclic trisaccharide. According to the NMR studies we proposed the structure of above mentioned cyclic trisaccharide **Cy-3c** (**Figure 3-2**).

The above-mentioned structures and their selectivity of coupling make us curious about the reaction mechanism of this electrochemical polyglycosylation. The activation of thioglycosides produces triflate intermediate. The participation of neighboring group can convert them into 1,6-anhydrosugars **Cy-1a, Cy-1b** which we observed in using 2-Nphth group. But the α-selectivity prevents the formation of 1,6-anhydrosugar **Cy-1** while using 2-azido group. The activation of linear disaccharide **L-2** also produces triflate intermediate of disaccharide which has a high possibility to be converted to cyclic disaccharide **Cy-2**. But only β-selectivity can convert the activated disaccharide **L-2** to the corresponding cyclic disaccharides **Cy-2**. The activated α-selective disaccharide **L-2** couples another monosaccharide **1** to form more longer oligosaccharides **L-n**. That's the way longer cyclic oligosaccharide **Cy-n** is formed. But here the cyclic trisaccharide **Cy-3c** formation was possible because of the activation of α,β mixed linear oligosaccharide **L-3c** which formed α, β, β- cyclic trisaccharide **Cy-3c** (**Figure 3-3**).

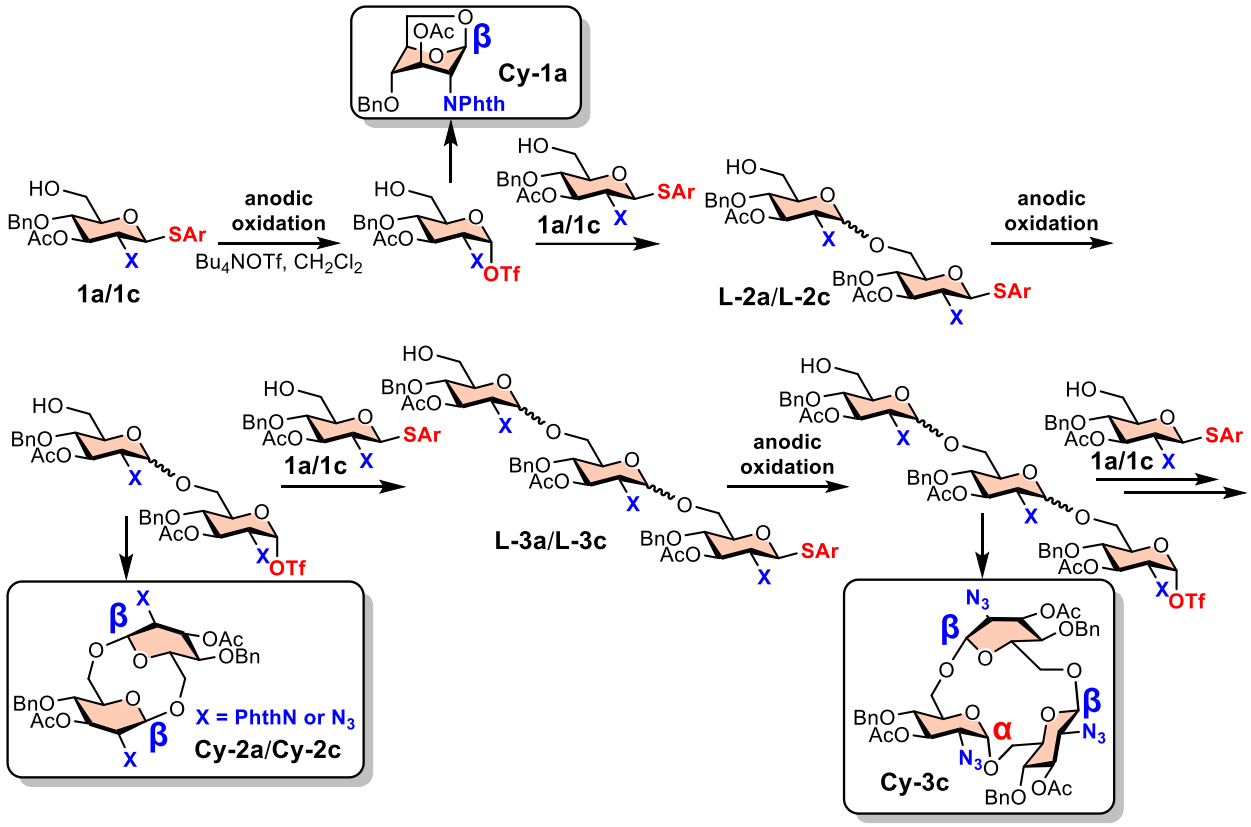


Figure 3-3. Proposed reaction mechanism for the formation of cyclic oligosaccharide under electrochemical polyglycosylation condition.

The provided amount of electricity plays an important role in the formation of linear and cyclic oligosaccharide **L-n**, **Cy-n**. In the lower amount of electricity usually linear oligosaccharide **L-n** is formed. With the increasing amount of electricity, the monosaccharide **1c** is decreased to be converted to

corresponding linear oligosaccharides **L-n** and the oligosaccharides **L-n** are also starts to be activated. The activation of oligosaccharides form both longer linear oligosaccharides **L-n** and cyclic oligosaccharides **Cy-n**. Here we described the formation of linear to cyclic oligosaccharide **L-n**, **Cy-n** by the increasing amount of electricity (**Figure 3-4**). Here we started with the optimized amount of electricity for the synthesis of chitin oligosaccharide precursor. The MS indicates the formation of linear oligosaccharides **L-n** only. Gradually we increased the total amount of electricity as 0.6, 0.7, 0.8, 0.9, 1.0, 1.05, 1.10 F/mol and with the increasing amount of electricity the peaks of linear oligosaccharides **L-n** are gone and the peaks of cyclic oligosaccharides **Cy-n** are raised. According to MALDI TOF MS (**Figure 3-4**) all the linear oligosaccharide **L-n** was converted to corresponding cyclic oligosaccharide **Cy-n** at 1.05 F/mol electricity.

