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Efficient Synthesis of Sugar Oxazolines from Unprotected *N*-Acetyl-2-amino Sugars by Using Chloroformamidinium Reagent in Water

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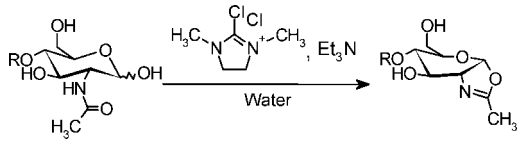

Sugar oxazoline derivatives were directly synthesized from the corresponding *N*-acetyl-2-amino sugars in aqueous media by using a chloroformamidinium-type dehydrating reagent. The present method could successfully be applied to chito-oligosaccharides, saccharides with acid functions, and a complex-type oligosaccharide derived from a glycopeptide.

Chemo-enzymatic glycosylations based on glycosidases have become a powerful tool for stereoselective synthesis of various glycosidic compounds.[1] In principle, the process of a chemo-enzymatic glycosylation consists of the following two steps: (1) the synthesis of a glycosyl donor by introducing an appropriate leaving group such as fluoride ion or *p*-nitrophenyl group to the anomeric center and (2) the subsequent enzyme-catalyzed transglycosylation of the glycosyl donor to an alcohol.[2] The latter step (transglycosylation) can be achieved without using any protecting groups in an aqueous solution. To the contrary, the former step (glycosyl donor synthesis) always necessitates the protection and deprotection of the hydroxy groups by use of various kinds of synthetic reagents as well as organic solvents, which makes the chemo-enzymatic process laborious and time consuming.

Sugar oxazolines are known to be useful glycosyl donors for both chemical glycosylations[3] and chemo-enzymatic glycosylations.[4] In particular, nonprotected oxazoline derivatives have extensively been employed for the chemo-enzymatic construction of an *N*-acetylglucosaminide unit in various oligosaccharides, polysaccharides, and glycoproteins.[4] To synthesize these nonprotected sugar oxazolines, per-acetylated 2-acetamido-2-deoxy sugars must first be prepared and converted to the corresponding sugar oxazolines by using Lewis acids such as ferric(III) chloride, tin(IV) chloride, boron trifluoride, and trimethylsilyl triflate,[5] followed by the removal of the acetyl groups by a base. In addition, the use of strong Lewis acids often damages the glycosidic bonds in the starting oligosaccharides, resulting in a complex reaction mixture. It is, therefore, absolutely necessary to develop an efficient and simple method for synthesizing sugar oxazolines directly from the corresponding 2-acetamido-2-deoxy sugars without employing any protecting groups and Lewis acids.

We already reported the direct synthesis of a sugar oxazoline by using a water-soluble carbodiimide as dehydrating reagent.[6] However, the yield was at most 37%, and the method required longer reaction time, because of the lower reactivity of the carbodiimide reagent as well as decomposition of the resulting oxazoline under a prolonged reaction time. Meanwhile, very recently, we have developed a novel method for direct activation of the anomeric center of various saccharides[7] by using 4-(4,6-dimethoxy-1,3,5-triazine-2-yl)-4-methylmorpholinium chloride (DMT-MM).[8] When the method was applied to *N*-acetylglucosamine (GlcNAc) aiming at the improvement of the oxazoline yield, the required oxazoline derivative (GlcNAc-oxa) was obtained in only 33% yield, while a stable adduct with α-configuration, DMT-α-GlcNAc, was produced as the main product (62%) (Scheme 1).[9]

We postulated that the use of a more reactive reagent than DMT-MM would lead to the formation of an unstable α-adduct, from which GlcNAc can be regenerated by hydrolysis. On the basis of this hypothesis, we screened cation-type formamidinium salts as an appropriate candidate for the more reactive reagent.

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(7) For example, a novel glycosyl donor for enzymatic glycosylation, 4,6-dimethoxy-1,3,5-triazin-2-yl β-lactoside, can be prepared directly from lactose in water: Tanaka, T.; Noguchi, M.; Kobayashi, A.; Shoda, S. *Chem. Commun.* **2008**, *201*, 6–2018.

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SCHEME 1. Direct Synthesis of GlcNAc-oxa and Triazine Adduct (DMT-α-GlcNAc) by DMT-MM in Water

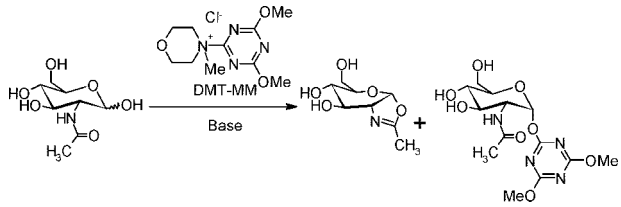
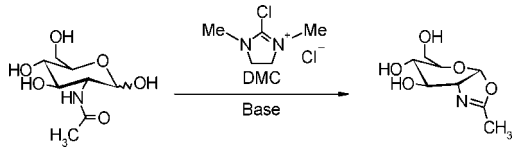


