ChemPubSoc Europe

DOI: 10.1002/open.201900185

ChemistryOPEN Communications

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

Sujit Manmode,[a] Shichidai Tanabe,[a] Takashi Yamamoto,[a] Norihiko Sasaki,[a] Toshiki Nokami,*[a, b] and Toshiyuki Itoh*[a, b]

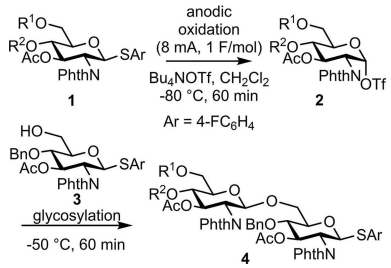
Electrochemical glycosylation of a linear oligosaccharide with a protecting-group-free primary hydroxyl group afforded cyclic oligo-saccharides, up to hexasaccharides, in high yields. Precursors of the cyclic oligosaccharides were prepared by automated electro-chemical assembly-a method for the automated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

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

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

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

Table 1. Optimization of monosaccharide terminal building block.



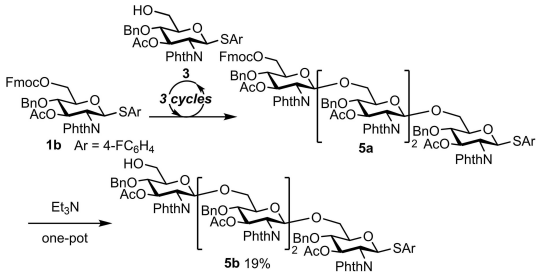
Building block	R^1	R^2	Product	Yield (%)	Selectivity
1a	Ac	Ac	4a	92[a]	β only
1b	Fmoc	Bn	4b (R^1 = H)	59[b]	β only
1c	MOM	Bn	4c	ND[c]	–
1d	ClAc	Bn	4d	45	β only
1e	ClAc	Ac	4e	57	β only

[a] Reference 13a. [b] Glycosylation at −40 °C and yield after Fmoc deprotec-tion (R^1 = H). [c] Not detected.

[a] *Dr. S. Manmode, Mr. S. Tanabe, Mr. T. Yamamoto, N. Sasaki, Prof. T. Nokami, Prof. T. Itoh*
Department of Chemistry and Biotechnology, Graduate School of Engineering, Tottori University
4-101 Koyamachominami, Tottori city 680-8552 Tottori Japan
E-mai:
E-mail: tnokami@tottori-u.ac.jp
titoh@tottori-u.ac.jp
[b] *Prof. T. Nokami, Prof. T. Itoh*
Center for Research on Green Sustainable Chemistry, Faculty of Engineering, Tottori University
4-101 Koyamachominami, Tottori city 680-8552 Tottori, Japan

as reported previously for the synthesis of oligoglucosamines.[14a] To our delight, glycosylation of **1b** afforded the desired product **4b** in 59% yield with perfect β-selectivity (entry 2); however, the yield was much lower than that achieved with **1a** (entry 1). Building block **1c** with methoxymethyl (MOM) protection at 6-OH and benzyl (Bn) protection at the C-4 hydroxyl group (4-OH) equipped donor did not afford even a trace amount of the desired disaccharide **4c**. Building block **1d** bearing chloroacetyl (ClAc) and Bn groups at 6-OH and 4-OH, respectively, gave a decreased glycosylation yield of 45% (entry 4). Finally, building block **1e** bearing ClAc and Bn groups at 6-OH and 4-OH, respectively, afforded disaccharide **4e** in 57% yield (entry 5). These results may be attributed to tolerance of the protecting groups under the electrochemical conditions and stability of the glycosyl triflate formed during the reactions.[15]

The best building block **1b** with 9-fluorenylmethoxycarbonyl (Fmoc) at 6-OH was then used in the automated electrochemical assembly with building block **3** for chain elongation for the synthesis of tetrasaccharides (Scheme 1). Subsequent



