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Unique cleavage of 2-acetamido-2-deoxy-D-glucose from the reducing end of biantennary complex type oligosaccharides

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ABSTRACT

Basic treatment of a biantennary complex-type sialyloligosaccharide, as well as its asialo form, was found to lead to the specific cleavage of 2-acetamido-2-deoxy-D-glucose (GlcNAc) from the reducing end. The resultant oligosaccharides were identical to those prepared by treatment with endo-β-glycosidase-M, which cleaves the glycosidic bond between two GlcNAc residues at the reducing end of N-linked oligosaccharides. In addition, mechanistic studies suggested that an elimination reaction in the reducing-end terminal GlcNAc residue causes this specific cleavage reaction.

1. Introduction

Acid hydrolysis is a common reaction for the cleavage of glycosidic bonds, which are linked via acid-labile acetal functional groups, and this method has been used for the structural analysis of oligosaccharides or the preparation of suitable sugar materials.[1] The hydrolysis velocity can be regulated by optimizing acid concentration; however, acid hydrolysis of oligosaccharides appears to occur nonspecifically. When considering basic hydrolysis of oligosaccharides, the peeling reaction has been used in the sequence analysis of polysaccharides.[2] However, this reaction usually causes epimerization at the C-2 position of the reducing end sugar in the resultant oligosaccharides.

During our studies on the hydrazinolysis of complex-type Fmoc-asparaginyl disialyloligosaccharide **1**[1a] followed by Kochetkov amination,[3] we found a cleavage reaction of 2-acetamido-2-deoxy-D-glucose (GlcNAc) from the reducing end of biantennary complex-type disialylundecasaccharide **2** as well as asialo nonasaccharide **3** (Fig. 1). Based on our finding, we have studied this undesired—but unique—cleavage reaction of the GlcNAc residue. This degradation reaction is of interest as the resultant oligosaccharide can be converted into the oxazoline form at the reducing end and can, in turn, be used for enzymatic oligosaccharide transfer reaction using endo-β-*N*-acetylglucosaminidase (EndoM). Endo M can transfer the oligosaccharide to the 4 position of single GlcNAc residues linked to the asparagine side chain of a protein.[4]

The Ito group has reported a similar detachment reaction of N-linked high mannose-type oligosaccharides.[5] However, the reaction mechanism was not studied in detail. In this paper, we report the synthesis of oligosaccharides **4** and **5** as potent glycosyl donors through the unique and selective cleavage reaction of the reducing end GlcNAc residue from complex-type oligosaccharides **2** and **3**.

2. Results and discussion

During Kochetkov amination of oligosaccharide **2** and **3**, prepared by hydrazinolysis reaction of Fmoc-asparaginyl disialyloligosaccharide **1**, we unexpectedly found the GlcNAc cleavage reaction of oligosaccharide **2** and **3** (Fig. 1). When oligosaccharide **2** or **3** was treated with a saturated ammonium bicarbonate solution at 37 °C, these conditions caused the undesired decomposition reaction. Monitoring of Kochetkov amination using ESI-mass spectrometry indicated the cleavage of the reducing end GlcNAc residue. However, the product was obtained only in a small amount (<10%), which we postulated because the reaction being quenched by the amination of the anomeric position at the reducing end, and the equilibrium state might bias the amination product rather than the cleaved product. In order to obtain an appropriate amount of the product, we optimized the conditions of the reaction. Thus, oligosaccharide **2** was dissolved in a 100 mM NaOH solution instead of a saturated ammonium bicarbonate solution and this solution was stirred at 50 °C (Scheme 1).

To monitor this reaction, after 16 h, an aliquot of the reaction mixture was desalted by cation-exchange resin and then examined by ESI-mass spectrometric analysis. The ESI-mass spectra (Fig. 2)

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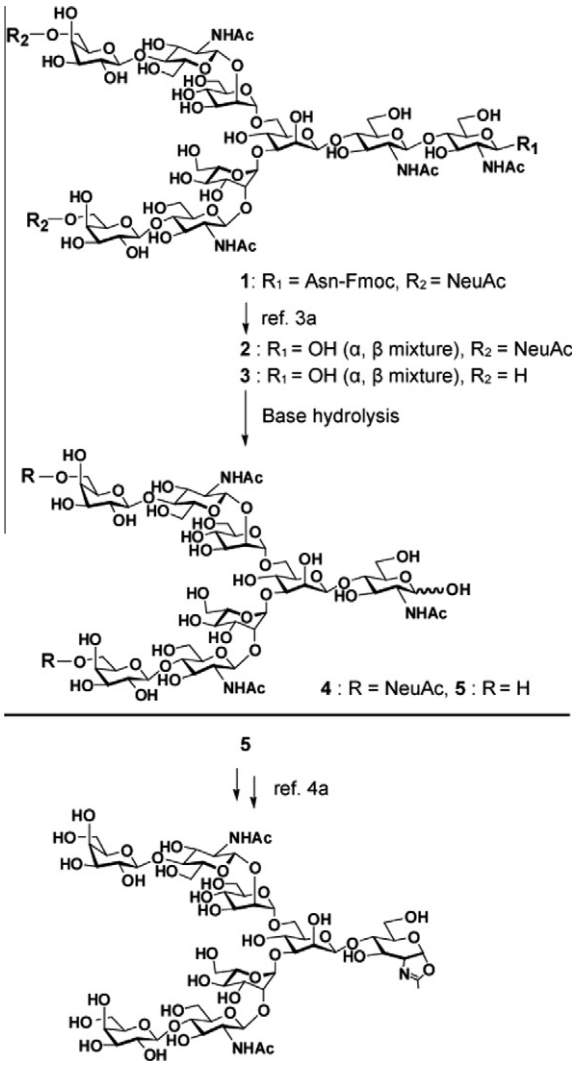
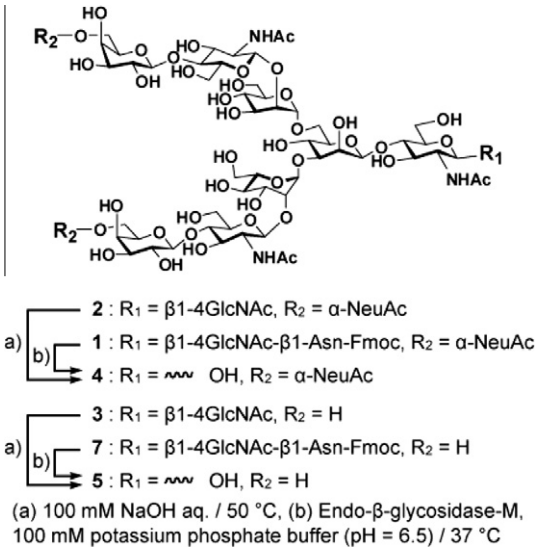


