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Facile oligosaccharide-oxazoline synthesis

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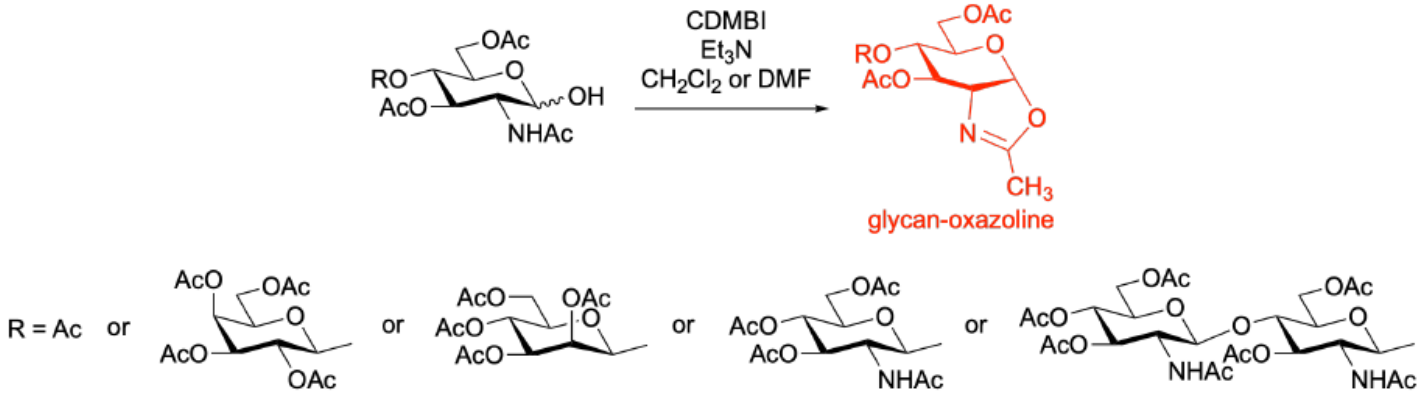
Abstract

This study improved the synthetic method for oligosaccharide-oxazolines, which are key intermediates in chemo-enzymatic glycan transfer reactions. By using protected glycan substrates, the required reagent amounts were reduced, and the corresponding oxazolines were obtained in good yield. Subsequent deprotection provided efficient access to short-chain oligosaccharide-oxazolines.



Keywords: Dehydration reaction, oligosaccharide, glycoengineering, oxazoline

Introduction

Glycan- and oligosaccharide oxazolines were originally developed as glycosyl donors for enzymatic glycosylation. They have also been diversified into a broad range of glycosides, including esters,[1] thioglycosides,[2] glycosyl azides,[3] and dithiocarbamates.[4–6] Their utility as substrates for glycosidic enzymes was first demonstrated in the chemo-enzymatic synthesis of artificial chitin via chitinase.[7] Subsequently, sulfated sialyl-Lewis X oligomers were prepared using sulfated oligosaccharide-oxazolines in combination with keratanase.[8] More recently, glycan-oxazolines have attracted considerable attention as privileged donor substrates in glycan-remodeling strategies designed to generate structurally homogeneous N-glycans on Immunoglobulin G (IgG).[9–11]

IgG universally carries a pair of N-linked glycans in its Fc region, and these glycans exert a profound influence on effector functions—including antibody-dependent cellular cytotoxicity (ADCC)—as well as on pharmacokinetic and pharmacodynamic properties.[12] Owing to the inherent complexity of N-glycan biosynthesis, Fc glycans occur as heterogeneous mixtures that are difficult to isolate as homogeneous, well-defined species, thereby complicating quantitative studies of structure–function relationships. A versatile chemo-enzymatic platform combining engineered endo-β-*N*-acetylglucosaminidase (ENGase) mutants with glycan-oxazolines as donor substrates has enabled the generation of IgGs bearing precisely defined, homogeneous glycans. Within this context, the use of glycan-oxazolines as highly efficient ENGase donor substrates represents a cornerstone of contemporary glycoengineering. Consequently, the development of robust, broadly applicable methods for glycan-oxazoline synthesis remains central to advancing homogeneous glycoprotein production.

