A Practical One-Step Synthesis of 1,2-Oxazoline Derivatives from Unprotected Sugars and Its Application to Chemoenzymatic β-*N*-Acetylglucosaminidation of Disialo-oligosaccharide

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Dedicated to Professor *Dieter Seebach* on the occasion of his 75th birthday

A facile and practical method for synthesis of sugar oxazolines (=dihydrooxazoles) from the corresponding *N*-acetyl-2-amino sugars has been developed by using 2-chloro-1,3-dimethyl-1*H*-benzimidazol-3-ium chloride (CDMBI) as a dehydrative condensing agent. The intramolecular dehydrative reaction between the 2-acetamido group and the anomeric OH group of unprotected *N*-acetyl-2-amino sugars took place smoothly in H_2O, leading to the formation of a 1,2-oxazoline (=4,5-dihydrooxazole) moiety in good yield. Since the reaction proceeds in H_2O without using any protecting groups, the resulting oxazolines can be utilized as effective glycosyl donors for the subsequent enzymatic glycosylation. We have successfully demonstrated a highly efficient chemoenzymatic transglycosylation of a disialo-oligosaccharide moiety to *p*-nitrophenyl *N*-acetylglucosaminide catalyzed by a mutant endo-*N*-acetylglucosaminidase without isolating disialo-oligosaccharide oxazoline as synthetic intermediate.

Introduction. – Chemoenzymatic glycosylation has become one of the most promising synthetic tools for regio- and stereoselective construction of glycosidic bonds in carbohydrate chemistry [1–6]. 1,2-Oxazolines (=4,5-dihydrooxazoles) derived from unprotected *N*-acetyl-2-amino sugars are known to be recognized by a variety of *N*-acetylglucosaminidases, and can be utilized as efficient glycosyl donors for chemoenzymatic construction of various *N*-acetylglucosaminide structures [7–13]. Although several synthetic methods have so far been developed for preparation of sugar oxazoline derivatives, these methods require multi-step procedures including protection and deprotection of the OH groups, as well as selective introduction of an appropriate leaving group to the anomeric center, which makes oxazoline synthesis extremely laborious [14–16]. In addition, these processes necessitate the use of organic solvents, strong acids, and bases. It had long been believed to be impossible to prepare sugar oxazolines in H_2O directly from the corresponding unprotected sugars.

Recently, we have discovered a one-step route for conversion of unprotected *N*-acetyl-2-amino sugars to the corresponding sugar oxazolines in H_2O by using 2-chloro-1,3-dimethyl-1*H*-imidazolin-3-ium chloride (DMC) as a H_2O-soluble dehydrative condensing agent [17]. According to this method, sugar oxazoline derivatives of unprotected oligosaccharides such as disialo-oligosaccharides and chito-oligosaccharides could be easily prepared and employed as efficient glycosyl donors for enzymatic

synthesis of glycoproteins and chitoheptaose catalyzed by an endo-β-*N*-acetylglucosaminidase and a chitinase, respectively [18][19].

Although the one-step synthesis of sugar oxazolines by DMC has greatly simplified the chemoenzymatic process of *N*-acetylglucosaminidation, there were still several disadvantages of DMC concerning its handling and control of reactivity. First, DMC reagent was difficult to handle, because it is unstable and extremely hygroscopic, which made exact weighing difficult. Second, a considerable amount of DMC was decomposed by the attack of H_2O before being converted to the intermediate, glycosyldimethylimidazolinium salt, giving rise to the hydrolyzate, 1,3-dimethyl-2-imidazolidin-2-one (DMI). Consequently, excess amount of DMC was needed in order to realize satisfied yields of oxazolines. In addition, it was difficult to establish a one-pot chemoenzymatic process, because the activity of enzyme catalysts was strongly inhibited by DMI that was produced during the course of the oxazoline formation and was hard to remove from the aqueous mixture.