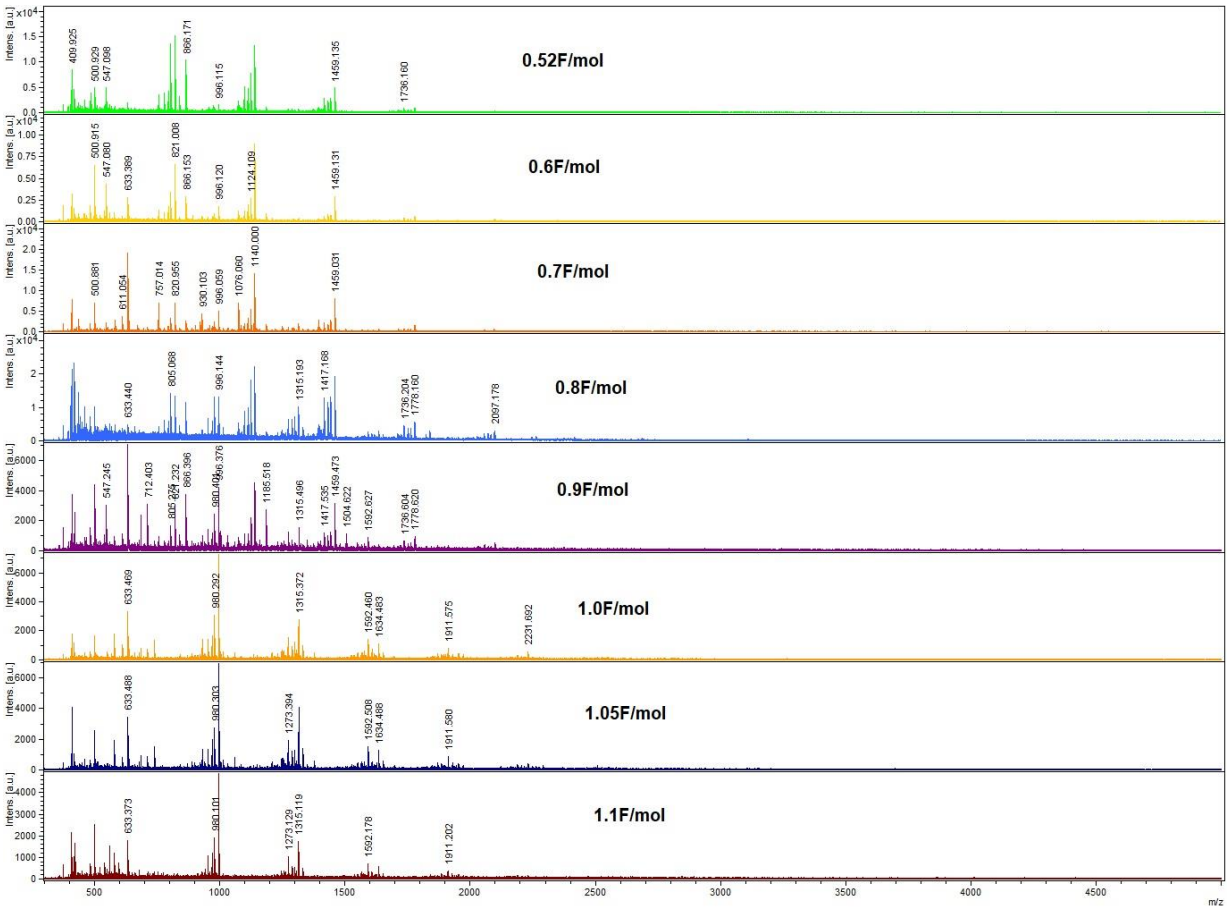


Figure 3-4. Effect of electricity in electrochemical polyglycosylation for building block **1c**.

According to the MS there is a possibility to obtain both linear and cyclic oligosaccharides **L-n**, **Cy-n** in the range of 0.8-0.9 F/mol electricity.

Conclusion

In this chapter we investigated electrochemical polyglycosylation of monosaccharides with protecting-group-free 6-OH **1a**, **1b**. Product distribution was influenced by C-2 substituent and protecting group of 3-OH and 4-OH. 2-Azido group was useful to avoid formation of 1,6-anhydrosugar **Cy-1**; however, further optimization of reaction conditions is needed to prepare cyclic oligosaccharides **Cy-n** selectively. Therefore, further investigation of protecting group and reaction conditions is in progress.

References

(1) Rahman, M. A.; Kuroda, K.; Endo, K.; Sasaki, N.; Hamada, T.; Sakai, H.; Nokami, T. *Beilstein J. Org. Chem.* **2022**, *18*, 1133–1139.

(2) Nokami, T.; Hayashi, R.; Saigusa, Y.; Shimizu, A.; Liu, C. Y.; Mong, K. K. T.; Yoshida, J. I. *Org. Lett.* **2013**, *15* , 4520–4523.

(3) Manmode, S.: Tanabe, S.; Yamamoto, T.; Sasaki, N.; Nokami, T.; Itoh, T. *ChemistryOpen* **2019**, *8*, 869–872.

(4) Someya, H.; Seki, T.; Ishigami, G.; Itoh, T.; Saga, Y.; Yamada Y.; Aoki, S. *Carbohydr. Res.* **2020**, *487*, 107888.

(5) Nokami, T.; Isoda, Y.; Sasaki, N.; Takaiso, A.; Hayase, S.; Itoh, T.; Hayashi, R.; Shimizu, A.; Yoshida, J. *Org. Lett.* **2015**, *17*, 1525–1528.

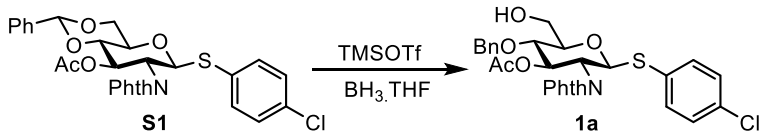
(6) Gening, M. L.; Titov, D. V.; Grachev, A. A.; Gerbst, A. G.; Yudina, O. N.; Shashkov, A. S.; Chizhov, A. O.; Tsvetkov, E.; Nifantiev, N. E. *Eur. J. Org. Chem.* **2010**, 2465–2475.

Experimental Section

General

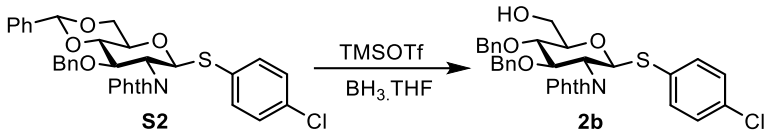
All reactions were carried out under argon atmosphere except notice. ^{1}H NMR and ^{13}C NMR spectra were recorded on JEOL JNM-ECZ600 (600 MHz for ^{1}H and 150 MHz for ^{13}C). ESI-MS spectra were recorded on Thermo Scientific Exactive spectrometer. MALDI-TOF MS spectra were recorded on Bruker Ultraflextreme spectrometer. Merck TLC (silica gel 60 F_{254}) was employed for TLC analysis. Gel permeation chromatography (GPC) was used with JAI Labo Ace LC-5060 recycling preparative HPLC (eluent: $CHCl_3$). Kanto silica gel (spherical, neutral, 63-210 μm) and Sephadex LH-20 were used for Silica gel chromatography and gel filtration chromatography, respectively. Compounds **S1**,[1] **S2**,[2] and **S3**, were synthesized according to the reported procedures. 1,6-anhydrosugars **4a**[3] and **4b**[4] were reported accordingly. Unless otherwise mentioned, all reagents were obtained from commercial suppliers and used without extra purification.