Therefore, efficient access to oligosaccharide-oxazolines is a pivotal step for this platform. Traditional methods that rely on trimethylsilyl triflate (TMSOTf) activation at elevated temperatures to produce acetyl-protected *N*-acetylglucosamine–derived oxazolines[13] are irreproducible and produce complex product mixtures, particularly for oligosaccharide substrates. To address these issues, Wang reported a TMSBr/BF_3·OEt_2-mediated conversion of fully acetylated glycans, although only low to moderate yields (35–67%) were obtained, likely due to inherently slow reaction kinetics.[14] Our own studies corroborated that this transformation selectively converts the β-anomer of the glycosyl acetate into the oxazoline, while leaving the α-anomer unchanged. Because this stereochemical bias arises from the anomeric effect, the overall yield is intrinsically limited by the α/β ratio and cannot be improved through conventional optimization.

Shoda *et al*. later developed a dehydration strategy enabling the direct conversion of unprotected glycans into oxazolines in aqueous media using excess amount of 1,3-dimethyl-1*H*-imidazol-3-ium chloride (DMC).[15] A less hygroscopic and more stable analogue, 2-chloro-1,3-dimethyl-1*H*-benzimidazol-3-ium chloride (CDMBI) **1**, was subsequently introduced.[16] Although Shoda's methodology has become the de facto standard for glycan-oxazoline synthesis, purification of short-chain glycans remains challenging because excess reagent is inefficiently removed by gel filtration. In this study, we report a mild, operationally simple, and high-yielding strategy for the synthesis of oligosaccharide-oxazolines that circumvents these limitations and provides a broadly applicable solution for diverse oligosaccharide substrates.