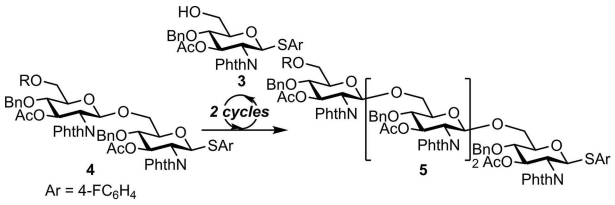
Scheme 1. Automated electrochemical assembly and subsequent one-pot Fmoc deprotection for synthesis of the precursor of the cyclic tetrasaccharide.

one-pot deprotection of Fmoc group afforded the desired tetrasaccharide **5b** in 19% overall yield, which was much lower than that of the tetrasaccharide assembled from the terminal building block **1a**.[14a] Therefore, improvement in the choice of terminal building block is highly desirable in efforts to address the precursors of cyclic oligosac-charides.

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

We then carried out the intramolecular cyclization of tetrasaccharide **5b** under conventional chemical glycosylation conditions (Scheme 2).[16] Tetrasaccharide **5b** was treated with

Table 2. Optimization of disaccharide terminal building block.

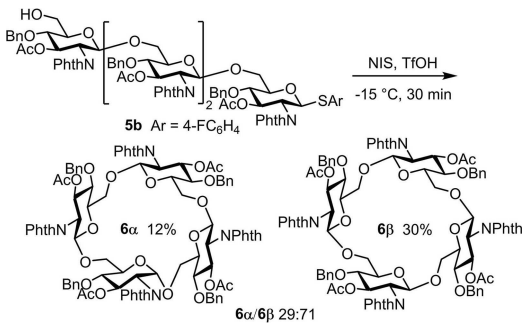


Building block	R	Product	Yield (%)	Selectivity
4f	Fmoc	5b	47	β only
4c	MOM	5c	7	β only
4d	ClAc	5d	ND[a]	–
4 g	Lev	5 g	12	β only

[a] Not detected.

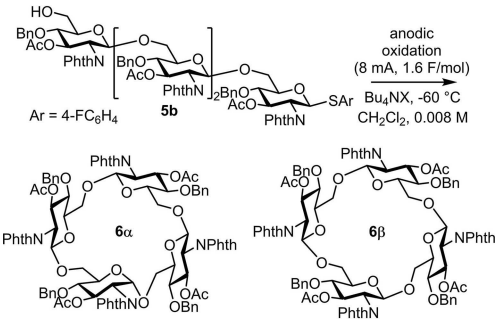
NIS and TfOH at −15 °C. The reaction afforded both α- and β-isomers of cyclic oligoglucosamine **6α** and **6β** in 12% and 30% yields, respectively. This result is in agreement with the finding reported by Nifantiev and coworkers.[12a] Therefore the choice of protecting groups at 4-OH and 3-OH is not crucial for obtaining cyclic oligoglucosamine stereoselectively.

To improve the efficiency of intramolecular glycosylation of tetrasaccharide **5b**, we optimized the reaction conditions of electrochemical glycosylation (Table 3). Tetrabutylammonium tetrafluoroborate (Bu_4NBF_4), a typical electrolyte for electrochemical reactions, afforded the corresponding β-isomer of cyclic oligosaccharide **6β** stereoselectively, but the yield was moderate and the corresponding glycosyl fluoride of the tetrasaccharide was observed by ESI-MS (*m/z* calc. for $C_{92}H_{85}FN_4O_{28}K$ $[M+K]^+$, 1751.4966; found, 1751.4931). Tetrabutyl bis(trifluoromethanesulfonylimide) (Bu_4NNTf_2) led to an improved yield of **6**; however, selectivity was not perfect. Finally, we determined tetrabutylammonium triflate (Bu_4NOTf) to be the best choice of electrolyte; it afforded **6β** in high yield and with high stereoselectivity. To investigate the influence of reaction temperature, we also performed the reaction at elevated temperature at −15 °C. Although the yield was decreased, complete β-selectivity was observed. Therefore, the lower reaction temperature was not crucial to obtain cyclic oligosaccharide **6β** stereoselectively. We propose the same reaction mechanism as the glycosyl triflate pool, which is an electrochemical con-



Scheme 2. Intramolecular glycosylation under the conventional chemical glycosylation conditions.