Synthesis of Monosaccharide building blocks

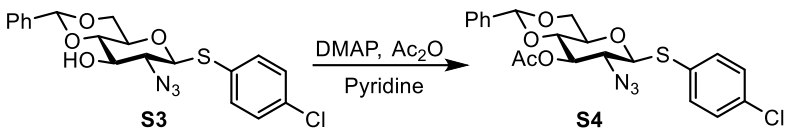


To stirred solution of **S1** (2.46 mmol, 1.3 g), and BH_3•THF (12.5 mL), TMSOTf (1.3 mL) was added dropwise at 0 °C. Then the mixture was stirred for four hours at room temperature. Reaction was quenched with saturated aqueous $NaHCO_3$ solution, and the reaction mixture was taken to ethyl acetate. The mixture was washed with saturated aqueous $NaHCO_3$ (3 times), water (3 times) and brine respectively. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified with silica gel chromatography (Hexane/EtOAc 2:1) to obtain **1a** (2.26 mmol, 1.20 g) as white solid form in 92% yield. **4-Chlorophenyl-3-*O*-acetyl-4-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside** (**1a**) TLC (Hexane/EtOAc 2:1) R_f 0.53; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.85 (*pseudo*-t, *J* = 6.6 Hz, 2 H), 7.74 (dd, *J* = 8.4, 2.4 Hz, 2 H), 7.33–7.30 (m, 3 H), 7.24–7.22 (m, 2 H), 5.78 (dd, *J* = 10.2, 9.3 Hz, 1 H), 5.73 (d, *J* = 10.8 Hz, 1 H), 4.66 (d, *J* = 11.7 Hz, 1 H), 4.62 (d, *J* = 11.7 Hz, 1 H), 4.20 (*pseudo*-t, *J* = 10.5 Hz, 1 H), 3.94 (dd, *J* = 12.3, 2.7 Hz, 1 H), 3.79–3.76 (m, 1 H), 3.75–3.73 (m, 1 H), 3.64 (ddd, *J*

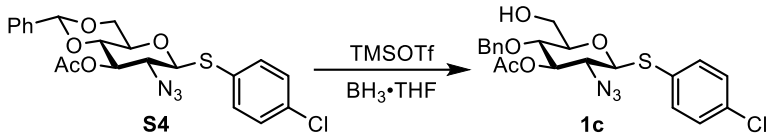
= 9.9, 3.9, 2.7 Hz, 1 H), 1.79 (dd, J = 8.4, 5.4 Hz, 1 H), 1.75 (s, 3 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.2, 167.9, 167.4, 137.7, 134.9, 134.62, 134.55, 134.3, 131.7, 131.2, 129.6, 129.3, 128.6, 128.1, 127.9, 123.9, 123.7, 82.9, 79.5, 76.0, 74.9, 73.9, 61.8, 54.2, 20.6; HRMS (ESI) *m/z* calcd for $C_{29}H_{26}ClKNO_7S$ $[M+K]^+$, 606.0750; found, 606.0728.



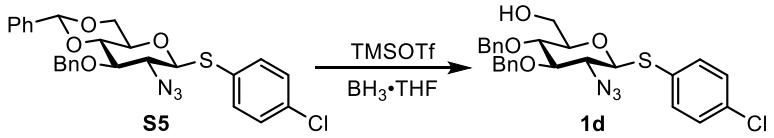
To stirred solution of **S2** (1.38 mmol, .849 g), and BH_3•THF (7 mL), TMSOTf (0.7 mL) was added dropwise at 0 °C. Then the mixture was stirred for four hours at room temperature. Reaction was quenched with saturated aqueous $NaHCO_3$ solution, and the reaction mixture was taken to ethyl acetate. The mixture was washed with saturated aqueous $NaHCO_3$ (3 times), water (3 times) and brine respectively. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified with silica gel chromatography (Hexane/EtOAc 3:1) to obtain **2b** (1.17 mmol, 0.73 g) as white solid form in 85% yield. **4-Chlorophenyl-3,4-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (2b)** TLC (Hexane/EtOAc 2:1) R_f 0.50; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.81–7.60 (m, 5 H), 7.31–7.25 (m, 3 H), 7.19 (d, J = 8.4 Hz, 2 H), 6.98 (d, J = 7.2 Hz, 2 H), 6.89–6.82 (m, 3 H), 5.54 (d, J = 10.5 Hz, 1 H), 4.87 (d, J = 10.8 Hz, 1 H), 4.79 (d, J = 12.3 Hz, 1 H), 4.72 (d, J = 10.8 Hz, 1 H), 4.44 (d, J = 12.3 Hz, 1 H), 4.37 (dd, J = 10.2, 9.0 Hz, 1 H), 4.19 (*pseudo*-t, J = 10.5 Hz, 1 H), 3.93 (dd, J = 12.3, 2.4 Hz, 1 H), 3.76 (dd, J = 12.3, 4.5 Hz, 1 H), 3.72–3.68 (m, 1 H), 3.59 (dd, J = 9.9, 2.4 Hz, 1 H), 2.16–2.04 (m, 1 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.2, 167.5, 138.0, 137.9, 134.3, 134.2, 134.1, 133.9, 131.7, 131.5, 130.6, 129.2, 128.7, 128.3, 128.2, 128.1, 127.6, 123.6, 123.6, 83.4, 80.2, 80.0, 79.3, 77.7, 77.5, 77.3, 75.2, 75.1, 61.8, 55.1; HRMS (ESI) *m/z* calcd for $C_{34}H_{30}ClNNaO_6S$ $[M+Na]^+$, 638.1375; found, 638.1357.



To stirred solution of **S3** (6.45 mmol, 2.70 g), CH_2Cl_2 (24 mL), DMAP (84 mg), pyridine (7.0 mL), and acetic anhydride (4.0 mL) was added dropwise. Then the mixture was stirred overnight at room temperature. Reaction was quenched with 1N HCl solution, and the reaction mixture was taken to ethyl acetate. The mixture was washed with 1N HCl solution (3 times), saturated aqueous $NaHCO_3$ (3 times), water (3 times) and brine respectively. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified with silica gel chromatography (Hexane/EtOAc 4:1) to obtain **S4** (6.20 mmol, 2.89 g) as white solid form in 96% yield. **4-Chlorophenyl 3-*O*-acetyl-2azido-4,6-*O*-benzylidene-2-deoxy-1-thio-β-D-glucopyranoside (S4)** TLC (Hexane/EtOAc 3:1) R_f 0.50; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.50–7.48 (m, 2 H), 7.44 (dd, J = 7.8, 1.5 Hz, 2 H), 7.36–7.30 (m, 6 H), 5.45 (s, 1 H), 5.27 (*pseudo*-t, J = 9.6 Hz, 1 H), 4.55 (d, J = 10.2 Hz, 1 H), 4.32 (dd, J = 10.5, 4.8 Hz, 1 H), 3.72 (*pseudo*-t, J = 10.2 Hz, 1 H), 3.51 (*pseudo*-t, J = 9.6 Hz, 1 H), 3.46 (td, J = 9.6, 4.8, Hz, 1 H), 3.40 (*pseudo*-t, J = 9.9 Hz, 1 H), 2.10 (s, 3 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.6, 137.0, 135.2, 135.2, 129.5, 129.3, 128.4, 126.3, 101.5, 86.6, 78.3, 77.7, 77.5, 77.3, 73.0, 70.7, 68.3, 63.6, 20.9. ; HRMS (ESI) *m/z* calcd for $C_{21}H_{20}ClKN_3O_5S$ $[M+K]^+$, 500.0444; found, 500.0425.