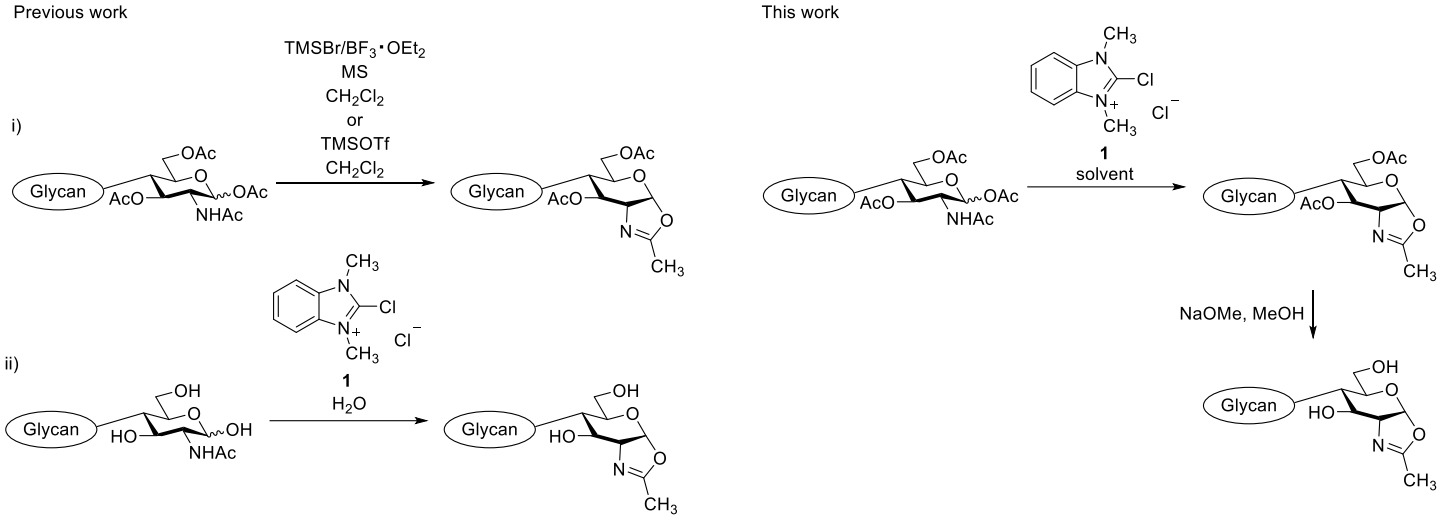


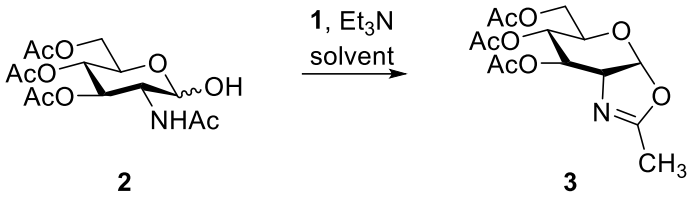
Figure 1. Comparison of previous and present methods for glycan and oligosaccharide-oxazoline formation. Traditional activation required (i) TMSBr/BF_3·OEt_2 or TMSOTf to generate protected oxazolines, followed by deacetylation, or (ii) a dehydrative procedure with CDMBI for unprotected oligosaccharide in water. The proposed method enables oxazoline formation using CDMBI under milder conditions, affording the product after NaOMe/MeOH deprotection.

Results and Discussion

We commenced our investigation by establishing the reaction conditions using the monosaccharide, *N*-acetylglucosamine hemiacetal **2.**[17] To optimize the transformation, we examined the effects of different solvents on reaction efficiency and selectivity. To hemiacetal **2**, two equivalents of CDMBI **1** and three equivalents of Et_3N were added. In aqueous oligosaccharide oxazoline formation, more than 10 equivalents of CDMBI are typically required. When only two equivalents of CDMBI **1** were used, in water, the reagent decomposed, and only a trace amount of product was obtained (run 1). In MeOH, EtOH, or 1-BuOH, the solvent alcohols reacted with the resulting oxazoline **3**[18], generating side products **4**–**6** (runs 2–4) and decreasing the yield of the desired compound **3**. Separation of oxazoline **3** from methyl glycoside **4** and ethyl glycoside **5** was difficult because of their similar polarity during silica gel column chromatography. The yields of **4** and **5** were calculated from ^{1}H-NMR integration. Methyl glycoside **4** and ethyl glycoside **5** were obtained in 10% yield and 20% yield, respectively as side-products. Butyl glycoside **6** was isolated in 48% yield. Tetrahydrofuran (THF), acetone, and acetonitrile (CH_3CN) gave acceptable yield (41-51%; runs 5-7). When dichloromethane (CH_2Cl_2) or dimethylformamide (DMF) was employed as the solvent (runs 8 and 9), oxazoline **3** was obtained in good yield (70–75%). Reducing the amount of CDMBI **1** to 1.1 equivalent lowered the yield (run 10).

NMR analysis used to monitor the reaction revealed that it proceeded quantitatively (Figure 2). Specifically, the signal observed for hemiacetal **2** at 5.2–5.3 ppm completely disappeared, and a new signal corresponding to the anomeric proton of oxazoline **3** was observed at 5.97 ppm (d, J = 7.3 Hz). No signals attributable to by-products were detected. These NMR observations showed a quantitative conversion in this transformation.

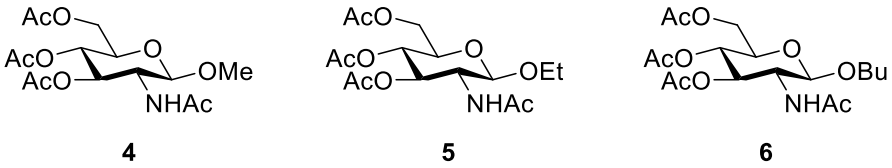
Table 1. Solvent effect on the dehydrative reaction. Hemiacetal **2:** 80 mg (20 mM), CDMBI 2 equiv., Et_3N 3 equiv



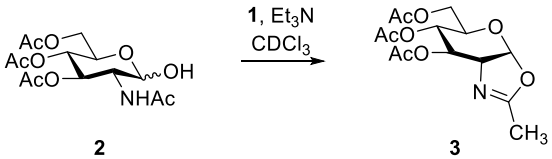
2 **3**

run	solvent	yield (%)
1	H_2O	trace
2	MeOH	77
3	EtOH	16
4	1-BuOH	9
5	THF	41
6	acetone	50
7	CH_3CN	51
8	CH_2Cl_2	70
9	DMF	75
10	DMF	40[a)]

a) 1.1 equiv. of CDMBI was used.