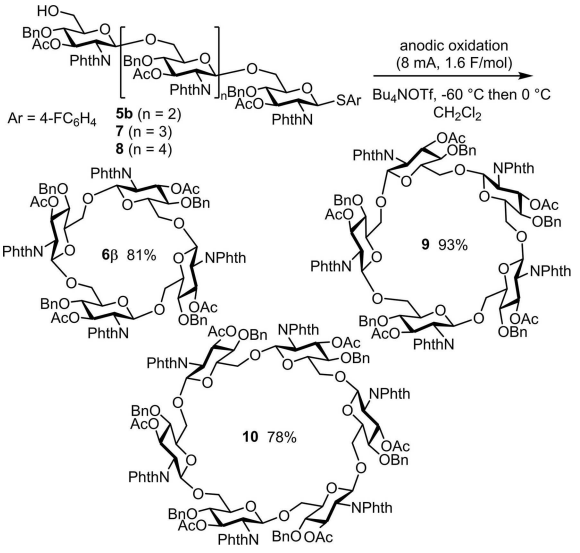
Table 3. Effect of electrolyte on intramolecular electrochemical glycosylation.



Electrolyte	Yield (%)	Selectivity
Bu_4NBF_4	17	**6β** only
Bu_4NNTf_2	66	**6α/6β** 15:85
Bu_4NOTf	65	**6β** only
Bu_4NOTf	52[a]	**6β** only

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

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



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

version of a thioglycoside into the glycosyl triflate at low temperature.[17] The reaction is initiated by single electron transfer from the linear oligosaccharide and thus-generated glycosyl cation is immediately trapped by triflate anion to afford the corresponding glycosyl triflate intermediate. The intramolecular glycosylation may occur from the glycosyl triflate gradually. Therefore, lower temperature is important to obtain the desired cyclic oligosaccharides in better yields.

Next the intramolecular cyclization of longer oligosaccharides under anodic oxidation conditions was investigated (Table 4). The following model reaction was carried out. An electrochemical cell charged with 0.008 M linear tetrasaccharide **5b** was electrochemically activated at −60 °C by means of 1.6 F/mol electricity. The reaction temperature was gradually increased to 0 °C over a period of 2.5 h. The reaction was finally quenched with Et_3N (See the Supporting Information for the detailed procedure). Purification of the reaction products by simple extraction with EtOAc avoiding tedious chromatographic methods, resulted cyclic tetrasaccharide **6β** as a single compound in 81% yield.[18] Increasing the concentration of substrate and then using a fourfold higher concentration than that used in the standard reaction conditions did not lead to a significant change in the glycosylation yield. This indicated high efficiency of the intramolecular glycosylation (Table 4). Similarly, cyclic pentasaccharide **9** and hexasaccharide **10** were also efficiently synthesized; yields were 93% and 78%, respectively.

The excellent results obtained for electrochemical cyclization encouraged us for detailed investigation of reactivity based selective cyclization in the presence of both primary and secondary sugar alcohols (Figure 1). We first prepared tetrasaccharide **5e** from **1a**.[14] Subsequent treatment with hydrochloric acid afforded the partially protected tetrasaccharide **5f** in quantitative yield.[19] This method was then used for the partially protected tetrasaccharide **5f**. After acetylation the cyclic tetra-saccharide **11** with complete stereoselectivity was obtained in 84% yield (3 steps from **5e**). Although further improvement of the procedures is necessary, global deprotection of the cyclic tetrasaccharide was also achieved in 24% (6 steps from **5e**, See the Supporting Information for the details).