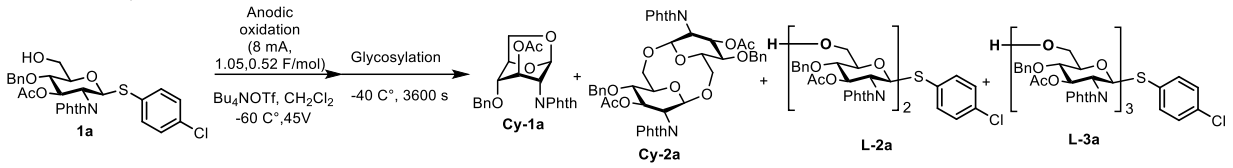


To stirred solution of **S4** (1.37 mmol, 0.63 g), and BH_3•THF (7 mL), TMSOTf (0.7 mL) was added dropwise at 0 °C. Then the mixture was stirred for four hours at room temperature. Reaction was quenched with saturated aqueous $NaHCO_3$ solution, and the reaction mixture was taken to ethyl acetate. The mixture was washed with saturated aqueous $NaHCO_3$ (3 times), water (3 times) and brine respectively. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified with silica gel chromatography (Hexane/EtOAc 5:1) to obtain **1c** (1.19 mmol, 0.55 g) as white solid form in 86% yield. **4-Chlorophenyl-3-*O*-acetyl-2-azido-4-*O*-benzyl-2-deoxy-1-thio-β-D-glucopyranoside (1c)** TLC (Hexane/EtOAc 2:1) R_f 0.47; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.49–7.46 (m, 2 H), 7.34–7.28 (m, 5 H), 7.24 (d, *J* = 8.1 Hz, 2 H), 5.15 (*pseudo*-t, *J* = 9.6 Hz, 1 H), 4.61 (d, *J* = 11.4 Hz, 1 H), 4.56 (d, *J* = 11.4 Hz, 1 H), 4.49 (d, *J* = 10.2 Hz, 1 H), 3.89 (ddd, *J* = 12.3, 5.4, 2.4 Hz, 1 H), 3.74–3.70 (m, 1 H), 3.57 (*pseudo*-t, *J* = 9.6 Hz, 1 H), 3.41 (ddd, *J* = 9.9, 3.9, 2.7 Hz, 1 H), 3.26 (*pseudo*-t, *J* = 9.9 Hz, 1 H), 2.00 (s, 3 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.9, 137.4, 135.2, 135.0, 129.5, 129.4, 128.7, 128.2, 128.1, 86.1, 79.7, 77.3, 77.1, 76.9, 75.9, 75.1, 74.8, 63.4, 61.6, 20.9; HRMS (ESI) *m/z* calcd for $C_{21}H_{22}ClNaN_3O_5S$ $[M+Na]^+$, 486.0861; found, 486.0842.



To stirred solution of **S5** (2.31 mmol, 1.18 g), and BH_3•THF (12 mL), TMSOTf (1.2 mL) was added dropwise at 0 °C. Then the mixture was stirred for six hours at room temperature. Reaction was quenched with saturated aqueous $NaHCO_3$ solution, and the reaction mixture was taken to ethyl acetate. The mixture was washed with saturated aqueous $NaHCO_3$ (3 times), water (3 times) and brine respectively. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified with silica gel chromatography (Hexane/EtOAc 5:1) to obtain **1d** (1.47 mmol, 0.75 g) as white solid form in 64% yield. **4-Chlorophenyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-1-thio-β-D-glucopyranoside (1d)**; TLC (Hexane/EtOAc 3:1) R_f 0.40; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.48–7.46 (m, 2 H), 7.36–7.32 (m, 6 H), 7.32–7.25 (m, 7 H), 4.88–4.86 (m, 2 H), 4.84–4.82 (m, 1 H), 4.64 (d, *J* = 11.1 Hz, 1 H), 4.41 (d, *J* = 10.2 Hz, 1 H), 3.87 (dd, *J* = 6.0, 2.7 Hz, 1 H), 3.71–3.67 (m, 1 H), 3.55–3.50 (m, 2 H), 3.36 (dd, *J* = 4.8, 2.7 Hz, 1 H), 3.30 (ddd, *J* = 10.2, 6.6, 2.7 Hz, 1 H), 1.82 (*pseudo*-t, *J* = 6.9 Hz, 1 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 137.6, 137.5, 135.1, 134.9, 129.6, 129.4, 128.69, 128.65, 128.32, 128.21, 128.19, 128.12, 86.1, 84.9, 79.8, 76.0, 75.2, 65.3, 61.9; HRMS (ESI) *m/z* calcd for $C_{26}H_{26}ClNaN_3O_4S$ $[M+Na]^+$, 534.1225; found, 534.1211.