To solve these problems related to the use of DMC, we modified the structure of dehydrating agent by introducing a benzene ring to the chloroformamidinium moiety of DMC. The present paper deals with a more facile and practical procedure for synthesis of sugar oxazolines by using a newly designed dehydrative condensing agent of 2-chloro-1,3-dimethyl-1*H*-benzimidazol-3-ium chloride (CDMBI) where the disadvantages derived from DMC have significantly been eliminated. We also reinvestigated optimal reaction conditions for CDMBI-mediated synthesis of sugar oxazolines, in particular, the effect of bases on the yield of an oxazoline, and demonstrated the first chemoenzymatic *N*-acetylglucosaminidation using disialo-oligosaccharide oxazoline as glycosyl donor without its isolation or purification.

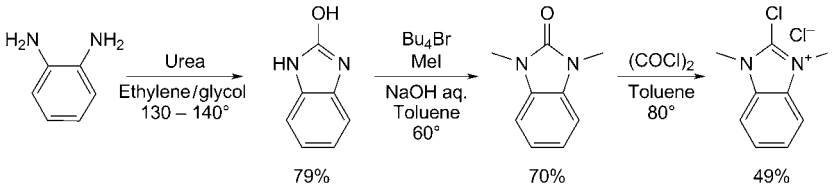
Results and Discussion. – One of the main purposes of modifying the structure of chloroformamidinium salt is to improve the yield of oxazoline formation by stabilizing the reactive intermediate of oxazoline synthesis. In the previous paper, we have proposed the mechanism of the DMC-mediated oxazoline ring formation which involves the preferential attack of the hemiacetal type OH group of *N*-acetyl-2-amino sugars to the chloroformamidinium moiety of DMC, giving rise to a reactive intermediate, glycosyl dimethylimidazolinium salt with β-configuration. Then, an intramolecular attack of the CO O-atom of the 2-acetamido group as well as the abstraction of the amide H-atom by a base takes place, affording a sugar oxazoline and DMI. We postulated that the reactivity of a chloroformamidinium moiety toward nucleophiles such as H_2O as solvent would be reduced by changing the imidazolidine ring of DMC to a more electron-rich benzimidazole ring, making the reactive intermediate of glycosyl intermediate more stable.

Another purpose of structure modification of the reagent is to facilitate the chemoenzymatic procedure by removing the hydrolyzate of the dehydrative condensing agent from the mixture, eliminating an enzyme inhibition caused by the hydrolyzate. We postulated that the existence of the benzene ring should decrease the solubility of the 1,3-dimethyl-1*H*-benzimidazol-2-one produced as a result of oxazoline formation. Based on these hypotheses, we have designed 1,3-dimethylbenzimidazol-2(3*H*)-one (=1,3-dihydro-1,3-dimethyl-2*H*-benzimidazol-2-one; DMBI) as a basic structure for dehydrative agent. In fact, the log D_{ow} value, octan-1-ol/H_2O partition

coefficient parameter, of DMBI was 1.33, indicating that DMBI is much more hydrophobic than DMI [20].

A new dehydrative condensing agent, 2-chloro-1,3-dimethyl-1*H*-benzimidazol-3-ium chloride (CDMBI), has been prepared *via* three steps starting from benzene-1,2-diamine (*Scheme 1*). Benzene-1,2-diamine was carbonylated by urea to afford 1*H*-benzimidazol-2-ol whose N-atoms were methylated by the action of MeI in the presence of NaOH [21][22]. The resulting 1,3-dimethylbenzimidazol-2(3*H*)-one (DMBI) was converted to CDMBI by using oxalyl chloride in moderate yield. The resulting CDMBI was found to be considerably soluble in H_2O (1.2 mg CDMBI/1 µl H_2O at r.t.) and not hygroscopic like DMC (*Fig. 1*).