Figure 1. Unique detachment reaction under basic condition.



Scheme 1. Preparation of oligosaccharides **4** and **5**.

indicated $m/z = 1009.1$ $[M-2H]^{2-}$ (original oligosaccharide **2**: $m/z = 1110.7$ $[M-2H]^{2-}$). This difference of mass value enabled us to speculate that the byproduct was oligosaccharide **4** generated by the cleavage of the GlcNAc residue from the reducing end. To date, the peeling reaction is known as a decomposition reaction of reducing oligosaccharides under basic conditions.[2] However, the resultant products are frequently epimerized at the C-2 position. To confirm the structure of the resultant oligosaccharide generated by basic treatment, we prepared an authentic sample of oligosaccharide **4** by the enzymatic hydrolysis of oligosaccharide **1** (Scheme 1). This enzyme, endo-β-glycosidase-M, can cleave the glycosyl bond between the two GlcNAc residues at the reducing end of N-linked oligosaccharides.[4a,6] Enzymatic digestion of oligosaccharide **1** was performed in a solution of 100 mM $KHPO_4$ buffer (pH 6.5) and this reaction successfully afforded oligosaccharide **4** as a mixture of α and β isomers at the reducing end.[7] To compare both structures, ^1H, HMQC, and TOCSY NMR spectra of oligosaccharides **4** were measured and then compared. As shown in Figure 3A and B, the NMR spectra were in good agreement. In particular, all cross peaks observed by HMQC are perfectly identical.

In addition, we carried out an additional NMR experiment to confirm the configuration at the 2 position of the reducing end, because the basic peeling reaction usually causes epimerization at C-2 in the reducing sugar. To analyze the product easily, an anomeric mixture of oligosaccharide **4** was fixed in its β configuration by conversion to the corresponding hydrazide[8] (Scheme 2).

This conversion reaction smoothly afforded homogeneous oligosaccharide **6** in which the reducing end was fixed in the β form. The 1D-selective TOCSY spectrum of **6** clearly showed 1D spectrum of the reducing end GlcNAc. Fortunately, this experiment showed that the $J_{1,2}$ value was 9.7 Hz and other coupling constants were reasonable values for the *gluco* configuration, meaning that the acetamido group at the C-2 position adopts the equatorial configuration (Fig. 4).

These NMR data proved that the cleavage product from **2** was oligosaccharide **4** (analytical yield: 38%) and that no epimerization of the GlcNAc residue at the reducing end had occurred. Similarly, we examined the cleavage reaction using oligosaccharide **3** under the same conditions (Scheme 1). This reaction also successfully afforded an analogous product, oligosaccharide **5**, in 16% yield (isolated yield; analytical yield: 29%). In this case, we also confirmed the product structure by comparison with authentic oligosaccharide **5**[7] prepared by endo-β-glycosidase-M treatment using Fmoc-asparaginyl asialooligosaccharide **7**.[1a] Both NMR spectra show very good agreement (Supplementary data—Fig. 1A–D). These data clearly suggested that the detachment reaction specifically occurred under basic conditions without epimerization of the 2 position of GlcNAc residue at the reducing end.

Although we examined the optimization of this reaction to obtain oligosaccharides **4** and **5** in good yields, this reaction formed multiple products depending on the length of reaction time. Because the product might not be stable under basic condition for an extended period, increasing the length of the reaction may cause decomposition. Of all the conditions we examined, those shown in Scheme 1 provided the best results.

A plausible mechanism for the cleavage of the chitobiose residue is shown in Figure 5. Because both ammonium bicarbonate and NaOH conditions generated the same product, and basic conditions caused the cleavage reaction. Therefore, we propose that the *N*-acetyl group of GlcNAc at the reducing end (**8**) may be converted into imino form **11** through keto-enol tautomerism (between **9** and **10**) and subsequent abstraction of H-3 by base, causing β-elimination and resulting in the formation of the product **13**.

3. Conclusion

Although the decomposition reaction of oligosaccharides under basic conditions (peeling) has long been known, we found the specific conditions that led to the selective cleavage of a single Glc-