4. Synthesis of Cyclic and linear oligosaccharides by electrochemical poly-glycosylation (building block 1)



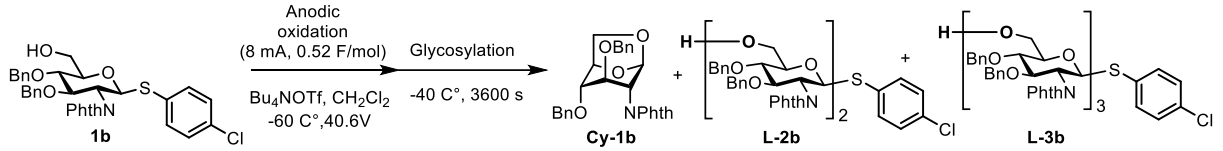
The electrochemical polymerization synthesis of linear and cyclic oligosaccharides **Cy-1a**, **Cy-2a**, **L-2a**, **L-3a** was carried out on an H-type divided cell (4G glass filter). The cell had a carbon felt anode (Nippon Carbon JF-20-P7) and platinum square plate (20 mm×20 mm). Building block **1a** (0.40 mmol, 226 mg), Bu_4NOTf (1.00 mmol, 391.6 mg), and CH_2Cl_2 (10 mL) were added to the anodic chamber. Trifluoromethanesulfonic acid (0.40 mmol, 36 μL), Bu_4NOTf (1.00 mmol, 391.6 mg), and CH_2Cl_2 (10 mL) were added to the cathodic chamber. The constant current (8 mA (current density: 2.0 mA/cm^2), 45 V (electrode distance: 4.5 cm)) was employed at –60 °C with magnetic stirring until 0.52 F/mol and 1.05F/mol of the electricity was consumed. After the electrolysis, the reaction was kept stirring at –40 °C for 1 h. After that, triethylamine (0.3 mL) was added to both chambers. The solution in both chambers was collected in eggplant flask, and the solvent was removed under reduced pressure. The mixture was dissolved in EtOAc and washed with water (3 times) and brine, respectively. The solution was dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified with preparative-GPC to afford linear oligosaccharides **L-2a** (*n* = 2, 13%), **L-3a** (*n* = 3, 6%), 1-6 anhydro sugar **Cy-1a** (*n* = 1, 25%) and cyclic disaccharide **Cy-2a** (*n* = 2, 7%), **4-Chlorophenyl (3-*O*-acetyl-4-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→6)-3-*O*-acetyl-4-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside** (**L-2a**) TLC (Hexane/EtOAc 1:1) R_f = 0.33; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.81 (*pseudo*-t, *J* = 5.1 Hz, 2 H), 7.74–7.70 (m, 4 H), 7.61–7.58 (m, 2 H), 7.34–7.30 (m, 2 H), 7.29–7.27 (m, 3 H), 7.25–7.24 (m, 5 H), 7.20 (dd, *J* = 5.1, 1.8 Hz, 2 H), 7.02–7.01 (m, 2 H), 5.79 (dd, *J* = 10.5, 9.0 Hz, 1 H), 5.66 (dd, *J* = 10.2, 9.0 Hz, 1 H), 5.56 (d, *J* = 10.5 Hz, 1 H), 5.53 (d, *J* = 8.4 Hz, 1 H), 4.68 (dd, *J* = 20.1, 11.7 Hz, 2 H), 4.34 (dd, *J* = 21.6, 11.4 Hz, 2 H), 4.28 (dd, *J* = 10.8, 8.4 Hz, 1 H), 4.10 (*pseudo*-t, *J* = 10.5 Hz, 1 H), 4.05 (dd, *J* = 11.1, 1.5 Hz, 1 H), 3.95 (d, *J* = 10.5 Hz, 1 H), 3.84–3.75 (m, 3 H), 3.67–3.62 (m, 2 H), 3.52 (*pseudo*-t, *J* = 9.6 Hz, 1 H), 1.99 (s, 1 H), 1.79 (s, 3 H), 1.66 (s, 3 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.3, 170.1, 167.8, 167.3, 137.8, 137.5, 134.6, 134.5, 134.4, 134.3, 131.7, 131.2, 129.8, 129.2, 128.6, 128.5, 128.1, 127.9, 127.9, 127.5, 123.8, 123.7, 123.6, 123.6, 107.4, 98.3, 82.6, 78.5, 77.3, 77.1, 76.9, 76.6, 76.4, 75.4, 74.8, 74.7, 73.9, 73.2, 68.5, 61.8, 55.2, 53.9, 29.8, 20.7, 20.5; HRMS (ESI) *m/z* calculated for $C_{52}H_{47}ClKN_2O_{47}S$ $[M+K]^+$, 1029.2068; found, 1029.2040.

4-Chlorophenyl (3-*O*-acetyl-4-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→6)-(3-*O*-acetyl-4-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→6)-3-*O*-acetyl-4-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**L-3a**) TLC (Hexane/EtOAc 1:1) R_f 0.23; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.83–7.80 (m, 2 H), 7.72–7.70 (m, 2 H), 7.62–7.57 (m, 4 H), 7.29 (d, *J* = 7.2 Hz, 5 H), 7.24 (d, *J* = 5.7 Hz, 8 H), 7.22–7.18 (m, 6 H), 7.01–6.97 (m, 4 H), 5.78 (dd, *J* = 10.8, 9.0 Hz, 1 H), 5.66-5.62 (m, 2 H), 5.56–5.53 (m, 2 H), 5.45 (d, *J* = 8.4 Hz, 1 H), 4.67 (d, *J* = 11.7 Hz, 1 H), 4.61 (d, *J* = 11.7 Hz, 1 H), 4.35–4.28 (m, 4 H), 4.25–4.22 (m, 1 H), 4.21–4.19 (m, 1 H), 4.13–4.09 (m, 2 H), 4.03 (*pseudo*-t, *J* = 10.5 Hz, 2 H), 3.97–3.94 (m, 1 H), 3.87 (dd, *J* = 11.4, 4.2 Hz, 1 H), 3.80–3.75 (m, 2 H), 3.70 (dd, *J* = 11.4, 4.8 Hz, 1 H), 3.67–3.64 (m, 2 H), 3.63–3.57 (m, 2 H), 3.45 (*pseudo*-t, *J* = 9.6 Hz, 1 H), 2.03 (s, 1 H), 1.76 (d, *J* = 3.8 Hz, 3 H), 1.70 (d, *J* = 6.5 Hz, 3 H), 1.62 (s, 3 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.3, 170.1, 167.9, 167.3, 137.9, 137.6, 134.9, 134.7, 134.5, 134.3, 134.2, 134.1, 131.8, 131.2, 129.3, 128.6, 128.5, 128.4, 128.4, 127.9, 127.8, 127.6, 127.5, 123.8, 123.7, 123.6, 98.2, 82.0, 78.3, 76.7, 76.5,

76.4, 75.5, 74.8, 74.7, 74.66, 74.65, 73.8, 73.3, 73.25, 73.2, 68.2, 68.16, 61.6, 60.5, 55.3, 55.0, 31.7, 22.7, 21.2, 20.7, 20.6, 20.5, 14.3, 14.2; HRMS (ESI) *m/z* calcd for $C_{75}H_{68}ClNaN_3O_{21}S$ $[M+Na]^+$, 1436.3647; found, 1436.3628.

Cyclobis-(1→6)-(3-*O*-acetyl-4-*O*-benzyl-2-Phthalimide-2-deoxy-2-β-D-glucopyranosyl) (Cy-2a) TLC (Hexane/EtOAc 1:1) R_f 0.25; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.87 (dd, *J* = 5.4, 3.0 Hz, 2 H), 7.74–7.72 (m, 2H), 7.39–7.38 (m, 1 H), 7.32–7.26 (m, 4 H), 5.70 (dd, *J* = 10.8, 8.7 Hz, 1 H), 4.93 (d, *J* = 1.8 Hz, 1 H), 4.78 (d, *J* = 11.7 Hz, 1 H), 4.70 (d, *J* = 11.7 Hz, 1 H), 4.48–4.45 (m, 2 H), 4.16–4.14 (m, 1 H), 3.88 (dd, *J* = 12.0, 2.7 Hz, 1 H), 3.80 (d, *J* = 11.7 Hz, 1 H), 1.90 (s, 3 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.5, 167.7, 138.3, 134.4, 131.7, 129.4, 128.5, 127.9, 123.7, 100.1, 75.0, 73.4, 71.8, 68.3, 57.7, 20.9; HRMS (ESI) *m/z* calcd for $C_{46}H_{42}KN_2O_{14}$ $[M+K]^+$, 885.2268; found, 885.2217.