Scheme 1



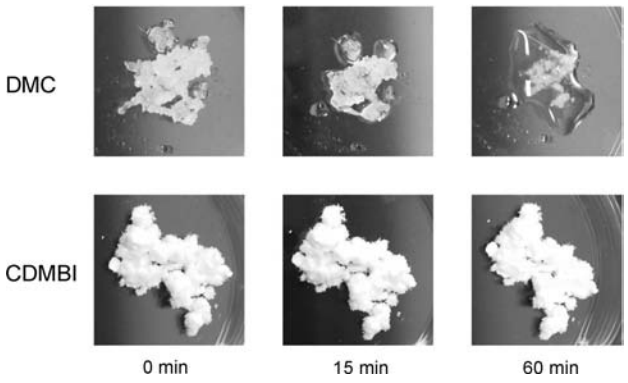


Fig. 1. *Hygroscopicity of DMC and CDMBI*

When *N*-acetylglucosamine (GlcNAc) was treated with CDMBI in the presence of a base in H_2O, an intramolecular dehydrative condensing reaction between the 2-acetamido group and the anomeric OH group took place smoothly, giving rise to the corresponding 1,2-oxazoline derivative (GlcNAc-oxa; *Scheme 2*). In this reaction, more than 2 equiv. of a base is required to enhance the nucleophilicity of the anomeric OH group as well as to scavenge HCl liberated. We investigated the effect of bases on the yield of GlcNAc-oxa (*Table 1*). The use of Et_3N that was the most suitable base for the oxazoline formation by using DMC was also found to be effective for the present reaction using CDMBI (*Entry 1*), although the yield decreased when $EtNMe_2$ was used (*Entry 2*). However, it has also been found that the existence of trialkylammonium chlorides derived from these tertiary amines was not suitable for the subsequent enzymatic glycosylation, because these organic salts inhibited enzyme activity. These

Scheme 2

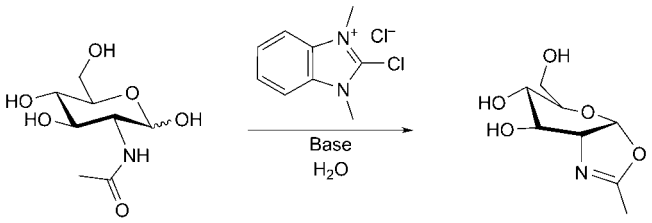


Table 1. *Synthesis of GlcNAc-oxa by Using CDMBI in the Presence of Various Bases*[a])

Entry	CDMBI [equiv.]	Base ([equiv.])	Time [h]	Yield [%][b])
1	3	Et_3N (7.5)	1	79
2	3	$EtNMe_2$ (15)	45	58
3	3	Na_3PO_4 (7.5)	1	81
4	3	K_3PO_4 3 H_2O (7.5)	1	76
5	3	Na_2CO_3 (7.5)	1	64
6	3	K_2CO_3 (7.5)	1	65
7	3	NaOH (7.5)	1	24

[a]) Reaction conditions; GlcNAc 150 mM, 0°. [b]) Determined by ^{1}H-NMR.

results prompted us to investigate inorganic bases as promising candidates for an acid scavenger. Among several inorganic bases screened, Na_3PO_4 gave the best result concerning the yield of GlcNAc-oxa (*Entries 3* and *4*). The use of carbonate salts and NaOH was not effective for the synthesis of GlcNAc-oxa (*Entries 5–7*).

Various *N*-acetyl-2-amino sugars have successfully been converted to the corresponding sugar oxazolines (*Table 2*). It is noteworthy that GlcNAc possessing a sulfuric acid can also be converted to the corresponding oxazoline derivative without affecting the sulfate (*Entry 1*). The present method could sucessfully be applied to oligosaccharides, affording the corresponding oxazoline derivatives in sufficient yields (*Entries 2–7*). It should also be noted that disialo-oligosaccharide oxazoline that is difficult to prepare by the conventional method of employing protecting groups could easily be obtained in good yield without affecting the carboxylic acid (*Entry 7*).

Table 2. *Synthesis of Sugar Oxazolines from Various Oligosaccharides*[a])

Entry	Saccharide ([mM])	CDMBI [equiv.]	Base ([equiv.])	Time [h]	Yield [%][b])
1	GlcNAc 6-sulfate (125)	5	K_2CO_3 (15)	1	88
2	Lacto-*N*-biose (100)	3	Na_3PO_4 (7.5)	1.5	93
3	$(GlcNAc)_2$ (150)	3	Na_3PO_4 (7.5)	2	89
4	$(GlcNAc)_2$ (10)	5	Na_3PO_4 (12.5)	1	90
5	$(GlcNAc)_3$ (50)	5	Na_3PO_4 (12.5)	2	87
6	$(GlcNAc)_5$ (10)	5.2	Na_3PO_4 (12.5)	10	82[c])
7	Disialo-oligosaccharide (50)	5	K_3PO_4 (15)	2	91

[a]) Reaction condition: 0°. [b]) Determined by ^{1}H-NMR. [c]) Yield of isolated product.