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

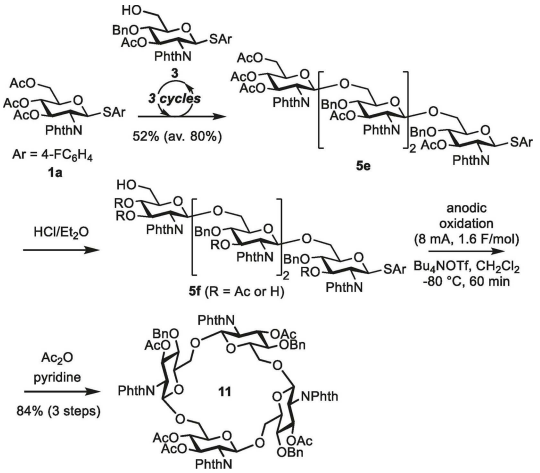


Figure 1. Selective intramolecular electrochemical glycosylation of the 6-OH primary alcohol.

method will enable the large-scale synthesis of natural and unnatural cyclic oligosaccharides. Consideration of the scope and limitations of this protocol is under investigation in our laboratory.

Experimental Section

The electrochemical synthesis of cyclotetrakis-(1→6)-(3-*O*-acetyl-4-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucop-yranosyl) (**6β**) was carried out in an H-type divided cell (4G glass filter) equipped with a carbon felt anode (Nippon Carbon JF-20-P7) and a platinum plate cathode (10 mm × 20 mm). In the anodic chamber were placed 4-Fluorophenyl-3-*O*-acetyl-4-*O*-benzyl-2-deoxy-2-phthalimido-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-β-D-glucopyranosyl-(1→6)-3-*O*-acetyl-4-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside **5b** (0.072 g, 0.039 mmol), Bu_4NOTf (0.195 g, 0.50 mmol) and CH_2Cl_2 (5.0 mL). In the cathodic chamber were placed trifluoromethanesulfonic acid (3.5 μL), Bu_4NOTf (0.195 g, 0.50 mmol) and CH_2Cl_2 (5.0 mL). The constant current electrolysis (8.0 mA (current density: 2.0 mA/cm^2), 43 V (electrode distance: 4.5 cm)) was carried out at −80 °C with magnetic stirring until 1.6 F/mol of electricity was consumed. After the electrolysis, the temperature was raised to 0 °C and Et_3N (0.5 mL) was added and the mixture was evaporated under reduced pressure and extracted five times with ethyl acetate. The extract is then evaporated under reduced pressure and kept under strong vacuum for extreme dryness to furnish desired cyclic oligosaccharide **6β** in 81% isolated yield (54 mg, 0.031 mmol). ^{1}H NMR (pyridine-d_5, 600 MHz) δ 1.61 (s, 3 H), 3.94 (pseudo-d, J = 7.8 Hz, 1 H), 4.08 (t, J = 7.8 Hz, 1 H), 4.31 (d, J = 10.8 Hz, 1 H), 4.38 (*pseudo*-d, J = 10.8 Hz, 1 H), 4.47 (*pseudo*-d, J = 10.8 Hz, 1 H), 4.60 (*pseudo*-d, J = 9.6 Hz, 1 H), 4.76 (td, J = 11.4, 5.4 Hz, 1 H), 6.04 (d, J = 7.8 Hz, 1 H), 6.13 (td, J = 12.0, 3.0 Hz, 1 H), 7.00 (bd, J = 2.4 Hz, 2 H), 7.14 (bd, J = 1.8 Hz, 2 H), 7.35 (s, 1 H), 7.42 (s, 1 H), 7.58 (s, 1 H), 7.64 (s, 1 H), 7.77 (s, 1 H); ^{13}C NMR (pyridine-d_5, 150 MHz) δ 170.9, 169.1, 168.4, 138.6, 136.1, 128.9, 128.3, 124.2, 99.1, 77.5, 75.5, 75.3, 74.2, 69.6, 56.0, 20.6; HRMS (ESI) *m/z* calcd for $C_{92}H_{84}N_4O_{28}K$ $[M+K]^+$, 1731.4904; found, 1731.4893.

