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Preparation of pseudo glycoamino acid and its application to glycopeptide synthesis

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ABSTRACT

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Oligosaccharides on cell surfaces play important roles in biological processes such as cell-cell interactions, cell adhesion, and immunogenic recognition.¹ These glycoconjugates are generated by cells through a complicated synthesis. In addition, there is a growing demand for chemo-enzymatic synthesized O- and N-glycans² because their peptide conjugates act as useful probes for studying glycoprotein-mediated biological reactions. Moreover, these glycopeptides may have therapeutic uses because it has been reported that addition of carbohydrates could potentiate the effects of drugs.³ Glycoamino acid is a key compound in glycopeptide and glycoprotein syntheses. Unfortunately, glycoamino acid synthesis is also a lengthy and complex process.

Endo- β -*N*-acetylglucosaminidase (ENGase, EC 3.2.1.96) is used for catalyzing the hydrolysis of the β -(1-4)-glycosidic linkages between *N*,*N*'-diacetylchitobiose moiety of N-glycans. This enzyme is isolated from *Mucor hiemalis* (Endo-M) and is unique in that it can act on all types of N-glycans including high-mannose, complex, and hybrid-type oligosaccharides, catalyzing the hydrolysis and transglycosylation of appropriate acceptors containing N-acetylglucosamine (GlcNAc) residues.⁴ Consequently, Endo-M is an effective tool for reconstructing and remodeling glycopeptides and glycoproteins.⁵ Further efforts are necessary for optimizing the transglycosylation efficiency of the enzyme.⁶ Moreover, the transglycosylation reaction was limited and could not be promoted by the enzyme in the absence of GlcNAc (sugar) as an acceptor.

We have studied the acceptor recognition site of Endo-M in detail and previously reported its structure.⁷ Our work demonstrated

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A pseudo glycoamino acid composed of a 1,3-diol structure and an amino acid was synthesized. This

amino acid analog can act as an alternative acceptor to an amino acid containing GlcNAc for transglyco-

sylation by Endo-M. A pseudo glycopeptide was synthesized using the pseudo glycoamino acid by a solid

phase procedure. We attempted transglycosylation of N-glycan to the peptide using Endo-M.

Based on these results, we designed the amino acid containing 1,3-diol structure that can be modified with a sugar chain using Endo-M. To connect a 1,3-diol moiety to the amino groups of a substrate by an amide linkage, we designed the 1,3-diol structure with a carboxylic acid group and a '1,3-diol tag' according to the previous report (Fig. 1). This amino acid analog can act as an alternative acceptor to an amino acid containing GlcNAc. Consequently, we refer to the compound as a 'pseudo glycoamino acid'.

The pseudo glycoamino acid was prepared from a 1,3-diol tag and an amino acid with an amino group side chain, such as Fmoc-Lys(1,3-diol tag)-OH (1), Fmoc-Orn(1,3-diol tag)-OH (2), and Fmoc-Dab(1,3-diol tag)-OH(3) (Fig. 2). Next, the pseudo glycoamino acid was used in solid phase synthesis and a pseudo glycopeptide was synthesized. Lastly, we attempted transglycosylation of complex-type oligosaccharide blocks using Endo-M for obtaining the pseudo glycopeptide.

First, the 1,3-diol tag was synthesized on the basis of procedures in the literature (Scheme 1).⁸ The 1,3-diol tag derivative 4

Figure 1. Structure of '1,3-diol tag'.



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that the oxygen atom on the pyranose ring was not essential for its activity; however, the narrow regions of the 1,3-diol structure from 4-OH to 6-OH of GlcNAc, primary, and secondary hydroxyl groups, were essential for transglycosylation using Endo-M. Furthermore, we determined that a functional group causing significant steric effects resided in the C-5 position of GlcNAc. This group interrupted transglycosylation.

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Figure 2. Structure of Fmoc-AA(1,3-diol tag)-OH.



Scheme 1. Preparation of the '1,3-diol tag' derivative **4**. Reagents and conditions: (a) (CF₃CO)₂O, rt; (b) MeOH, rt; (c) BH₃-THF, 0 °C to rt; (d) PhCH(OMe)₂, *p*-TsOH, DMF, 50 °C, 63% (4 steps); (e) Na₂CO₃, dioxane-water (1:1), rt.



Scheme 2. Preparation of pseudo glycoamino acids **1–3**. Reagents and conditions: (a) **4**, PyBOP, Et₃N, DMF, rt; (b) H₂ atmosphere, Pd/C, AcOH, rt; (c) FmocOSu, Na₂CO₃, dioxane-water (1:1), rt.

was prepared from p-malic acid. The acid was reacted with trifluoroacetic anhydride, subsequently the cyclic anhydride was opened by MeOH. Carboxylic acid **5** was reduced to a hydroxyl group using BH₃–THF, and thereby producing a dihydroxy derivative **6**. Two hydroxyl groups of **6** were protected by a benzylidene group using benzaldehyde dimethyl acetal and a catalytic amount of *p*-toluenesulfonic acid, thereby producing **7** in 63% yield (4 steps). The methyl ester of **7** was hydrolyzed by Na₂CO₃ solution to obtain the desired product **4**. Product **4** was used without further purification.