In the previous investigation for synthesis of oxazolines using DMC as dehydrative condensing agent, at least 15 equiv. of the reagent were required. The present method with CDMBI requires only 3–5.2 equiv. of the reagent. These results clearly show that the key intermediate of glycosyl dimethylbenzimidazolium salt is more stable and can consequently be converted to the corresponding oxazoline in a more effective manner than the case with DMC.

In this reaction, the hydrolyzate of DMBI precipitated from the aqueous mixture as the reaction proceeded, due to its higher hydrophobicity. This finding enabled us to remove the resulting DMBI simply by filtration, and the filtrate could be utilized for the subsequent enzymatic transglycosylation without purification of the synthetic oxazoline intermediate. Disialo-oligosaccharide derived from sialoglycopeptide (SGP) was converted to the corresponding oxazoline derivative by the action of CDMBI in the presence of K_3PO_4 in good yield. The resulting disialo-oligosaccharide oxazoline was then reacted with *para*-nitrophenyl β-2-acetamido-2-deoxyglucopyranoside (*p*NP-GlcNAc) as an acceptor substrate by using a mutant endo-*N*-acetylglucosaminidase (Endo-M N175Q) as a catalyst (*Scheme 3*). The transglycosylation proceeded smoothly, affording the corresponding disialo-oligosaccharide having a *p*-nitrophenyl group at the reducing end. The HPLC of the mixture showed that the peak derived from the glycosyl acceptor, *p*NP-β-GlcNAc, was smoothly consumed, and the peak due to the transglycosylated product was clearly observed as the main peak after 180 min (*Fig. 2*). This is the first one-solution chemoenzymatic transglycosylation of disialo-oligosaccharide without isolating the corresponding 1,2-oxazoline derivative as a glycosyl donor.

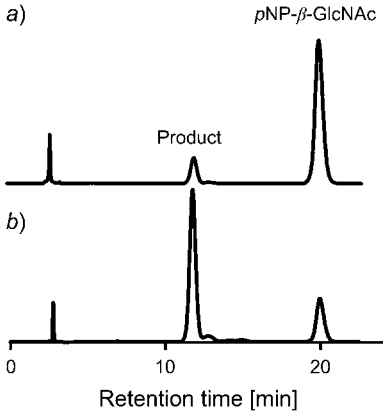
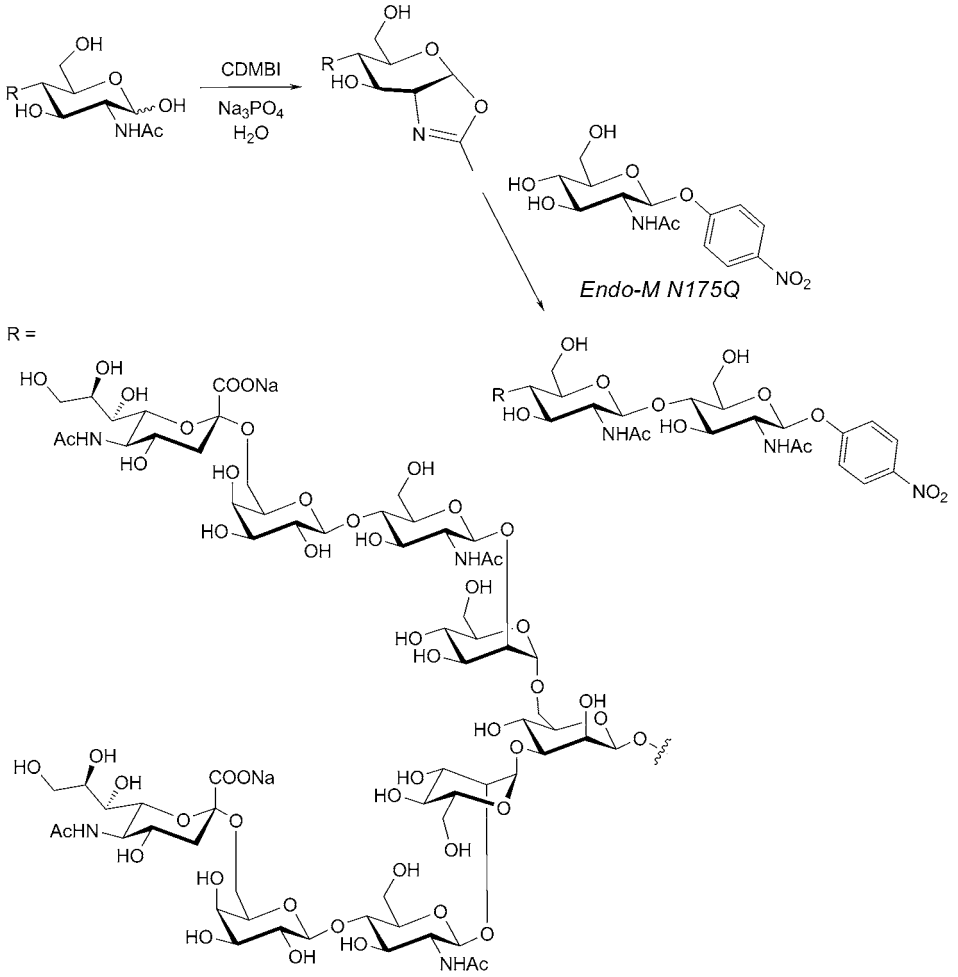