Acknowledgements

The study has been financially supported by the Grant-in-Aid for Scientific Research on Innovative Areas: "Middle Molecular Strategy" (No. 2707) from the JSPS (No. JP15H05844) and the Presidential Fund of Tottori University. The authors acknowledge fruitful suggestion of Professor Mahito Atobe of Yokohama National University. The authors also thank KOGANEI corporation for the development of the latest version of the automated electrochemical synthesizer.

Conflict of Interest

The authors declare no conflict of interest.

Keywords: cyclic oligosaccharides · electrochemical glycosylation · glucosamine · automated synthesis · stereoselectivity

[1] J. F. Stoddart, *Carbohydr. Res.* **1989**, *192*, xii–xvi.
[2] *Comprehensive Supramolecular Chemistry*, (Eds.: J. L. Davies, J. E. D. MacNicol, D. D. Vögtl), Elsevier: Oxford, **1996**; vol. 3.
[3] G. Gattuso, S. A. Nepogodie, J. F. Stoddart, *Chem. Rev.* **1998**, *98*, 1919–1958.
[4] H. Bender, *Advances in Biotechnological Process,* (Ed. A. Mizrahi), Alan R. Liss, Inc., New York, **1986**; *6*, 31–71.
[5] a) J. Szejtli, *Chem. Rev.* **1998**, *98*, 1743–1754; b) J. Szejtli, *Pure Appl. Chem.* **2004**, *76*, 1825-; c) G. Crini, *Chem. Rev.* **2014**, *114*, 10940–10975.
[6] a) Y. Takahashi, T. Ogawa, *Carbohydr. Res.* **1987**, *169*, 127–149; b) M. Mori, Y. Ito, T. Ogawa, *Tetrahedron Lett.* **1989**, *30*, 1273–1276; c) M. Mori, Y. Ito, T. Ogawa, *Tetrahedron Lett.* **1990**, *31*, 3029–3030; d) M. Mori, Y. Ito, J. Uzawa, T. Ogawa, *Tetrahedron Lett.* **1990**, *31*, 3191–3194.
[7] G. Excoffier, M. Paillet, M. Vignon, *Carbohydr. Res.* **1985**, *135*, C10-C11.
[8] P. M. Collins, M. H. Ali, *Tetrahedron Lett.* **1990**, *31*, 4517–4520.
[9] T. Nakagawa, K. Ueno, M. Kashiwa, J. Watanabe, *Tetrahedron Lett.* **1994**, *35*, 1921–1924.
[10] M. Wakao, K. Fukase, S. Kusumoto, *J. Org. Chem.* **2002**, *67*, 8182–8190.
[11] a) M. Nishizawa, M. Imagawa, Y. Kan, H. Yamada, *Tetrahedron Lett.* **1991**, *32*, 5551–5554; b) M. Nishizawa, H. Imagawa, E. Morikumi, S. Hatakeyama, H. Yamada, *Chem. Pharm. Bull.* **1994**, *42*, 1365–1366; c) A. Dondoni, M. C. Scherrmann, *J. Org. Chem.* **1994**, *59*, 6404–6412; d) A. Dondoni, A. Marra, *Chem. Commun.* **1999**, 2133–2145; e) A. Dondoni, A. Marra, M. C. Scherrmann, V. Bertolasi, *Chem. Eur. J.* **2001**, *7*, 1371–1382; f) L. Fan, O. Hindsgaul, *Org. Lett.* **2002**, *4*, 4503–4506; g) K. Kim, B. Y. Lee, S. H. Yoon, H. Jeon, J. Y. Baek, K. S. Jeong, *Org. Lett.* **2008**, *10*, 2373–2376.