5. Synthesis of Cyclic and linear oligosaccharides by electrochemical poly-glycosylation (building block 1b)

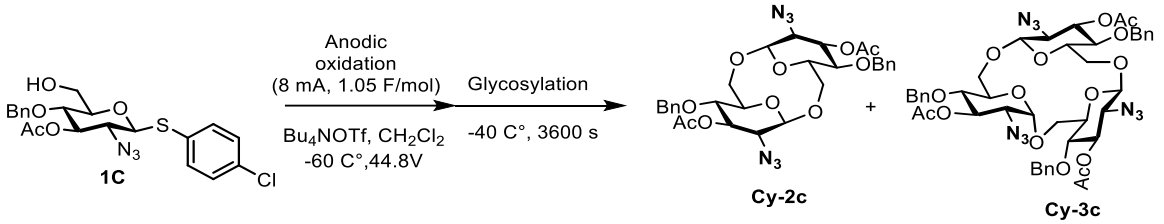


The electrochemical polymerization synthesis of linear and cyclic oligosaccharides **Cy-1b**, **L-2b, L-3b** was carried out on an H-type divided cell (4G glass filter). The cell had a carbon felt anode (Nippon Carbon JF-20-P7) and platinum square plate (20 mm × 20 mm). Building block **1b** (0.40 mmol, 246 mg), Bu_4NOTf (1.00 mmol, 391.6 mg), and CH_2Cl_2 (10 mL) were added to the anodic chamber. Trifluoromethanesulfonic acid (0.40 mmol, 36 μL), Bu_4NOTf (1.00 mmol, 391.6 mg), and CH_2Cl_2 (10 mL) were added to the cathodic chamber. The constant current (8 mA (current density: 2.0 mA/cm^2), 40.6 V (electrode distance: 4.5 cm)) was employed at –60 °C with magnetic stirring until 0.52 F/mol and 1.05 F/mol of the electricity was consumed. After the electrolysis, the reaction was kept stirring at –40 °C for 1 h. After that, triethylamine (0.3 mL) was added to both chambers. The solution in both chambers was collected in eggplant flask, and the solvent was removed under reduced pressure. The mixture was dissolved in EtOAc and washed with water (3 times) and brine, respectively. The solution was dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified with preparative-GPC to afford linear oligosaccharides **L-2b** (*n* = 2, 3.6%), **L-3b** (*n* = 3, 2.4%), 1,6-anhydro sugar **Cy-1b** (*n* = 1, 25%)

4-Chlorophenyl (3,4-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→6)-3,4-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (L-2b) TLC (Hexane/EtOAc 2:1) R_f 0.23. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.76–7.62 (m, 4 H), 7.56–7.48 (m, 3 H), 7.36–7.33 (m, 4 H), 7.32–7.28 (m, 1 H), 7.24–7.17 (m, 6 H), 7.09–7.07 (m, 2 H), 7.00–6.99 (m, 2 H), 6.89–6.84 (m, 5 H), 6.84–6.78 (m, 5 H), 5.31 (d, *J* = 10.5 Hz, 1 H), 5.28 (d, *J* = 8.4 Hz, 1 H), 4.89 (d, *J* = 10.8 Hz, 1 H), 4.81 (d, *J* = 12.3 Hz, 1 H), 4.74 (d, *J* = 11.1 Hz, 1 H), 4.64 (d, *J* = 12.3 Hz, 1 H), 4.52 (d, *J* = 10.8 Hz, 1 H), 4.45 (d, *J* = 12.3 Hz, 1 H), 4.37–4.33 (m, 2 H), 4.28 (d, *J* = 12.3 Hz, 1 H), 4.21 (dd, *J* = 10.8, 8.7 Hz, 2 H), 4.03 (*pseudo*-t, *J* = 10.5 Hz, 1 H), 3.99 (dd, *J* = 10.8, 1.5 Hz, 1 H), 3.91 (d, *J* = 10.2 Hz, 1 H), 3.78–3.72 (m, 2 H), 3.67 (dd, *J* = 11.1, 5.1 Hz, 1 H), 3.56–3.51 (m, 2 H), 3.44–3.40 (m, 1 H), 2.09–2.07 (m, 1 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.23, 167.21, 138.0, 137.9, 137.7, 137.5, 134.3, 134.0, 133.98, 133.97, 133.95, 133.9, 131.5, 131.49, 130.2, 129.1, 128.7, 128.6, 128.5, 128.4, 128.2, 128.15, 128.1, 128.07, 128.04, 128.01, 127.96, 127.5, 123.5, 123.48, 123.45, 123.4, 98.4, 82.9, 80.1, 79.6, 79.2, 79.1, 78.6, 75.5, 75.2, 75.0, 68.2, 62.0, 55.8, 54.6, 29.8; HRMS (ESI) *m/z* calcd for $C_{62}H_{55}ClNaN_2O_{12}S$ $[M+Na]^+$, 1109.3056; found, 1109.3041.