TABLE 1. Synthesis of GlcNAc-oxa by Using DMC in the Presence of Various Bases[a]



entry	base	yield[b] (%)
1	4.3 M Me_3N aq	74
2	$(Me)_2NEt$	72
3	Et_3N	90
4	4.3 M Et_3N aq	86
5	*n*-BuN$(Me)_2$	78
6	$(i\text{-Pr})_2NEt$	61
7	$(n\text{-Pr})_3N$	4

[a] Reaction conditions: GlcNAc, 250 mM; GlcNAc:DMC:base = 1:3:9; temperature, 0 °C; reaction time, 15 min. [b] Determined by ^{1}H NMR with sodium benzenesulfonate as an internal standard.

In this paper, we wish to report an extremely facile and practical method for direct synthesis of sugar oxazoline derivatives starting from 2-acetamido-2-deoxy sugars by using 2-chloro-1, 3-dimethylimidazolinium chloride (DMC) as dehydrating reagent in water.[10]

We assumed that DMC would be gradually hydrolyzed in an aqueous solution because of its high reactivity before reacting with the hemiacetal-type hydroxy group of GlcNAc.[11] In addition, two molecules of HCl are liberated during the course of the reaction, which requires the use of at least 2 equiv of an acid scavenger to promote the reaction effectively. On the basis of these considerations, we investigated the amount of DMC and bases and found that the optimized ratios to GlcNAc were 3 and 9, respectively. It should be noted that the oxazoline formation took place very rapidly; GlcNAc was completely consumed and converted GlcNAc-oxa within 15 min at 0 °C. Table 1 summarizes the influence of various bases on the yield of GlcNAc-oxa in D_2O. Among several bases screened, triethylamine gave the best result concerning the yield of GlcNAc-oxa (entry 3). When trimethylamine solution or *N*,*N*-dimethylethylamine was used, the yield decreased due to their lower basicities (entries 1 and 2). When tri-*n*-propylamine was used, a good result could not be obtained due to its lower solubility (entry 7).

Table 2 summarizes the synthesis of various sugar oxazolines from the corresponding 2-acetamido-2-deoxy sugars. The present method could be applied to other monosaccharides like *N*-acetylgalactosamine and *N*-acetylmannosamine (entries 2 and 3) and oligosaccharides (entries 4−9). It is noteworthy that chitooligosaccharides that are difficult to derivatize by the conventional method of employing protecting groups could easily be converted to the corresponding oxazoline derivatives in good yields. The monosaccharides possessing a sulfuric acid moiety or phosphoric acid moiety can also be converted to GlcNAc-6-sulfate and GlcNAc-6-phospate, respectively without affecting the structures of these functional groups (entries 10 and 11).

TABLE 2. Synthesis of Sugar Oxazolines from Various Mono- and Oligosaccharides

entry	saccharide	DMC (equiv)	base (equiv)	yield[a] (%)
1	GlcNAc: 250 mM	3	9	90
2	GalNAc: 50 mM	10	30	47
3	ManNAc: 50 mM	10	30	76
4	LacNAc: 50 mM	10	30	90
5	$(GlcNAc)_2$: 50 mM	10	30	77
6	$(GlcNAc)_3$: 10 mM	15	45	75
7	$(GlcNAc)_4$: 10 mM	15	45	83
8	$(GlcNAc)_5$: 10 mM	15	45	69
9	$(GlcNAc)_6$: 10 mM	15	45	81
10	GlcNAc-6-sulfate: 125 mM	3	9	84
11	GlcNAc-6-phosphate: 125 mM	3	9	79
12	Man_{5-7} GlcNAc:[b] 45 mM	10	30	64
13	disialooligo saccharide:[c] 50 mM	15	45	92

[a] Yields for entries 1−11 were determined by ^{1}H NMR with sodium benzenesulfonate as an internal standard. Yields for entries 12 and 13 were determined by comparing the integration of the anomeric proton of the reducing end in the oxazoline and that of the methyl protons of the NAc group in unreacted oligosaccharides. [b] Ovalubmin oligosaccharides cleaved by *endo*-*N*-acetylglucoasminidase A. [c] Fetuin-type biantenary oligosaccharide cleaved by *endo*-*N*-acetylglucoasminidase M.

The present method could successfully be applied to a high mannose-type hepta-saccharide (entry 12)[12] and disialo complex-type deca-saccharide having two *N*-acetylneuramic acid moieties (entry 13).[13] It has been well-known that *N*-acetylneuramic acid-containing oligosaccharides play an important role in glycobiology.[14] Recent progress in organic synthesis and enzymology has enabled us to develop an efficient chemo-enzymatic route toward synthesis of glycoconjugates possessing these oligosaccharide chains.[15] However, there has been no report concerning the synthesis of sugar oxazolines having an *N*-acetylneuramic acid moiety.