4 **5** **6**



2 **3**

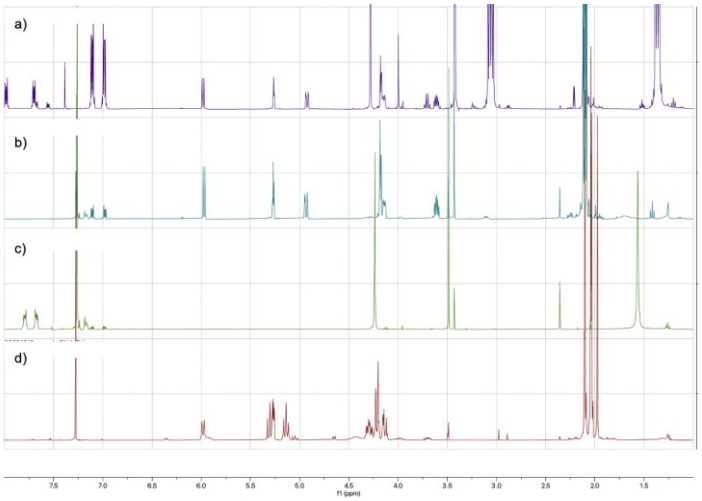
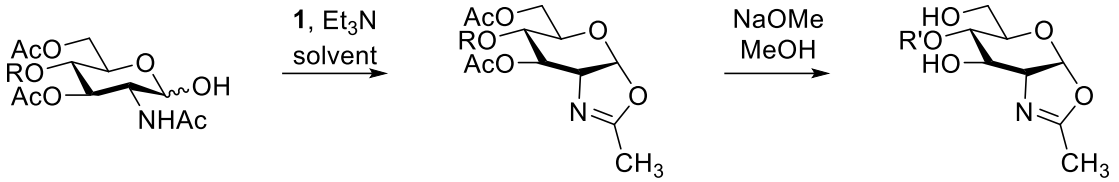
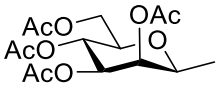
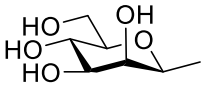
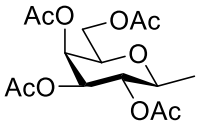
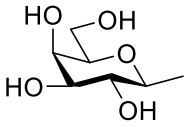
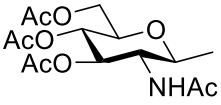
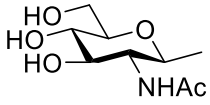
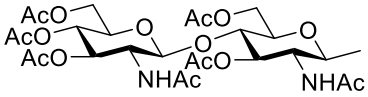
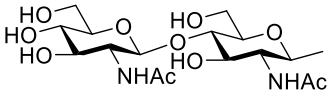


Figure 2. ^{1}H-NMR spectra used to monitor the reaction progress are shown. a) reaction mixture; b) oxazoline **3**; c) CDBMI, and d) hemiacetal **2**.

Table 2. Scope of oligosaccharide-oxazoline preparation



2, 8, 11, 14, 17 **3, 9, 12, 15, 18** **7, 10, 13, 16, 19**

run	Hemi acetal	R	oxazoline	yield (%)	de Ac oxazoline	R’	yield (%)
1	**2**	Ac	**3**	70	**7**	H	quant.
2	**8**		**9**	85	**10**		quant.
3	**11**		**12**	70	**13**		quant.
4	**14**		**15**	90	**16**		quant.
5	**17**		**18**	quant.	**19**		quant.

The scope of the reaction was investigated under the optimized reaction conditions (Table 2). Although DMF afforded a better yield (Table 1, run 9), CH_2Cl_2 was considered a more suitable solvent because of the ease of product isolation. Because our study focused on N-glycans on IgG, disaccharide **8** was chosen as substrate for IgG glycan remodeling (run 2)[19]. In addition, the repeating unit of the polylactosamine derivative **11**[20], which is associated with stem cell differentiation[21], also furnished oxazoline **12**[22] in 70% yield (run 3). The substrate for chitinase **14**[23] also afforded the corresponding oxazoline **15**[24] in 90% yield (run 4). In addition to the disaccharide, chitotriose **17**[25] afforded corresponding oxazoline **18**[26] in quantitative yield (run 5). De-*O*-acetylation afforded all unprotected oxazolines **7**, **10**, **13**, **16**, and **19** in quantitative yields.

