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DIRECT CONVERSION OF 2-ACETAMIDO-2-DEOXYSUGARS TO 1,2-OXAZOLINE DERIVATIVES BY DEHYDRATIVE CYCLIZATION IN WATER

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Abstract – Sugar oxazoline derivatives were directly prepared by dehydrative cyclization of 2-acetamido-2-deoxysugars using a water-soluble carbodiimide in water. *N*-Acetyl-D-glucosamine, *N*-acetyl-D-galactosamine, and *N,N'*-diacetylchitobiose were converted to the corresponding 1,2-oxazoline derivatives in 37, 28, and 40 % yields, respectively.

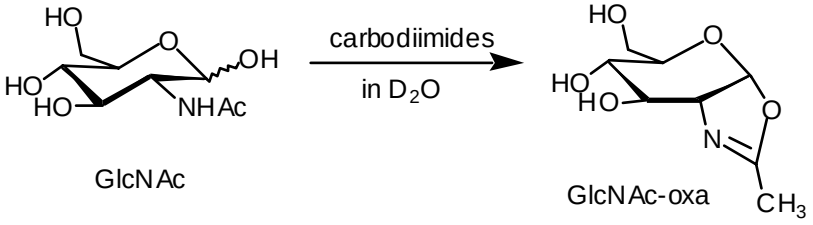
Formation of a glycosidic linkage between two sugar residues is a key step for architecture of oligo- and polysaccharides, as well as saccharide chains in glycoproteins and the related materials.[1] Enzymatic glycosylation catalyzed by glycosidases is a very powerful tool for synthesis of such glyco-materials, which has often been an advantageous reaction compared with chemical glycosylation method, because of the high regio and stereoselectivities.[2] Although we can employ sugar substrates having no protecting groups for the enzymatic glycosylations, the introduction of an activating group at the anomeric position is necessary to proceed the reaction efficiently. For example, glycosyl fluorides and *p*-nitrophenyl glycosides have been used as glycosyl donors for the enzymatic glycosylations, because these substrates can be recognized well by glycosidases. Recently, 1,2-oxazoline derivatives of mono- and disaccharides with no protecting groups were found to be recognized by *N*-acetylglucosaminidases and utilized as efficient glycosyl donors for the enzymatic glycosylations.[3] This indicates that sugar oxazoline derivatives are promising key compounds for construction of various oligo- and polysaccharide chains with well-defined structures.

For preparation of sugar oxazolines, fully acetylated 2-acetamido-2-deoxysugars have generally been used as starting materials, which can be converted to the corresponding oxazolines using Lewis acids

such as ferric(III) chloride, tin(IV) chloride, or trimethylsilyl triflate.[4] Then, deprotection of acetyl groups gives the free sugar oxazolines. These reactions are not the sufficient route for synthesis of the free sugar oxazolines from oligosaccharides having a 2-acetamido-2-deoxysugar residue at a reducing end. Especially, the use of Lewis acids induces a cleavage of the glycosidic linkages in oligosaccharide chains, resulting in low yield. Other methods for oxazoline synthesis also require the protection and deprotection of the hydroxy groups.[5] To generalize the enzymatic glycosylation using the sugar oxazolines of oligosaccharides as glycosyl donors, therefore, it is absolutely necessary to develop efficient and simple methods for preparation of the free sugar oxazolines from the corresponding 2-acetamido-2-deoxysugars without the protection and deprotection of the hydroxy groups, as well as the use of Lewis acids.

Based on the above viewpoints, here we report a direct method for preparation of the free sugar oxazoline derivatives from 2-acetamido-2-deoxysugars by dehydrative cyclization using a carbodiimide as the condensing agent. It should be noted that the present dehydrative reaction proceeds in water. We believe that this reaction can be applied to prepare the sugar oxazolines from various oligo- and polysaccharides in further investigation.

First, screening of carbodiimides for efficient formation of a sugar oxazoline (GlcNAc-oxa) from *N*-acetyl-D-glucosamine (GlcNAc) was carried out (Scheme 1). An equimolar mixture of GlcNAc, carbodiimide, and triethylamine in D_2O was stirred for 5 h at room temperature and the yield of GlcNAc-oxa was directly determined by ^1H NMR spectral measurement of the reaction mixture. Two water-soluble carbodiimides, 1-[3-(dimetylamino)propyl]-3-ethylcarbodiimide and its hydrochloride (WSC and WSC-HCl, respectively), as well as a more common carbodiimide, 1,3-dicyclohexylcarbodiimide (DCC), were tested. Although the yields obtained by using both water-soluble carbodiimides were comparable, we selected WSC-HCl because WSC-HCl was more stable and resists hydrolysis for longer reaction time in aqueous media.