4-Chlorophenyl (3,4-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→6)-(3,4-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→6)-3,4-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (L-3b) TLC (Hexane/EtOAc 1:1) R_f 0.50; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.90–7.85 (m, 4 H), 7.79–7.75 (m, 2 H), 7.73–7.70 (m, 3 H), 7.69–7.65 (m, 2 H), 7.40–7.30 (m, 11 H), 7.24–7.17 (m, 4 H), 7.13–7.02 (m, 2 H), 7.01–6.95 (m, 4 H), 6.92–6.77 (m, 10 H), 5.56 (s, 1 H), 5.53 (d, *J* = 11.4 Hz, 1 H), 5.47 (d, *J* = 10.8 Hz, 1 H), 5.31 (d, *J* = 6.9 Hz, 1 H), 5.26–5.23 (m, 1 H), 5.17–5.15 (m, 1 H), 4.89–4.84 (m, 1 H), 4.78 (d, *J* = 12.6 Hz, 1 H), 4.75–4.71 (m, 3 H), 4.70–4.67 (m, 3 H), 4.64 (dd, *J* = 11.6, 2.9 Hz, 3 H), 4.61–4.55 (m, 2 H), 4.49–4.44 (m, 2 H), 4.43–4.40 (m, 1 H), 4.38–4.31 (m, 4 H), 4.29–4.24 (m, 2 H), 4.21–4.13 (m, 3 H), 4.08–4.04 (m, 2 H), 3.98-3.92 (m, 2 H), 3.80–3.69 (m, 9 H), 3.63–3.55 (m, 2 H), 3.46–3.35 (m, 5 H), 2.11 (d, *J* = 4.3 Hz, 1 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.2, 168.0, 137.6, 137.4, 134.8, 134.6, 134.3, 134.2, 133.9, 131.8, 131.4, 129.2, 129.14, 129.1, 128.8, 128.7, 128.6, 128.59, 128.57, 128.5, 128.4, 128.3, 128.25, 128.2, 128.15, 128.13, 128.10, 128.09, 128.05, 128.04, 128.02, 127.99, 127.93, 127.91, 127.9, 127.6, 127.4, 127.36, 123.9, 123.6, 123.4, 102.2, 101.1, 85.4, 79.0, 76.5, 75.1, 74.8, 72.5, 71.8, 70.4, 70.3, 68.8, 68.2, 64.7, 57.8, 51.9, 29.8, 26.5; HRMS (ESI) *m/z* calcd for $C_{90}H_{80}ClKN_3O_{18}S$ $[M+K]^+$, 1596.4478; found, 1596.4441.

6. Synthesis of Cyclic and linear oligosaccharides by electrochemical polyglycosylation (building block 1c)

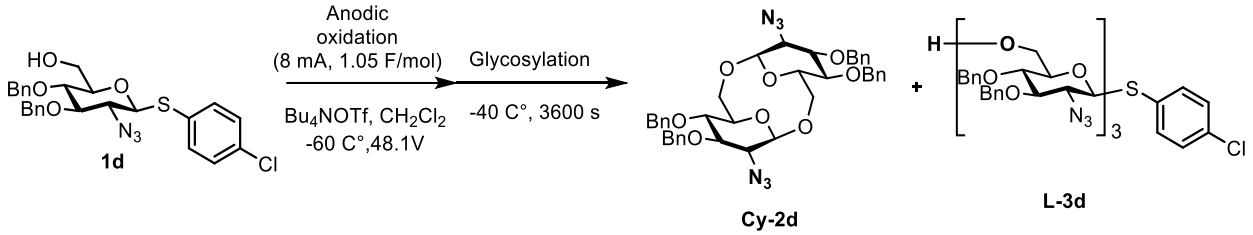


The electrochemical polymerization synthesis of cyclic oligosaccharides **Cy-2c** and **Cy-3c** was carried out by an H-type divided cell (4G glass filter). The cell had a carbon felt anode (Nippon Carbon JF-20-P7) and platinum square plate (20 mm×20 mm). Building block **1c** (0.40 mmol, 185.24 mg), Bu_4NOTf (1.00 mmol, 391.6 mg), and CH_2Cl_2 (10 mL) were added to the anodic chamber. Trifluoromethanesulfonic acid (0.4 mmol, 36 μL), Bu_4NOTf (1.00 mmol, 391.6 mg), and CH_2Cl_2 (10 mL) were added to the cathodic chamber. The constant current (8 mA (current density: 2.0 mA/cm^2), 44.8 V (electrode distance: 4.5 cm)) was employed at –60 °C with magnetic stirring until 0.52 F/mol and 1.05F/mol of the electricity was consumed. After the electrolysis, the reaction was kept stirring at –40 °C for 1 h. After that, triethylamine (0.3 mL) was added to both chambers. The solution in both chambers was collected in eggplant flask, and the solvent was removed under reduced pressure. The mixture was dissolved in EtOAc and washed with water (3 times) and brine, respectively. The solution was dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified with preparative-GPC to afford cyclic oligosaccharides **Cy-2c** (*n* = 2, 49%), **Cy-3c** (*n* = 3, 17%), and a trace amount of cyclic oligosaccharide **Cy-4c** (*n* = 4) and linear tetrasaccharide **L-4c** (*n* = 4).

Cyclobis-(1→6)-(3-*O*-acetyl-2-azido-4-*O*-benzyl-2-deoxy-2-β-D-glucopyranosyl) (Cy-2c) TLC (Hexane/EtOAc 3:1) R_f 0.28; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.35–7.29 (m, 5 H), 5.05 (dd, *J* = 9.0, 3.6 Hz, 1 H), 4.72 (d, *J* = 1.8 Hz, 1 H), 4.68 (dd, *J* = 16.2, 11.7 Hz, 2 H), 4.44 (dd, *J* = 10.2, 9.6 Hz, 1 H), 4.11 (dd, *J* = 12.3, 1.2 Hz, 1 H), 3.80 (d, *J* = 10.2 Hz, 1 H), 3.71 (dd, *J* = 12.6, 1.2 Hz, 1 H), 3.65 (dd, *J* = 3.6, 1.8 Hz, 1 H), 1.96 (s, 3 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.2, 138.1, 128.6, 128.2, 128.0, 100.3, 75.7, 74.7, 74.5, 73.7, 70.9, 65.3, 21.0; HRMS (ESI) *m/z* calcd for $C_{30}H_{34}KN_6O_{10}$ $[M+K]^+$, 677.1968; found, 677.1933.

Cyclotris-(1→6)-(3-*O*-acetyl-2-azido-4-*O*-benzyl-2-deoxy-2-α,β,β-D-glucopyranosyl) (Cy-3c) TLC (Hexane/EtOAc 2:1) R_f 0.32; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.37–7.28 (m, 9 H), 7.26 (d, *J* = 7.8 Hz, 2 H), 7.24 (s, 1 H), 7.22–7.21 (m, 3 H), 5.61 (dd, *J* = 10.8, 9.0 Hz, 1 H), 5.08 (dd, *J* = 10.2, 9.6 Hz, 1 H), 4.93 (*pseudo*-t, *J* = 9.9 Hz, 1 H), 4.79 (d, *J* = 3.6 Hz, 1 H), 4.71 (d, *J* = 11.4 Hz, 1 H), 4.61 (*pseudo*-t, *J* = 11.4 Hz, 3 H), 4.52–4.49 (m, 4 H), 4.31 (dd, *J* = 12.3, 1.8 Hz, 1 H), 4.20 (d, *J* = 8.1 Hz, 1 H), 4.11 (dd, *J* = 14.4, 7.2 Hz, 1 H), 4.02 (dd, *J* = 13.2, 8.7 Hz, 1 H), 3.91-3.89 (m, 1 H), 3.80–3.72 (m, 4 H), 3.71–3.68 (m, 1 H), 3.45–3.40 (m, 2 H), 3.29 (*pseudo*-t, *J* = 9.6 Hz, 1 H), 3.17–3.12 (m, 2 H), 3.05 (dd, *J* = 10.8, 3.6 Hz, 1 H), 2.08 (s, 3 H), 2.06 (s, 3 H), 1.96 (s, 3 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.0, 169.7, 137.9, 137.2, 137.15, 128.8, 128.7, 128.5, 128.48, 128.3, 128.0, 127.9, 127.74, 127.7, 105.7, 101.3, 97.9, 77.7, 76.4, 75.3, 75.28, 74.9, 74.3, 74.2, 74.0, 73.9, 73.5, 71.8, 71.6, 69.6, 68.8, 64.9, 63.7, 61.2, 21.1, 21.0, 20.96, 14.3; HRMS (ESI) *m/z* calcd for $C_{45}H_{51}KN_9O_{15}$ $[M+K]^+$, 996.3136; found, 996.3195.