Conclusions

In summary, this study established an efficient and mild protocol for the preparation of oligosaccharide-oxazolines in organic media, followed by a streamlined de-*O*-acetylation step. Because CDMBI **1** functions as a dehydrating reagent, its use in organic solvent systems is mechanistically rational and operationally advantageous. This method provides reliable access to oligosaccharide-oxazolines and is broadly applicable to chemoenzymatic glycan synthesis.

Experimental Section

General. All commercial reagents were used without further purification. Flash column chromatography was performed on silica gel 60N (spherical, neutral, 40–100 μm Kanto Co.). ^{1}H- and ^{13}C- NMR spectra were recorded on JEOL JMM-ECA600II and JEOL JNM-ECZL400S. Chemical shifts (δ) are reported in ppm relative to internal TMS (δ = 0.00 ppm) in $CDCl_3$, or remaining solvent peak (δ = 3.31 ppm for CD_3OD) for ^{1}H-NMR spectra. ^{13}C-NMR chemical shifts (δ) are reported in ppm relative to remaining solvent peak $CDCl_3$ (δ = 77.0 ppm) or CD_3OD (δ = 49.0 ppm).

Typical experimental procedure for oxazoline preparation

To a mixture of hemiacetal in anhydrous CH_2Cl_2 or CH_2Cl_2-DMF (1:1) (20-200 mM), Et_3N (3 equiv.) and CDMBI (2 equiv.) were added under Ar atmosphere. The reaction mixture was stirred at room temperature. After starting material was consumed by TLC analysis, the mixture was concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$: MeOH containing 1% Et_3N), or gel filtration (Bio-Beads SX-8: toluene or Sephadex LH-20: $CHCl_3$: MeOH) to give a corresponding oxazoline.

Typical experimental procedure for deacetylation

To a solution of oxazoline in anhydrous MeOH-THF (1:1), a drop of NaOMe (28% in MeOH) was added. After stirring the mixture overnight, the mixture was concentrated and dried under high vacuum.

Compound 7. quant. ^{1}H-NMR (400 MHz, CD_3OD): δ 5.99 (d, *J* = 7.3 Hz, 1H, H-1), 3.96 (ddq, *J* = 6.9, 3.2, 1.6 Hz, 1H, H-2), 3.83 (t, *J* = 3.6 Hz, 1H, H-3), 3.73 (dd, *J* = 12.1, 2.6 Hz, 1H, H-6_a), 3.60 (dd, *J* = 12.0, 6.0 Hz, 1H, H-6_b), 3.50 (ddd, *J* = 9.0, 3.5, 1.1 Hz, 1H, H-4), 3.32 – 3.23 (m, 1H, H-5), 1.99 (d, *J* = 1.6 Hz, 3H, CH_3); ^{13}C-NMR (101 MHz, CD_3OD): δ 168.6, 102.6, 75.1, 74.2, 70.8, 68.4, 63.6, 13.8.

Compound 10. quant. ^{1}H-NMR (600 MHz, CD_3OD): δ 6.02 (d, *J* = 7.3 Hz, 1H, H-1), 4.63 (s, 1H, H-1'), 4.27 (t, *J* = 3.4 Hz, 1H), 4.10 – 4.05 (m, 1H, H-2), 3.92 (dd, *J* = 11.7, 2.3 Hz, 1H), 3.85 (d, *J* = 2.7 Hz, 1H), 3.78 – 3.68 (m, 3H), 3.66 – 3.60 (m, 1H), 3.57 – 3.51 (m, 1H), 3.48 – 3.42 (m, 1H), 3.35 (tt, *J* = 5.3, 2.8 Hz, 1H), 3.29 – 3.23 (m, 1H), 2.04 (d, *J* = 1.6 Hz, 3H, CH_3); ^{13}C-NMR (151 MHz, CD_3OD): δ 169.0, 102.7, 102.1, 78.9, 78.7, 75.2, 73.1, 72.3, 71.6, 68.6, 67.6, 63.3, 62.9, 13.8.

