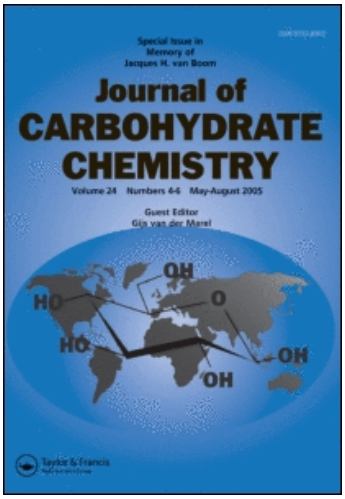

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Regioselective Synthesis of β-D-Gal-(1⟶3)-D-Glcnac Using β-Galactosidase from *Xanthomonas Manihotis*

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COMMUNICATION

REGIOSELECTIVE SYNTHESIS OF β-D-Gal-(1→3)-D-GlcNAc USING β-GALACTOSIDASE FROM *XANTHOMONAS MANIHOTIS*

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The carbohydrate chains of glycoconjugates are involved in a variety of molecular recognition events. For example, the tetrasaccharide sialyl Lewis a (sLe^a) plays a pivotal role in the metastasis of cancer cells.[1] In order to elucidate the biological function of carbohydrate chains, many researchers have synthesized components of carbohydrate chains. 2-Acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyl)-D-glucopyranose (**1**) is an important constituent of sLe^a in complex type carbohydrate chains. This disaccharide has been synthesized using the transglycosylation activity of bovine testes β-galactosidase.[2] In this case the product mixtures contained unwanted isomers and had to be treated with *Escherichia coli* β-galactosidase in order to hydrolyze the undesired isomers. Eventually, **1** was obtained in 12 % yield. This method is far from ideal as it requires two steps to obtain **1** and bovine testes are expensive and not easily available.

In this paper, I describe the synthesis of β-D-Gal-(1→3)-D-GlcNAc (**1**) using β-galactosidase from *Xanthomonas manihotis*. There were two reasons for the choice of this enzyme source: 1) it is easy to culture a large amount of *X. manihotis*, which is a gram-negative bacterium and 2) this enzyme hydrolyzes two linkages in the following order of preference; β-D-Gal-(1→3)-D-GlcNAc >> β-D-Gal-(1→4)-D-GlcNAc.[3,4] In previous studies, the regioselectivity of transglycosyation reactions has been shown to

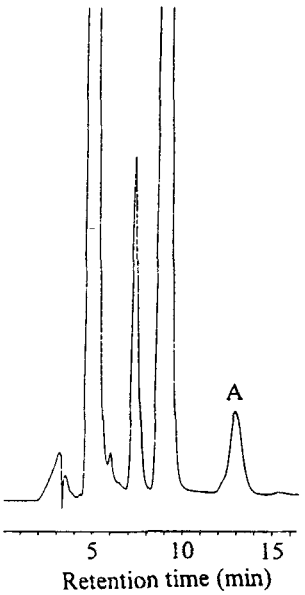

Figure 1. HPLC of the transglycosylation reaction.

be strongly related to the specificity of hydrolysis.[5,6] Therefore, the transglycosylation products were expected to be regioselective and provide the β1→3 linkage product preferentially.

p-Nitrophenyl β-D-galactopyranoside (pNP-β-Gal) was used as the galactose donor and 2-acetamido-2-deoxy-D-glucopyranose (GlcNAc) was used as the acceptor in the first transglycosylation reaction. The HPLC plot of the reaction mixture is shown in Figure 1. A new peak A was determined to be disaccharide (**1**) by comparing its ^{13}C NMR spectral data with that reported in the literature.[7] No other disaccharide was observed in the reaction mixture. After purification using an activated carbon column, **1** was isolated in a yield of 22.4%. This yield was twice that obtained using Hedbys's method.[2]

When 2-acetamido-2-deoxy-D-galactopyranose (GalNAc) was used as the acceptor instead of GlcNAc, a different result was obtained. After purification using an activated carbon column, the disaccharide fractions were concentrated. The β1→3 and β1→6 linked disaccharides were obtained in a combined yield of 24.7%. According to

^{1}H NMR spectral analysis, the major product was β-D-Gal-(1→3)-D-GalNAc (**2**) and the minor product was β-D-Gal-(1→6)-D-GalNAc (**3**) by comparison with the literature data.[7] The ratio of **2** to **3** was 7:3. This result was similar to that obtained using β-galactosidase from bovine testes.[8]

In conclusion, only the β1→3 transfer product was formed when GlcNAc was used as the acceptor, whereas both the β1→3 and β1→6 transfer products were obtained when GalNAc was used the acceptor. Therefore, the regioselectivity of galactosyl transfer catalyzed by β-galactosidase from *X. manihotis* appears to be dependent on the structure of the acceptor. β-Galactosidase from *X. manihotis* which selectively cleaves β1→3 linked galactooligosaccharides, should be a useful tool not only in sequence determination variety of glycoconjugate but also for the regioselective synthesis of **1**. I plan to use this disaccharide in the construction of sLe^a or complex type sugar chains.

EXPERIMENTAL

General methods. β-Galactosidase (EC 3.2.1.23) from *X. manihotis* was the product of New England Biolab (USA). Oligosaccharides in the transglycosylation reaction were analyzed by HPLC using a Pharmacia P-3500 system equipped with an Asahipak NH2P50 column (4.6 x 250 mm, Showa Denko) and UV-monitor (215 nm). Elution was performed at the flow rate of 0.8 mL/min using 75% acetonitrile. The ^{1}H and ^{13}C NMR spectra were measured at 500 and 125 MHz, respectively, on a Varian Unity-500 spectrometer using D_2O as solvent and a small amount of acetonitrile (δ2.00 for ^{1}H spectra and 1.27 ppm for ^{13}C spectra) as the internal standard.

β-D-Gal-(1→3)-D-GlcNAc (1). A reaction mixture consisting of 72 mg of pNP-β-Gal, 160 mg of GlcNAc, and 20% (v/v) of acetonitrile, and β-galactosidase (210 units) in 0.1 M sodium acetate buffer (pH 5.0, 1 mL) was incubated at 37 °C. After 5 h, the enzyme was denatured by heating the mixture in boiling water for 5 min. The reaction mixture was applied to an activated carbon column (1.6 x 40 cm). The transfer product was eluted using a gradient from zero to 30% aqueous ethanol solution (1 L each), at the flow rate of 2 mL/min. The eluent was collected in 20 mL fractions and the amount of

sugar was measured by the phenol-sulfuric acid method.[9] After concentration of the disaccharide fractions, 20.5 mg of **1** was obtained (22.4% yield).

β-D-Gal-(1→3)-D-GalNAc (2) and β-D-Gal-(1→6)-D-GalNAc (3). A reaction mixture consisting of 72 mg of pNP-β-Gal, 160 mg of GalNAc, and 20% (v/v) of acetonitrile, and β-galactosidase (200 units) in 0.1 M sodium acetate buffer (pH 5.0, 1 ml) was incubated at 37 °C. After 5 h, the enzyme was denatured by heating the mixture in boiling water for 5 min. The reaction mixture was applied to an activated carbon column (1.6 x 40 cm). The transfer product was eluted using a gradient from zero to 30% aqueous ethanol solution (1 L each), at the flow rate of 2 mL/min. The eluent was collected in 20 mL fractions. After concentration of the disaccharide fractions, 22.6 mg of Gal-GalNAc was obtained for a combined yield of 24.7%. The ratio of **2** and **3** was 7:3 by ^{1}H NMR spectral analysis.

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