7. Synthesis of Cyclic and linear oligosaccharides by electrochemical polyglycosylation (building block 1d)



The electrochemical polymerization synthesis of linear and cyclic disaccharides **Cy-2d** and linear trisaccharide **L-3d** was carried out by an H-type divided cell (4G glass filter). The cell had a carbon felt anode (Nippon Carbon JF-20-P7) and platinum square plate (20 mm×20 mm). Building block **1d** (0.4 mmol, 185.24 mg), Bu_4NOTf (1.00 mmol, 391.6 mg), and CH_2Cl_2 (10 mL) were added to the anodic chamber. Trifluoromethanesulfonic acid (0.4 mmol, 36 μL), Bu_4NOTf (1.00 mmol, 391.6 mg), and CH_2Cl_2 (10 mL) were added to the cathodic chamber. The constant current (8 mA (current density: 2.0 mA/cm^2), 48.1 V (electrode distance: 4.5 cm) was employed at –60 °C with magnetic stirring until 0.52 F/mol and 1.05 F/mol of the electricity was consumed. After the electrolysis, the reaction was kept stirring at –40 °C for 1 h. After that, triethylamine (0.3 mL) was added to both chambers. The solution in both chambers was collected in eggplant flask, and the solvent was removed under reduced pressure. The mixture was dissolved in EtOAc and washed with water (3 times) and brine, respectively. The solution was dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified with preparative-GPC to afford Cyclic disaccharides **Cy-2d** (*n* = 2, 14%), and linear trisaccharide **L-3d** (*n* = 3, 13%).

Cyclobis-(1→6)-(2-azido-3,4-di-benzyl-2-deoxy-2-β-D-glucopyranosyl) (Cy-2d); TLC (Hexane/EtOAc 3:1) R_f 0.63; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.36–7.34 (m, 2 H), 7.33–7.31 (m, 4 H), 7.30–7.28 (m, 4 H), 4.85 (*pseudo*-t, *J* = 11.4 Hz, 2 H), 4.78 (d, *J* = 11.1 Hz, 1 H), 4.67 (d, *J* = 11.4 Hz, 1 H), 4.58 (d, *J* = 1.2 Hz, 1 H), 4.20 (*pseudo*-t, *J* = 9.6 Hz, 1 H), 3.92 (dd, *J* = 12.0, 2.4 Hz, 1 H), 3.76 (dd, *J* = 9.6, 1.8 Hz, 1 H), 3.67-3.62 (m, 2 H), 3.50 (dd, *J* = 6.6, 1.2 Hz, 1 H), 3.23–3.20 (m, 2 H), 1.63–1.61 (m, 2 H), 1.43 (dd, *J* = 15.0, 7.5 Hz, 2 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 138.2, 137.8, 128.54, 128.49, 128.13, 128.09, 127.98, 127.97, 100.9, 81.7, 75.0, 74.5, 74.2, 69.2, 67.7, 58.9, 24.0, 19.7, 13.7; HRMS (ESI) *m/z* calcd for $C_{40}H_{42}KN_6O_8$ $[M+K]^+$, 773.2696; found, 773.2650.

4-Chlorophenyl (2-azido-2-deoxy-3,4-di-*O*-benzyl-β-D-glucopyranosyl)-(1→6)-(2-azido-2-deoxy-3,4-di-*O*-benzyl-β-D-glucopyranosyl)-(1→6)-2-azido-2-deoxy-3,4-di-*O*-benzyl-1-thio-β-D-glucopyranoside (L-3d) TLC (Hexane/EtOAc 2:1) R_f 0.40; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.54 (dd, *J* = 6.6, 2.1 Hz, 1 H), 7.37–7.25 (m, 23 H), 4.91–4.75 (m, 12 H), 4.64–4.61 (m, 3 H), 4.42 (d, *J* = 10.2 Hz, 1 H), 4.29-4.26 (m, 2 H), 4.16 (dd, *J* = 11.4, 1.8 Hz, 1 H), 4.04 (dd, *J* = 11.4, 1.5 Hz, 1 H), 3.79 (dd, *J* = 11.4, 5.1 Hz, 1 H), 3.66–3.62 (m, 2 H), 3.54–3.45 (m, 4 H), 3.43-3.40 (m, 1 H), 3.38–3.36 (m, 1 H), 3.31 (dd, *J* = 10.2, 9.3 Hz, 1 H), 3.21 (*pseudo*-t, *J* = 8.7 Hz, 4 H), 1.65–1.60 (m, 5 H), 1.43 (dd, *J* = 15.0, 7.5 Hz, 5 H), 1.25 (s, 1 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 137.9, 137.82, 137.79, 137.6, 135.0, 129.3, 128.82, 128.77, 128.66, 128.62, 128.61, 128.54, 128.49, 128.3, 128.21, 128.19, 128.18, 128.15, 128.13, 128.09, 128.08, 128.05, 128.02, 127.99, 127.98, 129.92, 127.9, 127.7, 102.6, 102.4, 86.0, 85.0, 83.2, 82.9, 78.9, 77.8, 77.6, 75.9, 75.8, 75.5, 75.4, 75.13, 75.10, 75.09, 74.8, 66.5, 66.4, 65.1, 61.7, 58.9, 24.0, 19.8, 13.7; HRMS (ESI) *m/z* calcd for $C_{66}H_{68}ClNaN_9O_{12}S$ $[M+Na]^+$, 1268.4289; found, 1268.4249.

Reference

(1) Yano, K.; Itoh, T.; Nokami, T. *Carbohydr. Res.* **2020**, *492*, 108018.

(2) Hevey, R.; Morland, A.; Ling, C. C. *J. Org. Chem.* **2012**, *77* , 6760–6772.

(3) Nokami, T.; Hayashi, R.; Saigusa, Y.; Shimizu, A.; Liu, C. Y.; Mong, K. K. T.; Yoshida, J. *Org. Lett.* **2013**, *15*, 4520–4523.

(4) Garcia, J.; Vilarrasa, J.; Bordas, X.; Banaszek, A. *Tetrahedron Lett.* **1986**, *27*, 639–640.