Fig. 2. *HPLC* (UV 300 nm) *of the mixture for one-solution chemoenzymatic glycosylation reaction catalyzed by* Endo-M N175Q. *a*) 5 min, *b*) 180 min.

Conclusions. – We have designed and prepared a new H_2O-soluble dehydrating agent, 2-chloro-1,3-dimethyl-1*H*-benzimidazol-3-ium chloride (CDMBI), for the direct synthesis of sugar oxazolines from the corresponding unprotected *N*-acetyl-2-amino sugars in H_2O. This dehydrative condensing agent was stable in air and did not show hygroscopicity. Since the hydrolyzate of CDMBI, DMBI, precipitates during the course of the reaction, DMBI can be removed easily from the mixture by filtration. The

Scheme 3



development of the new agent CDMBI paved a way for one-pot or one-solution chemoenzymatic transglycosylation of disialo-oligosaccharides for the first time catalyzed by *N*-acetylglucosaminidases by using sugar oxazolines as synthetic intermediates.

Experimental Part

General. ^{1}H-NMR Spectra: *Bruker DPX-400* (400 MHz), *AV-400* (400 MHz), or *DRX-500* (500 MHz) at r.t. in D_2O, CD_3OD, (D_6)DMSO, or $CDCl_3$. ^{13}C-NMR Spectra: *Bruker DPX-400* (100 MHz), *AV-400* (100 MHz), or *DRX-500* (125.7 MHz) at r.t. in D_2O or CD_3OH with complete H-atom decoupling. Assignments of ^{1}H- and ^{13}C-NMR spectra were performed by ^{1}H,^{1}H-COSY, HSQC, and HMQC experiments. MALDI TOF-MS: *Shimadzu Axima CFR-plus* spectrometer, with 3,5-dihydroxybenzoic acid (DHB) as a matrix.

*Preparation of 2-Chloro-1,3-dimethyl-1*H*-benzimidazol-3-ium Chloride* (CDMBI). *2-Hydroxybenzimidazole* (=*1*H*-Benzimidazol-2-ol*). A mixture of benzene-1,2-diamine 250 g (2.31 mol) and urea 166.7 g (2.78 mol) in ethylene glycol (1.17 l) was heated at 130–140° for 25 h. During the course of the reaction, an additional amount of urea (13.9 g (231 mmol) in total) was supplied after 10 and 18 h. The mixture was cooled to r.t., and H_2O was added with stirring to precipitate the product. After washing with H_2O and subsequently with toluene, the precipitate was dried *in vauo* to give 245.4 g of 2-hydroxybenzimidazole (79%).

