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An Efficient Synthesis of a Galβ1-3GalNAc-Serine Derivative Using β-Galactosidase

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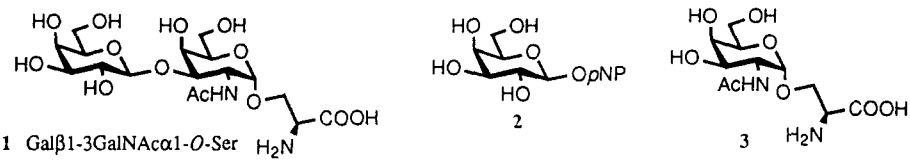
Abstract: An efficient route for the synthesis of Galβ1-3GalNAcα1-*O*-Serine derivative was developed using a novel β1-3 galactosidase obtained from *Bacillus circulans*. By a transglycosylation reaction with *p*NP-β-Gal **2** as a donor and GalNAcα1-*O*-Z-Ser-OAll **4** as an acceptor, Galβ1-3GalNAcα-1-*O*-Z-Ser-OAll **5** was synthesized in 68% yield. © 1997, Elsevier Science Ltd. All rights reserved.

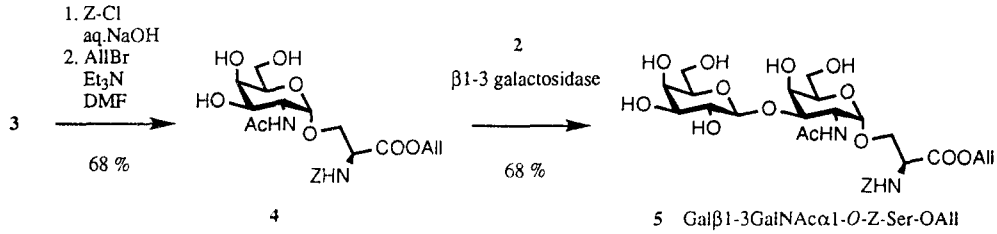
Development of an efficient procedure for the large-scale synthesis of oligosaccharides is necessary for the study of the biological phenomena mediated by glycoconjugates. Although a number of chemical synthetic procedures have been developed,[1] large-scale synthesis of the oligosaccharides in glycoconjugates is still difficult due to the multiple protection and deprotection steps required. Enzymatic synthesis using glycosyltransferases,[2] the biocatalysts responsible for the construction of oligosaccharides *in vivo*, is efficient, but the necessary glycosyltransferases and sugar nucleotides are too expensive to be practical for use in large-scale synthesis. In contrast, the synthesis of oligosaccharide-blocks using readily available and inexpensive glycosidases is increasingly being looked to as a practical alternative for the synthesis of glycoconjugates.[3] Recently, we succeeded in obtaining a large amount of a new β-galactosidase, by cloning the corresponding gene from a strain of *Bacillus circulans* and expressing it in *Escherichia coli* TG1.[4] This enzyme hydrolizes β1-3 linked galactosyl oligosaccharides preferentially, with only a slight activity towards β1-4 and β1-6 bonds. There are two general classes of glycosylation reaction mediated by glycosidases: reverse hydrolysis reaction and transglycosylation reaction.[5] We were able to synthesize Galβ1-3GalNAc or Galβ1-3GlcNAc using the transglycosylation activity of this enzyme (β1-3 galactosidase).[6] In this communication, we describe an efficient synthesis of the Galβ1-3GalNAcα1-*O*-Ser derivative **1**, a core structure of mucin-type glycopeptides.



Scheme 1

GalNAcα1-*O*-Ser **3** was synthesized by reverse hydrolysis from GalNAc and serine in the presence of *N*-acetyl-α-D-galactosaminidase.[7] The β1-3 galactosidase was prepared from the cells of a recombinant *E. coli* strain harboring the necessary gene. Following ultrasonic destruction of the cells, centrifugation and concentration, the activity of β1-3 galactosidase in the preparation was found to be 1.3 units / ml.

First, the transglycosylation of *p*NP-β-Gal **2** to GalNAcα1-*O*-Ser **3** was examined using the β1-3 galactosidase from *B. circulans*. The enzyme solution was added to a solution of GalNAcα1-*O*-Ser **3** and *p*NP-β-Gal **2** in 20% DMF-80% potassium phosphate buffer (KPB, pH 6.0, 0.1M). The reaction mixture was incubated at 37°C and monitored on a HPLC using a UV detector (215 nm) and a NH_2-column (CAPCELL PAK). After 3 hours the reaction had produced two small peaks but thereafter proceeded no further. The yield of the transglycosylation product was very low. We reasoned that the low reactivity and regioselectivity of this transglycosylation reaction might be due to the carboxyl and/or amino group of the serine residue interacting with the enzyme.



Scheme 2

Therefore, we decided to use a serine derivative in which the amino and carboxyl groups were protected as the acceptor in the transglycosylation reaction. The protection of the amino and carboxyl groups was performed by the procedure shown in Scheme 2. Treatment of GalNAcα1-*O*-Ser **3** with Z-Cl in aqueous NaOH afforded GalNAcα1-*O*-Z-Ser. Reaction of GalNAcα1-*O*-Z-Ser with allylbromide in the presence of triethylamine gave GalNAcα1-*O*-Z-Ser-OAll **4** in 77% yield. Transglycosylation using GalNAcα1-*O*-Z-Ser-OAll **4** and *p*NP-β-Gal **2** in the presence of β1-3 galactosidase from *B. circulans* gave Galβ1-3GalNAcα1-*O*-Z-Ser-OAll **5** as a single product in 68% yield.[8] The product was detected by HPLC (Figure 1). The structure of the product was determined by ^1H and ^{13}C NMR spectroscopy.[9] The β1-3 linkage was confirmed by an NOE interaction between the H-1' and H-3 protons (Figure 2).