[12] a) M. L. Gening, D. V. Titov, A. A. Grachev, A. G. Gerbst, O. N. Yudina, A. S. Shashkov, A. O. Chizhov, Y. E. Tsvetkov, N. E. Nifantiev, *Eur. J. Org. Chem.* **2010**, 2465–2475; b) D. V. Titov, M. L. Gening, A. G. Gerbst, A. O. Chizhov, Y. E. Tsvetkov, N. E. Nifantiev, *Carbohydr. Res.* **2013**, *381*, 161–178; c) M. L. Gening, Y. E. Tsvetkov, D. V. Titov, A. G. Gerbst, O. N. Yudina, A. A. Grachev, A. S. Shashkov, S. Vidal, A. Imberty, T. Saha, D. Kand, P. Talukdar, G. B. Pier, N. E. Nifantiev, *Pure Appl. Chem.* **2013**, *85*, 1879–1891.
[13] a) R. Noyori, I. Kurimoto, *J. Org. Chem.* **1986**, *51*, 4320–4322; b) C. Amatore, A. Jutand, J.-M. Mallet, G. Meyer, P. Sinaÿ, *J. Chem. Soc. Chem. Commun.* **1990**, 718–719; c) G. Balavoine, A. Gref, J.-C. Fischer, A. Lubineau, *Tetrahedron Lett.* **1990**, *31*, 5761–5764; d) T. Nokami, K. Saito, J. Yoshida, *Carbohydr. Res.* **2012**, *363*, 1–6.
[14] a) T. Nokami, R. Hayashi, Y. Saigusa, A. Shimizu, C. Y. Liu, K.-K. T. Mong, J. Yoshida, *J. Org. Lett.* **2013**, *15*, 4520–4523; b) T. Nokami, Y. Isoda, N. Sasaki, A. Takaiso, S. Hayase, T. Itoh, R. Hayashi, A. Shimizu, J. Yoshida, *Org. Lett.* **2015**, *17*, 1525–1528; c) Y. Isoda, N. Sasaki, K. Kitamura, S. Takahashi, S. Manmode, N. Takeda-Okuda, J. Tamura, T. Nokami, T. Itoh, *Beilstein J. Org. Chem.* **2017**, *13*, 919–924; d) S. Manmode, T. Sato, N. Sasaki, I. Notsu, S. Hayase, T. Nokami, T. Itoh, *Carbohydr. Res.* **2017**, *450*, 44–48; e) S. Manmode, M. Kato, T. Ichiyanagi, T. Nokami, T. Itoh, *Asian J. Org. Chem.* **2018**, *7*, 1802–1805; f) Y. Isoda, K. Kitamura, S. Takahashi, T. Nokami, T. Itoh, *ChemElectroChem* **2019**, *6*, *in press*. DOI: 10.1002/celc.201900215
[15] D. K. Hunt, P. H. Seeberger, *Org. Lett.* **2002**, *16*, 2751–2754.
[16] G. H. Veeneman, S. H. van Leeuwen, J. H. van Boom, *Tetrahedron Lett.* **1990**, *31*, 1331–1334.
[17] T. Nokami, A. Shibuya, H. Tsuyama, S. Suga, A. A. Bowers, D. Crich, J. Yoshida, *J. Am. Chem. Soc.* **2007**, *129*, 10922–10928.
[18] Solubility of cyclic oligoglucosamines in conventional organic solvents was so low that we could purify the com-pound by washing the crude product with EtOAc. The β-isomer of tetrasaccharide **6β** has the lowest solubility among them.
[19] HCl/Et_2O mediated hydrolysis of acetate resulted mixture of mono, di- and tri-acetate protected tetrasaccharide detected by ESI-MS. The rate of hydrolysis of the acetyl group of the primary hydroxyl group is faster than that of secondary hydroxyl group, thus we considered that the primary alcohol was fully hydrolyzed. Contrary, Zemplén's condition gave complete deacetalized product; however, insolubility of the compound in CH_2Cl_2 renders its use for cyclization.

Manuscript received: May 27, 2019