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Glycosylation of N^{α}-lauryl-*O*-(β-D-xylopyranosyl)-L-serinamide as a saccharide primer in cells

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ABSTRACT

N^{α}-Lauryl-*O*-(β-D-xylopyranosyl)-L-serinamide (Xyl-Ser-C12) was synthesized as a saccharide primer to obtain oligosaccharides of glycosaminoglycan using the glycan biosynthetic potential of mouse osteosarcoma FBJ-S1 cells and Chinese hamster ovary (CHO) cells. The glycosylated products secreted into the culture medium were collected and analyzed by liquid chromatography–mass spectrometry and glycosidase digestion. The structure of the Xyl-Ser-C12 derivatives was investigated. Several glycosaminoglycan-type oligosaccharides, such as GalNAc-(GlcA-GlcNAc)$_n$-GlcA-Gal-Gal-Xyl-Ser-C12, were detected, and identified as intermediates of the biosynthesis of heparan sulfate glycosaminoglycans. Xyl-Ser-C12 exhibited greater acceptor activity for the glycosylation of glycosaminoglycan-type oligosaccharides than *p*-nitrophenyl-β-D-xylopyranoside.

1. Introduction

Oligosaccharides, which play an important role in cellular functions, such as recognition, adhesion, and division, are attracting increasing interest in biotechnology and drug development.[1] This expanding interest has led to an increased demand for the construction of saccharide libraries to provide research materials. Several strategies involving direct extraction from natural resources, organic synthesis, and enzymatic synthesis, have been developed in order to construct oligosaccharide libraries.[2] Recently, a way to obtain oligosaccharides by administering saccharide primers to animal cells has been established. Dodecyl β-lactoside (Lac-C12),[3–5] dodecyl-acetamido-2-deoxy-β-D-glucopyranoside (GlcNAc-C12), dodecyl-β-D-galactopyranosyl-(1(4)-2-acetamido-2-deoxy-β-D-glucopyranoside (LacNAc-C12),[6] and azidododecyl β-lactoside (Lac-C12N3)[7] have been developed as saccharide primers to produce glycan libraries involving ganglio-, globo-, and neolacto-series oligosaccharides.

Glycosaminoglycans (GAGs) are known to participate in specific biological and physiological functions in cancer progression, angiogenesis, cell adhesion, and anticoagulation.[8,9] However, due to the heterogeneity and diversity of GAG structures, the relationship between their biological function and structure is not well understood.[10] Therefore, it is important to develop a method of reconstructing GAG oligosaccharides and to know the GAG biosynthetic pathway in cells for understanding their structure–function relationships.

The biosynthesis of GAGs is initiated by the formation of a tetrasaccharide linkage region composed of glucuronic acid-galactose-galactose-xylose (β-GlcA-(1→3)-β-Gal-(1→3)-β-Gal(1→4)-β-Xyl-), where Xyl is attached to a serine residue in the core protein.[10] It has been found that exogenous β-D-Xylosides such as *p*-nitrophenyl-β-D-xylopyranoside[11,12] (Xyl-pNP), methylumbelliferyl-β-D-xylopyranoside (Xyl-MU),[13] β-estradiol xylopyranoside,[14] and naphthol xyloside derivatives (XylNapOH)[15–17] could act as artificial initiators for the elongation of glycosaminoglycan chains, and primed the synthesis of glycosaminoglycan-type oligosaccharides in several types of cells. Furthermore β-D-xylosides have been found to inhibit the endogenous biosynthesis of glycosaminoglycan.[12,18]

In the present study, a novel saccharide primer, N^{α}-lauryl-*O*-(β-D-xylopyranosyl)-L-serinamide (Xyl-Ser-C12, Fig. 1), was employed as an initiator to synthesize glycosaminoglycan (GAG) oligosaccharides using the GAG biosynthesis system of cells. Xyl-Ser-C12, mimicking the region where xylose attached to the specific serine residues of the core protein, was suggested to prime the elongation of GAG chains. The glycosylation of Xyl-Ser-C12 by mammalian cells was examined.