Compound 13. quant. ^{1}H-NMR (600 MHz, CD_3OD): δ 6.02 (d, *J* = 7.4 Hz, 1H, H-1), 4.31 (d, *J* = 7.6 Hz, 1H, H-1'), 4.26 (t, *J* = 3.0 Hz, 1H), 4.04 (ddt, *J* = 7.4, 3.1, 1.5 Hz, 1H, H-2), 3.84 – 3.79 (m, 1H), 3.81 – 3.73 (m, 2H), 3.71 (d, *J* = 4.8 Hz, 1H), 3.70 – 3.67 (m, 2H), 3.54 (ddd, *J* = 7.7, 4.3, 1.1 Hz, 1H), 3.51 (dd, *J* = 9.7, 7.6 Hz, 1H), 3.45 (ddd, *J* = 9.7, 3.4, 0.7 Hz, 1H), 3.42 – 3.39 (m, 1H), 2.03 (d, *J* = 1.8 Hz, 3H, CH_3); ^{13}C-NMR (151 MHz, CD_3OD): δ 168.7, 106.3, 102.2, 80.1, 77.0, 74.9, 72.8, 72.6, 72.4, 70.5, 68.0, 63.1, 62.8, 13.8.

Compound 16. quant. ^{1}H-NMR (600 MHz, CD_3OD): δ 6.02 (d, *J* = 7.4 Hz, 1H, H-1), 4.53 (d, *J* = 8.4 Hz, 1H, H-1'), 4.34 (t, *J* = 2.6 Hz, 1H), 4.06 (ddt, *J* = 7.8, 3.1, 1.6 Hz, 1H, H-2), 3.92 (dd, *J* = 11.8, 2.3 Hz, 1H), 3.70 (dd, *J* = 12.0, 2.3 Hz, 1H), 3.69 – 3.50 (m, 4H), 3.46 (dd, *J* = 10.4, 8.5 Hz, 1H), 3.35 – 3.25 (m, 2H), 2.03 (d, *J* = 1.8 Hz, 3H, CH_3), 1.98 (s, 3H, Ac); ^{13}C-NMR (151 MHz, CD_3OD): δ 173.5, 168.6, 104.1, 101.8, 81.1, 77.9, 75.7, 72.3, 71.9, 71.3, 67.7, 63.0, 62.7, 57.5, 22.9, 13.6.

Compound 19. quant. ^{1}H-NMR (600 MHz, CD_3OD): δ 6.04 (d, *J* = 7.3 Hz, 1H, H-1), 4.53 (dd, *J* = 14.7, 8.2 Hz, 2H), 4.38 (dd, *J* = 3.1, 1.5 Hz, 1H), 4.15 (ddt, *J* = 7.1, 3.2, 1.7 Hz, 1H, H-2), 3.91 – 3.86 (m, 1H), 3.83 (dd, *J* = 12.2, 2.2 Hz, 1H), 3.74 – 3.69 (m, 2H), 3.67 (d, *J* = 7.8 Hz, 1H), 3.66 – 3.64 (m, 2H), 3.63 (dd, *J* = 4.0, 3.0 Hz, 1H), 3.62 – 3.56 (m, 2H), 3.55 – 3.53 (m, 1H), 3.53 – 3.50 (m, 1H), 3.50 – 3.47 (m, 1H), 3.47 – 3.44 (m, 1H), 3.42 (dd, *J* = 9.9, 8.4 Hz, 1H), 3.25 (ddd, *J* = 9.0, 6.6, 2.4 Hz, 1H), 2.04 (s, 3H), 2.02 (d, *J* = 1.9, 3H, CH_3), 1.99 (s, 3H, Ac); ^{13}C-NMR (151 MHz, CD_3OD): δ 173.8, 173.4, 168.7, 104.3, 103.3, 101.9, 81.6, 81.2, 78.2, 76.4, 75.9, 74.1, 72.5, 72.0, 71.0, 67.8, 63.1, 62.6, 61.8, 57.3, 56.8, 23.1, 23.0, 13.7.

Acknowledgements

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Supplementary Material

The characterized spectra (^{1}H-NMR & ^{13}C-NMR) have been provided in the supplementary file attached with this manuscript.

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