The mechanism of oxazoline ring formation involves the preferential attack of the hemiacetal-type hydroxy group to the 2-position of DMC to give a reactive intermediate **1** with β-configuration (Scheme 2). Then, an intramolecular attack of the carbonyl oxygen of the 2-acetamido group as well as the abstraction of the amide proton by triethylamine take place, affording a sugar oxazoline and 1,3-dimethyl-2-imidazolidinone. The α-hemiacetal-type hydroxy group, which is in equilibrium with the β one, may also react with DMC to form an α-adduct **2**.[16] However, the resulting intermediate is immediately hydrolyzed by the attack of water, regenerating the starting free sugar,

(10) Shoda, S.; Kobayashi, A.; Noguchi, M.; Tanaka, T.; Gyakushi, H. PCT, Int. Appl., WO2009/111526 A1.

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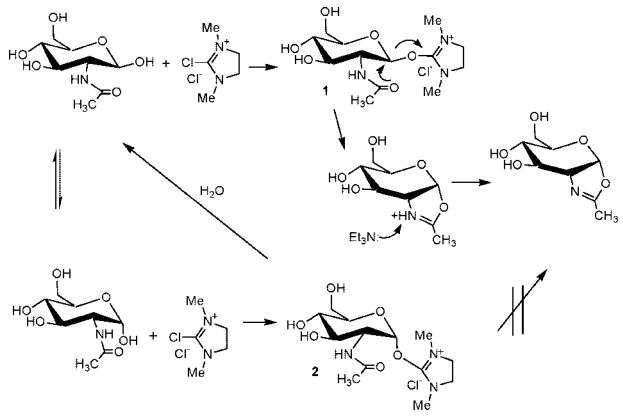
(16) The anomeric ratio (α/β) of GlcNAc in the presence of 9 equiv of triethylamine at room temperature in D_2O was determined to be 6/4 by ^{1}H NMR.

SCHEME 2. Plausible Mechanism for Sugar Oxazoline Formation via Reactive Intermediate 1



because intramolecular ring formation from the α-adduct is sterically prohibited.[17]

In conclusion, we have achieved a direct synthesis of various sugar oxazoline derivatives starting from the corresponding unprotected *N*-acetyl-2-amino sugars via an intramolecular dehydration process in water without using any protecting groups. One of the most significant characteristics of this method is that the oxazoline derivatives can be prepared efficiently in an aqueous solution. This fact will pave a way to the "one-pot chemo-enzymatic glycosylation"[18] without isolating the sugar oxazolines as synthetic intermediates. On the basis of this new concept, extensive investigation on the one-pot synthesis of various types of glycosyl compounds is in progress in our laboratory.

(17) Another route via the β-chloride intermediate that can be formed as a result of a nucleophilic attack of the chloride ion to the α-adduct **2** cannot be excluded.

(18) Tanaka, T.; Noguchi, M.; Kobayashi, A.; Shoda, S. *Polym. Prepr. Jpn.* **2008**, *57*, 1742.

Experimental Section

Formation of GlcNAc-oxa and Determination of the Yield by ^{1}H NMR and H−H COSY Method. *N*-acetylglucosamine (GlcNAc) and triethylamine were dissolved in D_2O and the resulting solution was cooled to 0 °C. DMC was added to the solution and the mixture was stirred for 15 min at the same temperature. After adding sodium benzenesulfonate to estimate the yield, the reaction mixture was directly subjected to NMR analysis. The yield was determined by comparing the integration of the aromatic protons of sodium benzenesulfonate and the anomeric proton of the oxazoline derivative (GlcNAc-oxa).

Isolation of LacNAc-oxa. *N*-Acetyllactosamine (LacNAc) (26.4 mg, 68.9 μmol) and triethylamine (104 μL, 750 μmol) were dissolved in H_2O and the resulting solution was cooled to 0 °C. DMC (44.6 mg, 264 μmol) was added to the solution and the mixture was stirred for 1 h at the same temperature. The reaction mixture was purified by using a preparative reversed phase HPLC column (Inertsil ODS-3 10 mm i.d., 250 mm length, eluent H_2O). The oxazoline fraction was collected and lyophilized, giving rise to 21.1 mg of LacNAc-oxa (84% yield). ^{1}H NMR (D_2O, 500 MHz) δ 5.98 (1H, d, H1, $J_{1,2}$ = 7.2 Hz), 4.32−4.30 (2H, m, H3 and H1′), 4.08 (1H, m, H2), 3.76 (1H, d), 3.75−3.54 (6H, m), 3.51 (1H, dd), 3.40 (1H, t), 3.34 (1H, t), 1.95 (3H, s, $-CH_3$). ESI-MS calcd for $[M + Na]^+$ 388.1214, found *m*/*z* $[M + Na]^+$ 388.1216.

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Supporting Information Available: Experimental details and ^{1}H NMR for all reaction mixture and isolated LacNAc-oxa, and H−H COSY spectra for a selected reaction mixture. This material is available free of charge via the Internet at http://pubs.acs.org.

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