1,3-Dimethylbenzimidazolone (=*1,3-Dihydro-1,3-dimethyl-2*H*-benzimidazol-2-one*; DMBI). To a mixture of 2-hydroxybenzimidazole (200 g, 1.49 mol), Bu_4NBr (24 g, 74 mmol), aq. NaOH soln. (40 wt-%, 382 ml), and toluene (994 ml) was added MeI (204 ml) dropwise at 60°, and the mixture was heated at the same temp. for 19 h. After cooling the mixture, the org. layer was washed with aq. HCl and aq. $NaHCO_3$, and dried (Na_2SO_4). After removing the solvent, the residue was recrystallized from acetone and hexane to give 169 g of DMBI as white solid (70%).

*2-Chloro-1,3-dimethyl-1*H*-benzimidazol-3-ium Chloride* (CDMBI). To a soln., of DMBI (10.2 g, 62.9 mmol) in toluene (83.5 ml), oxalyl chloride (13.4 ml, 157 mmol) was added at r.t., and the resulting mixture was heated to 80°. After 49 h, oxalyl chloride (5.0 ml, 59 mmol) was added, and the mixture was stirred at 80° for 23 h. The resulting precipitate was washed with toluene and dried *in vacuo* to give 6.73 g 2-chloro-1,3-dimethyl-1*H*-benzimidazol-3-ium chloride (49%).

General Procedure for the Synthesis of Oxazolines from N*-Acetylglucosamine* (GlcNAc). To a mixture of GlcNAc (20.9 mg, 94.6 μmol) and Et_3N (98.8 μl, 709 μmol) in H_2O (630 μl) was added CDMBI 61.5 mg (284 μmol) at 0°, and the resulting soln. was stirred for 1 h at the same temp. After adding D_2O and sodium mesitylene benzenesulfonate as an internal standard to estimate the yield, the mixture was directly subjected to NMR analysis. The yield was determined by comparing the integration of the arom. H-atoms of sodium benzenesulfonate and the anomeric H-atom of the oxazoline derivative (GlcNAc-oxa). ^{1}H-NMR (400 MHz, D_2O): 5.97 (*d*, *J* = 7.4, H–C(1)); 4.03–3.98 (*m*, H–C(2)); 3.86 (*t*, *J* = 3.5, H–C(3)); 3.70 (*dd*, *J* = 2.5, 12.5, 1 H of CH_2(6)); 3.55 (*dd*, *J* = 6.5, 12.5, 1 H of CH_2(6)); 3.51–3.46 (*m*, H–C(5)); 3.29–3.25 (*m*, H–C(4)); 1.94 (*s*, Me).

Synthesis of Various Sugar Oxazolines by Using CDMBI as Dehydrating Agent. 1,2-Oxazoline Derivative of GlcNAc-6-sulfate. N-Acetylglucosamine 6-sulfate sodium salt (GlcNAc-6-sulfate; 4.46 mg, 13.8 μmol) and K_2CO_3 (14.9 mg, 209 μmol) was dissolved in D_2O (110 μl), and the resulting soln. was cooled to 0°. CDMBI (60.1 mg, 68.6 μmol) was added to the soln., and the mixture was stirred for 1 h at the same temp. After adding sodium mesitylene sulfonate (=2,4,6-trimethylbenzenesulfonic acid sodium salt (1:1)) to estimate the yield, the mixture was directly subjected to NMR analysis. The yield was determined by comparing the integration of the signals of the arom. H-atoms of sodium mesitylenesulfonate and the anomeric H-atom of GlcNAc-6-sulfate-oxa. ^{1}H-NMR (500 MHz, D_2O): 6.09 (*d*, *J* = 7.4, H–C(1)); 4.29–4.25 (*m*, 1 H of CH_2(6)); 4.16 (*dd*, *J* = 5.9, 11.2, 1 H of CH_2(6)); 4.16–4.12 (*m*, H–C(2)); 3.99 (*t*, *J* = 3.6, H–C(3)); 3.71 (*dd*, *J* = 8.9, 3.5, H–C(4)); 3.64–3.60 (*m*, H–C(5)); 2.06 (*s*, Me).