2. Results

2.1. Chemical synthesis of Xyl-Ser-C12

The chemical synthesis of N^{α}-lauryl-*O*-(β-D-xylopyranosyl)-L-serinamide **(5)** was shown in Scheme 1. 2,3,4-Tri-*O*-acetyl-D-xylopyranosyl-trichloroacetimidate **(1)** served as the glycosyl donor. Glycosylation of **1** with *N*-α-carbobenzoxy-L-serinamide

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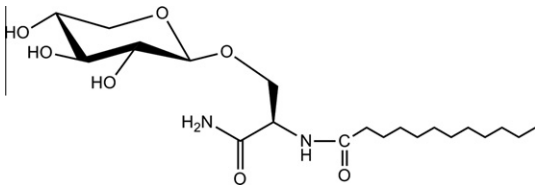


Figure 1. The structure of N^{α}-lauryl-*O*-(β-D-xylopyranosyl)-L-serinamide (Xyl-Ser-C12).

(Z-Ser-NH_2) was carried out in the presence of $BF_3{\cdot}Et_2O$. The obtained product **2** was considered to be an orthoester, and was subjected to TMSOTf-catalyzed isomerization to obtain β-anomer **3** predominantly. Conversion of **3** into **4** was carried out in a two-step synthesis involving the removal of a carbobenzyloxy group (Cbz) by catalytic hydrogenation over Pd/C and condensation with lauric acid in 41% overall yield for the two steps. Finally, N^{α}-lauryl-*O*-(β-D-xylopyranosyl)-L-serinamide **(5)** was obtained by *O*-deacetylation with methanolic NaOMe and purification by silica gel chromatography.

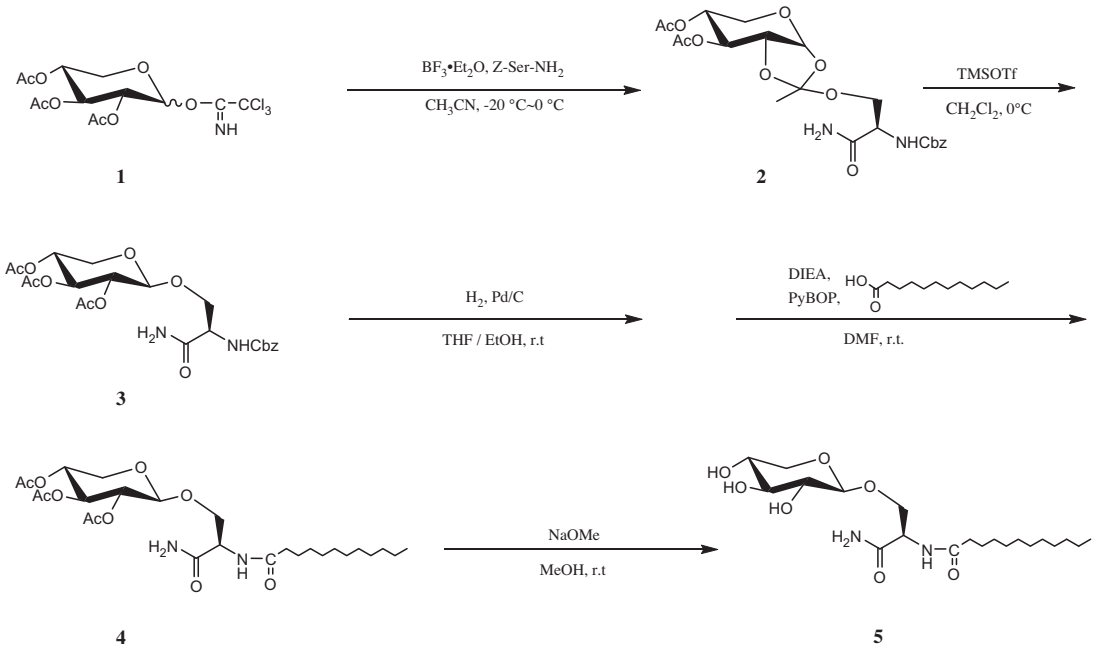
2.2. Glycosylation of Xyl-Ser-C12 by FBJ-S1 cells

The murine osteosarcoma cell line FBJ-S1[19,20] was employed to investigate the extent of the glycosylation of Xyl-Ser-C12. FBJ-S1 cells were incubated with 50 μM Xyl-Ser-C12 for 48 h, and the glycosylated products were isolated from the culture medium using a Sep-Pak C18 cartridge. These products were separated into neutral and acidic fractions with an aminopropyl SPE cartridge. The structures of the neutral and acidic products were analyzed by LC–MS. As shown in Table 1, two neutral products and six acidic products were detected and their structures were predicted based on MS/MS assignments (Tables 1 and 2 and Supplementary Fig. 1) and glycosidase digestion (Supplementary Fig. 3).