WSC = $CH_3CH_2-N=C=N-CH_2CH_2CH_2-N(CH_3)_2$

WSC-HCl = $CH_3CH_2-N=C=N-CH_2CH_2CH_2-\overset{+}{N}H(CH_3)_2 \; Cl^-$

Scheme 1. Conversion of GlcNAc to GlcNAc-oxa using various carbodiimides in D_2O

Table 1 shows the selected results on the yields of GlcNAc-oxa using WSC-HCl in D_2O. When the reaction was carried out in the presence of various bases, triethylamine gave a higher yield than that using other bases as well as that without base (entries 1 - 4). The use of larger amounts of triethylamine, however, was not effective for increasing the yield (entry 5). Although the reaction proceeded slower at lower temperature (4 ^oC), the yield reached 13 % for 1 day, which was higher than that obtained at room temperature under the same reaction conditions (9 %) (entries 6 - 8). The higher temperature like 60 ^oC gave very complicated products. The yields increased by using large excess WSC-HCl toward GlcNAc with prolonged reaction times (entries 9 - 11), and at most 37 % by using 7 equivalents of WSC-HCl under the conditions of entry 11.[6] However, the yield did not increase by increasing the amount of WSC-HCl than 7 equivalents (entry 12). This is probably due to that the excess amounts of WSC-HCl are gradually hydrolyzed for the long reaction time such as 5 days in water.

Table 1. Yields of GlcNAc-oxa from GlcNAc Using WSC-HCl in D_2O[a]

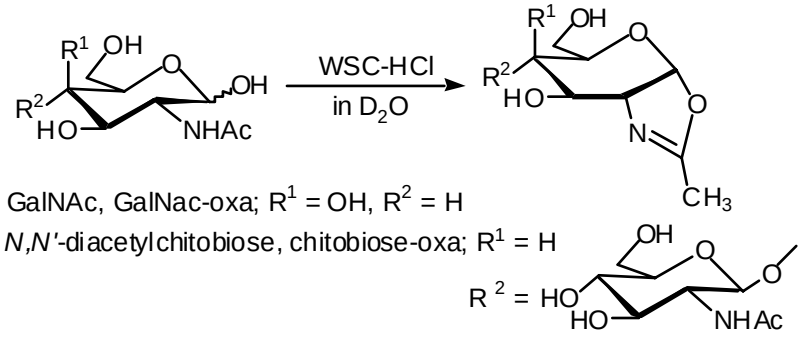
entry	base	GlcNAc: WSC-HCl :base	temp (^oC)	time	yield (%)[b]
1	-	1 : 1 : 0	rt	5 h	4
2	$NaHCO_3$	1 : 1 : 1	rt	5 h	3
3	Pyridine	1 : 1 : 1	rt	5 h	3
4	Et_3N	1 : 1 : 1	rt	5 h	7
5	Et_3N	1 : 1 : 2	rt	5 h	7
6	Et_3N	1 : 1 : 1	rt	1 day	9
7	Et_3N	1 : 1 : 1	4	5 h	4
8	Et_3N	1 : 1 : 1	4	1 day	13
9	Et_3N	1 : 3 : 1	4	2 days	26
10	Et_3N	1 : 5 : 1	4	3 days	32
11	Et_3N	1 : 7 : 1	4	4 days	37
12	Et_3N	1 : 10 : 1	4	5 days	36

a) Reaction conditions: GlcNAc (0.25 mmol), D_2O (0.50 mL).
b) Determined by ^1H NMR spectra of the reaction mixtures using sodium benzoate (0.10 mmol) as an internal standard. The yield was calculated by the integrated ratio of the peak due to C-1 proton of GlcNAc-oxa to the peak due to the aromatic protons of sodium benzoate.

As application of the present reaction to other sugar substrates, *N*-acetyl-D-galactosamine (GalNAc) and *N,N'*-diacetylchitobiose were employed (Scheme 2). Preparation of sugar oxazolines from these substrates was carried out using 7 equivalents of WSC-HCl under the same conditions to those of entry 11. The yields of GalNAc-oxa and chitobiose-oxa determined by ^1H NMR spectra were 28 and 40 %, respectively. These data indicate that the reaction mentioned here is sufficiently applicable to direct preparation of sugar oxazolines from various aminosugars.