N-α-Benzyloxycarbonyl-lysine α-benzyl ester (Z-Lys-OBn) and **4** were condensed using 1H-benzotriazol-1-yl-oxy-tris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP), producing the amide derivative **8** in 80% yield (Scheme 2). The benzyl, benzyloxy-carbonyl, and benzylidene groups of **8** were removed by hydrogenation in the presence of 10% Pd/C. The corresponding compound was reacted with *N*-(9-fluorenylmethoxycarbonyl) succinimide (FmocOSu) for obtaining the desired product **1** in 62% yield (2 steps). Pseudo glycoamino acids **2** and **3** were synthesized in the same manner as **1**.

Next, transglycosylation of the complex-type oligosaccharide block to **1** was attempted using Endo-M. Using **1** as the glycosyl acceptor, transglycosylation of a complex-type oligosaccharide block [(NeuAc-Gal-GlcNAc-Man)₂-Man-GlcNAc-] with sialylglycopeptide (SGP) as the glycosyl donor was examined in the presence of Endo-M under the same reaction conditions as described previously.⁷ The reversed-phase high-performance liquid chromatography (RP-HPLC) profile of transglycosylation of SGP to **1** after 2 h is shown in Figure 3. Characterization of the product was performed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), and the transglycosylated product was confirmed.

The time course of transglycosylation of **1**, **2**, and **3** is shown in Figure 4. The highest yield using **1** as an acceptor was 26% after 2 h. Glycosylated **2** and **3** gave maximum yields of 14% and 6%, respectively. These data showed that differences in the amino acid side chain length strongly influenced the transglycosylation yield. Moreover, these results indicated that the transglycosylation rate was so low that it probably shortened the length of the alkyl amino acid side chain. We also plan to further study transglycosylation to longer side chain derivatives. When the GlcNAc derivative was used as an acceptor, the transglycosylated product reached 18%



Figure 3. RP-HPLC profile of the reaction mixture incubated with Fmoc-Lys(1,3-diol tag)-OH (1) and SGP in the presence of Endo-M for 2 h, as detected by absorbance at 280 nm. The large peak at 18–19 min corresponds to the residual acceptor 1.



Figure 4. Time course of the transglycosylation reaction using Endo-M. ●: Fmoc-Lys(1,3-diol tag)-OH (1), ▲: Fmoc-Orn(1,3-diol tag)-OH (2), ■: Fmoc-Dab(1,3-diol tag)-OH (3), ♦: Fmoc-Asn(GlcNAc)-OH.



Figure 5. Structure of [Lys(1,3-diol tag)⁵]-vapreotide (11).



Scheme 3. Preparation of [Lys(1,3-diol tag)⁵]-vapreotide (11).

yield after 1 h. These results suggested that an efficient acceptor was required for flexibility. This demonstrates that transglycosylation activity of **1** is superior to that of the GlcNAc derivative.

The best performing pseudo glycoamino acid **1** was used in the solid-phase synthesis of the glycopeptide analog **11** of vapreotide (Fig. 5).⁹

Peptide resin **12** was prepared from Rink Amide ResinTM using an Fmoc strategy by the dimethylphosphinothioic mixed anhydride (Mpt–MA) method (Scheme 3).¹⁰ Resin **12** was treated with a mixture of trifluoroacetic acid (TFA)/phenol/water/ethanedithiol (EDT)/triisopropylsilane (TIS) = 81.5:5:5:2.5:1 for cleaving the resin peptide and removing the side chain protecting groups. Following this, crude peptide **13** was obtained. Purification of the peptide by RP-HPLC gave **13** in 17% yield (based on the amino-acid analysis).



Figure 6. RP-HPLC profile of the reaction mixture incubated with $[Lys(1,3-diol tag)^5]$ -vapreotide (**11**) and SGP in the presence of Endo-M after a reaction time of 8 h at an absorbance of 254 nm. The large peak at 18–19 min corresponds to residual amounts of acceptor **11**.



Figure 7. Time course of transglycosylation using Endo-M. ■: [Lys(1,3-diol tag)⁵]-vapreotide (11), ●: Fmoc-Lys(1,3-diol tag)-OH (1).

Peptide **13** was then dissolved in DMSO, and a solution of $AgNO_3$ and DIEA in H_2O was added.¹¹ Next, a mixture of 1 M HCl and DMSO (1:1, v/v) was added to the reaction mixture, and [Lys(1,3-diol tag)⁵]-vapreotide (**11**) was obtained in 40% yield from **13**.

Next, transglycosylation was performed using pseudo glycopeptide **11** as the acceptor, SGP as the glycosyl donor, and Endo-M (Fig. 6). Surprisingly, synthesis from **11** gave 28% yield of the transglycosylated product after 4 h (Fig. 7). The bioactivities of vapreotide and the transglycosylated product were similar.¹²

Studies of further application of the pseudo glycopeptide method are now in progress.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2011.09.121.

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