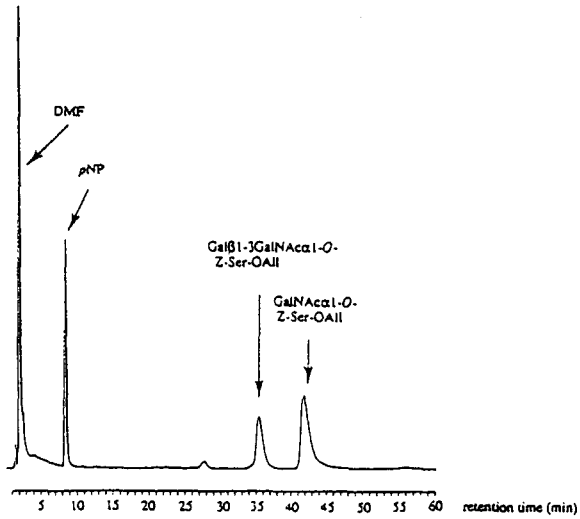


Figure 1. HPLC separation of the reaction mixture obtained using β1-3 galactosidase. Column: Waters AccQ TAG, 3.9 x 150 mm. Mobile phase: 30% MeOH-70% water. Flow rate 0.8 ml min^{-1}. Detector: UV (215 nm)

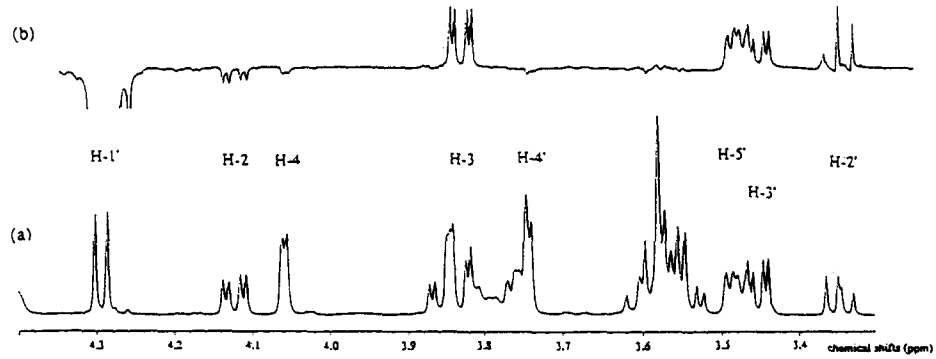


Figure 2. ^{1}H-NMR spectra for Galβ1-3GalNAcα1-*O*-Z-Ser-OAll: (a) 1D spectrum of Galβ1-3GalNAcα1-*O*-Z-Ser-OAll. (b) Differential NOE spectrum irradiated at H-1'.

In summary, we have developed an efficient synthesis of a Galβ1-3GalNAcα1-*O*-Ser derivative, an important synthon of mucin-type glycopeptides. Our investigations revealed that although changing the aglycon of the acceptor sugar was not beneficial, chemical protection of the serine resulted in a dramatic increase in reactivity and yield. The galactosylation of GalNAcα1-*O*-Z-Ser-OAll is exceptionally high yielding for a galactosidase mediated transglycosylation and should be useful for large-scale synthesis. We believe that this synthetic route will prove to be of great importance to those wishing to synthesize mucin-type glycopeptides on a large scale.

Acknowledgement

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8. Preparative synthetic procedure is as follows. The β1-3 galactosidase solution (100μl, 0.13 unit) was added to the 20% DMF-80% KPB buffer (600μl, pH 6.0, 0.1M) containing *p*NP-β-Gal (10.7mg, 35.3μmol) and GalNAcα1-*O*-Z-Ser-OAll (51.1mg, 106μmol). The mixture was incubated for 270 min at 37°C. After the reaction was stopped by heating at 100°C for 5 min, the solution was filtered and fractionated by HPLC (column: JAIGEL ODS A-343-10, 20 x 250mm; eluent: 30% MeOH-70% H_2O for 100min then 50% MeOH-50% H_2O; flow rate: 7 ml min^{-1}; detection: UV at 215 nm).
9. Selected spectral data for Galβ1-3GalNAcα1-*O*-Z-Ser-OAll **5**: ^1H NMR (D_2O) δ 4.81 (d, $J_{1,2}$=3.17Hz, H-1), 4.51 (br s, Ser α), 4.40 (d, $J_{1',2'}$=7.81Hz, H-1'), 4.23 (dd, $J_{2,3}$=10.99Hz, H-2), 4.17 (d, $J_{3,4}$=2.93Hz, H-4), 3.94 (dd, $J_{3,4}$=3.17Hz, H-3), 3.85 (d, $J_{3',4'}$=2.93Hz, H-4'), 3.59 (m, H-5'), 3.56 (dd, $J_{2',3'}$=10.01Hz, H-3'), 3.46 (dd, H-2'), 1.93 (s, Ac); ^{13}C NMR (D_2O) δ 103.57 (C-1'), 97.03 (C-1), 75.87 (C-3), 73.96 (C-5'), 71.49 (C-3'), 69.88 (C-5), 69.58 (C-2'), 67.55, 67.52 (C-4,4'), 60.06, 59.94 (C-6,6'), 47.51 (C-2).

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