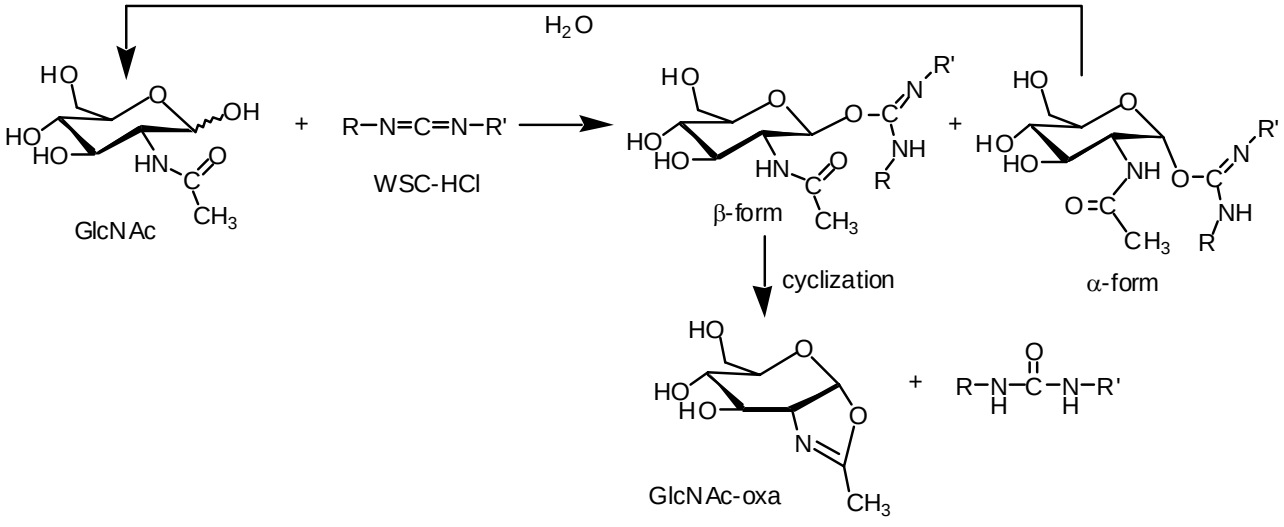
A typical experimental procedure for synthesis of oxazoline derivative of *N,N'*-diacetylchitobiose (GlcNAc-GlcNAc) was as follows. To a solution of GlcNAc-GlcNAc (0.212 g, 0.50 mmol) in D_2O (1.0

mL) were added triethylamine (0.050 g, 0.5 mmol) and WSC-HCl (0.096 g, 0.50 mmol) at 4 °C, and the solution was stirred for 1 days at 4 °C. After treating with Amberlite IR-120B NA (6.2 g) to remove the urea compound, the reaction mixture was further purified by chromatography (column: Inertsil ODS-3, eluent: water, detection: UV) to give 0.042 g of GlcNAc-GlcNAc-oxa (21 % yield).



Scheme 2. Conversion of GalNAc and *N,N'*-diacetylchitobiose to corresponding sugar oxazolines using WSC-HCl in D_2O.

The following mechanism is proposed for this reaction (Scheme 3, represented by GlcNAc). The first step is formation of a reactive intermediate by the reaction of an anomeric hydroxy group with WSC-HCl.[7] In the second step, the resulting intermediate cyclizes to form a sugar oxazoline and a urea derivative. The cyclization does not occur from the α-form of the intermediate as shown in Scheme 3, which is probably converted into β-anomer of GlcNAc by the nucleophilic attack of water. Existence of both anomers in GlcNAc and occurrence of hydrolysis of WSC-HCl imply the necessity of the large excess WSC-HCl toward the substrate.



Scheme 3. Proposed reaction mechanism.

In conclusion, we found that 2-acetamido-2-deoxysugars were directly converted to the corresponding

sugar oxazoline derivatives by dehydrative cyclization using the water-soluble carbodiimide in water. To our best knowledge, this is the first example of direct conversion of free 2-acetamido-2-deoxysugars to the corresponding oxazoline derivatives without using any protection-deprotection procedures. The detailed studies concerning improvement of the yields of oxazoline products are now in progress.

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6. When the reaction was carried out for longer reaction times than those indicated in Table 1 (entries 8 – 12), the yields gradually decreased, probably due to hydrolysis of GlcNAc-oxa.
7. The ^{1}H NMR spectrum of the reaction mixture indicated that in the α- and β-anomers, β-anomer predominantly reacted with WSC-HCl, because the ratio of the peak due to β-anomeric proton decreased with progress of the reaction.