*1,2-Oxazoline Derivative of Lacto-*N*-biose (Lacto-*N*-biose-oxa)*. Lacto-*N*-biose (20.4 mg, 53.2 μmol) and Na_3PO_4 (65.4 mg, 399 μmol) were dissolved in H_2O (532 μl), and the resulting soln. was cooled to 0°. CDMBI (34.7 mg, 160 μmol) was added to the soln., and the mixture was stirred for 1.5 h at the same temp. After adding sodium mesitylene sulfonate to estimate the yield, the mixture was directly subjected to NMR analysis. The yield was determined by comparing the integration of the signals of the arom. H-atoms of sodium mesitylenesulfonate and the anomeric H-atom of Lacto-*N*-biose-oxa. ^{1}H-NMR (500 MHz, D_2O): 6.00 (*d*, *J* = 7.4, H–C(1)); 4.48 (*d*, *J* = 7.8, H–C(1′)); 4.27–4.23 (*m*, H–C(2)); 4.04 (*t*, *J* = 3.0, H–C(3)); 3.81–3.54 (*m*, H–C(4′), H–C(4), H_a–C(6), H_a–C(6′), H_b–C(6′), H–C(5′), H_b–C(6), H–C(3′)); 3.48–3.44 (*m*, H–C(2′)); 3.33–3.29 (*m*, H–C(5)); 1.95 (*s*, Me).

1,2-Oxazoline Derivative of N,N′*-Diacetylchitobiose* ($(GlcNAc)_2$-oxa). *N,N′*-Diacetylchitobiose (20.0 mg, 47.1 μmol) and Na_3PO_4 (57.9 mg, 353 μmol) were dissolved in H_2O (314 μl), and the resulting soln. was cooled to 0°. CDMBI 30.7 mg (141 μmol) was added to the soln., and the mixture was stirred for 2 h at the same temp. After adding D_2O and sodium mesitylene sulfonate to estimate the yield, the mixture was directly subjected to NMR analysis. The yield was determined by comparing the integration of the signals for the arom. H-atoms of sodium mesitylenesulfonate and the anomeric H-atom of $(GlcNAc)_2$-oxa. ^{1}H-NMR (400 MHz, D_2O): 6.01 (*d*, *J* = 7.4, H–C(1)); 4.51 (*d*, *J* = 8.3, H–C(1′)); 4.37–

4.35 (*m*, H–C(2)); 4.15 – 4.12 (*m*), 3.90 – 3.20 (*m*, 10 H); 1.99 (*d*, *J* = 1.6, Me of oxazoline); 1.98 (*s*, Me of acetamide).

1,2-Oxazoline Derivative of N,N′,N″-*Triacetylchitotriose* ((GlcNAc)$_3$-oxa). *N,N′,N″*-Triacetylchitotriose (20.2 mg, 32.2 μmol) and Na_3PO_4 (66.0 mg, 403 μmol) was dissolved in H_2O (643 μl), and the resulting soln. was cooled to 0°. CDMBI (34.7 mg, 160 μmol) was added to the soln., and the mixture was stirred for 2 h at the same temp. After adding D_2O and sodium mesitylenesulfonate to estimate the yield, the mixture was directly subjected to NMR analysis. The yield was determined by comparing the integration of the signals of the arom. H-atoms of sodium mesitylenesulfonate and the anomeric H-atom of (GlcNAc)$_3$-oxa. ^{1}H-NMR (400 MHz, D_2O): 6.00 (*d*, *J* = 7.3, H–C(1)); 4.54 – 4.48 (*m*, H–C(1′), H–C(1″)); 4.36 – 4.34 (*m*, H–C(2)); 4.15 – 4.09 (*m*), 3.90 – 3.18 (*m*, 16 H); 2.01 (*s*, Me of acetamide), 1.98 (*d*, *J* = 1.7, Me of oxazoline), 1.97 (*s*, Me of acetamide).