2.3. Analyses of the structures of the glycosylated products derived from FBJ-S1 cells

As shown in Table 1, two neutral products with *m*/*z* 615.9 $[M+Cl]^-$ (SXN1) and 777.9 $[M+Cl]^-$ (SXN2) were detected. MS/MS spectra of SXN1 revealed peaks at *m*/*z* 178.7 corresponding to the C_1 fragment ion indicating that SXN1 was Hex-Xyl-Ser-C12. The C_2 fragment ion with *m*/*z* 340.6 in the MS/MS spectra of SXN2, suggested the existence of two hexose residues in SXN2 (Table 2). The non-reducing hexose residues of SXN1 and SXN2 were cleaved by an exo-β-galactosidase (Supplementary Fig. 3A). Thus, SXN1 and SXN2 were identified as Galβ-Xyl-Ser-C12 and Galβ-Galβ-Xyl-Ser-C12, respectively. Six products with *m*/*z* 659.7 $[M-H]^-$, 870.8 $[M-H]^-$, 1032.9 $[M-H]^-$, 917.9 $[M-H]^-$, 1120.8 $[M-H]^-$, and 749.4 $[M-2H]^{2-}$ were detected in the acidic fraction (Table 1). SXA1 with *m*/*z* 659.7 was suggested to be the product having a sulfated group in Gal-Xyl-Ser-C12. The B_1 fragment with *m*/*z* 240.7 of SXA1 showed that the Gal residue was sulfated (Table 2). SXA2 was considered to be NeuAc-Gal-Xyl-Ser-C12 based on the B_1 fragment (*m*/*z* 290.2) and the B_2 fragment (*m*/*z* 451.5). SXA3 was deduced to be NeuAc-Gal-β-Gal-β-Xyl-Ser-C12 from the B_1 fragment (*m*/*z* 289.9) and the B_2 fragment (*m*/*z* 451.5). Both of the *N*-acetylneuraminic acid residues of SXA2 and SXA3 were cleaved by α-(2→3)-neuraminidase (Supplementary Fig. 3B). Thus, linkages of *N*-acetylneuraminic acid to galactose in SXA2 and SXA3 were determined to be α-(2→3). SXA4 was deduced to be GlcA-β-Gal-β-Gal-β-Xyl-Ser-C12, based on the C_2 fragment with *m*/*z* 354.8 and the digestion by β-glucuronidase (Supplementary Fig. 3C). In the MS/MS spectra of SXA5, the elongation of HexNAc and HexA to SXN2 was indicated by the Z_4 fragment with *m*/*z* 899.6 and the Y_3 fragment with *m*/*z* 742.0 (Table 2). The structure of SXA5 was considered as HexNAc-HexA-Gal-β-Gal-β-Xyl-Ser-C12. SXA6 with *m*/*z* 749.4 $[M-2H]^{2-}$ was determined to be $(HexNAc\text{-}GlcA)_2$-Gal-β-Gal-β-Xyl-Ser-C12 from the Z_6 fragment with *m*/*z* 1278.5 and the Y_5 fragment with *m*/*z* 1120.6 (Table 2).

To identify the isomer of the non-reducing HexNAc in SXA5 and SXA6 and the linkage between the HexNAc and HexA, digestion experiments using exo-type β-acetylhexosaminase and α-acetylgalactosaminidase were carried out. Since SXA5 and SXA6 were not digested by β-acetylhexosaminase, it was suggested that HexNAc was conjugated to HexA by an α-linkage (Supplementary Fig. 3D). Furthermore, since SXA5 and SXA6 were also hydrolyzed by α-acetylgalactosaminidase, HexNAc of the non-reducing end was suggested to be α-GalNAc (Supplementary Fig. 3E). The major product was SXA5 (GalNAc-α-GlcA-β-Gal-β-Gal-β-Xyl-Ser-C12).



Scheme 1. Synthesis of N^{α}-lauryl-*O*-(β-D-xylopyranosyl)-L-serinamide (Xyl-Ser-C12).