1,2-Oxazoline Derivative of N,N′,N″,N‴,N⁗-*Pentaacetylchitopentaose* ((GlcNAc)$_5$-oxa). *N,N′,N″,N‴,N⁗*-Pentaacetylchitopentaose 8.20 mg (7.93 μmol) and Na_3PO_4 16.3 mg (99.4 μmol) were dissolved in H_2O (793 μl), and the resulting soln. was cooled to 0°. CDMBI 8.90 mg(41.0 mmol) was added to the soln., and the mixture was stirred for 10 h at the same temperature. After completion of the reaction, the mixture was filtered and lyophilized. The product was purified by HPLC (column, *Inertsil ODS-3* (10 × 250 mm); eluent, H_2O/MeCN 96 : 4, flow rate, 4.8 ml/min; column oven temp., 30°, detection, UV (214 nm)) and concentrated *in vacuo* to give 6.62 mg of (GlcNAc)$_5$-oxa (82%). ^{1}H-NMR (500 MHz, D_2O): 6.04 (*d*, *J* = 7.35, H–C(1)); 4.56 – 4.51 (*m*, H–C(1′), H–C(1″), H–C(1‴), H–C(1⁗)); 4.40 – 4.38 (*m*, H–C(3)); 4.18 – 4.14 (*m*, H–C(2)); 3.82 – 3.41 (*m*, 27 H); 3.28 – 3.24 (*m*); 2.06 – 2.00 (*m*, 5 Me).

1,2-Oxazoline Derivative of Disialo-Oligosaccharide (Disialo-oligosaccharide-oxa). Disialo-oligosaccharide 2.75 mg (1.36 μmol) was dissolved in D_2O containing CDMBI (0.5M, 13.6 μl), and the resulting soln. was cooled to 0°. To this mixture, a D_2O soln. of Na_3PO_4 (1.5M, 13.6 μl) was added, and the resulting mixture was stirred for 2 h at 4°. After adding D_2O, the mixture was directly subjected to NMR analysis. The yield was determined by comparing the integration of the signals of the anomeric H-atom of the oxazoline derivative and the axial H-atom at the 3-position of sialic acid. ^{1}H-NMR (500 MHz, D_2O): 6.07 (*d*, *J* = 7.35, H–C(1)); 5.12 (*s*, 1 H of CH_2(1) of α-Man); 4.94 (*s*, 1 H of CH_2(1) of α-Man); 4.72 (*s*, 1 H of CH_2(1) of β-Man); 4.61 – 4.57 (*m*, H–C(1) of two β-GlcNAc); 4.41 (*d*, *J* = 7.95, H–C(1) of two β-Gal); 4.38 – 3.36 (*m*); 2.66 – 2.62 (*m*, 2 H_{ax}–C(3) of sialic acid); 2.05 – 2.00 (*m*, Me of GlcNAc-4, Me of GlcNAc-4′, Me of NeuAc-6, Me of NeuAc-6′); 1.72 – 1.67 (*t*, *J* = 12.3, 2 H_{eq}–C(3) of sialic acid).

One-Solution Chemoenzymatic Glycosylation Catalyzed by Endo-M N175Q. Disialo-oligosaccharide (2.46 mg, 1.22 μmol) was dissolved in H_2O containing CDMBI (0.5M, 12.2 μl), and the resulting soln. was cooled to 4°. Aq. Na_3PO_4 soln. (1.5M, 12.2 μl) was added, and the mixture was stirred for 2 h at the same temp. After completion of the reaction, the mixture was neutralized with HCl (2.0M, 5.0 μl) and centrifuged by using centrifugal filter device to remove DMBI. To the filtrate, an aq. soln. *p*-nitrophenyl β-2-acetamido-2-deoxyglucopyranoside (10 mM, 41.5 μl) was added, and the mixture was incubated with *Endo-M N175Q* (100 mU/ml, 76.5 μl) in phosphate buffer (50 mM, pH 7.0) at 30°. The transglycosylation reaction was monitored, and the transglycosylated product was isolated by means of HPLC (column, *Inertsil ODS-3* (4.6 × 250 mm); eluent, H_2O/MeCN 90 : 10 containing 0.1% CF_3COOH; flow rate, 1.0 ml/min; column oven temp.; 30°, detection, UV (300 nm)). MALDI-TOF-MS: 2389.3 ($[M - H + 2\,Na]^+$, (M = *p*NP-(GlcNAc)$_2$-Man-(Man-GlcNAc-Gal-NeuAc)$_2$; $C_{90}H_{140}N_7Na_2O_{64}^+$; calc. 2388.8).

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