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Evaluation of fluorescently labeled xylopyranosides as probes for proteoglycan biosynthesis

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Abstract—A new fluorescent analog to the antiproliferative 2-(6-hydroxynaphthyl)-β-D-xylopyranoside has been synthesized and tested on a T24 cell line. The new analog was efficiently uptaken by the T24 cells but did not initiate priming of GAG chains. The results are similar to other fluorescently labeled analogs and we propose that these compounds are too large and unpolar to efficiently function as GAG-primers.

Proteoglycans, composed of glycosaminoglycan (GAG) chains covalently attached by a xylose residue to a core protein, are widely expressed in invertebrate and vertebrate tissues. Many biological functions of proteoglycans are due to interactions between GAG chains and a variety of pathogens and molecules, such as prion protein, viruses, growth factors, cytokines, and factors involved in blood coagulation.[1–3]

The first step of the GAG biosynthesis is xylosylation of a serine residue. A specific linker tetrasaccharide, GlcA(β1–3)Gal(β1–3)Gal(β1–4)Xylβ, is then assembled and serves as an acceptor for elongation of GAG chains (Fig. 1). Addition of GlcNAc or GalNAc to the nonreducing terminal GlcA residue determines whether heparan sulfate (HS) or chondroitin sulfate/dermatan sulfate (CS/DS) is initiated. It is still unclear what determines whether HS or CS/DS chains are attached to the core protein but repetitive Ser-Gly sequences and a high proportion of Phe, Tyr, or Trp promote the formation of HS.[4]

Xylose is an unusual structural component in mammalian cells and it has, so far, only been found in one unique position, that is, as the linker between protein and carbohydrate in proteoglycans. Interestingly, biosynthesis of GAG chains can also take place independently of core protein by using xylopyranosides as primers. Xylopyranosides with hydrophobic aglycons can penetrate cell membranes and initiate GAG synthesis by serving as acceptors in the first galactosylation step.[5–8] The composition of the GAG assembled on the xyloside primer depends on the structure of the aglycon, which may reflect selective partitioning of primers into different intracellular compartments or into different branches of biosynthetic pathways. In most cases, priming of CS dominates and synthesis of free HS chains is low or undetectable. Increased yields of HS can be obtained when the aglycon of the xylopyranosides comprises aromatic, polycyclic structures, such as naphthol-derivatives.[9]

The xyloside-primed GAG chains can be retained inside the cells but are usually mainly secreted into the medium. β-D-Xyloside primed HS chains have interesting biological properties, such as activation of basic fibroblast growth factor,[10] antithrombotic effects[11] growth inhibition of transformed cells,[8,12–14] and inhibition of scrapie prion protein infectivity.[15]

It has previously been shown that the GAG-priming 2-(6-hydroxynaphthyl)-β-D-xylopyranoside **1** (Fig. 2) selectively inhibits growth of transformed or tumor-derived cells in vitro as well as in vivo. Treatment with this xyloside reduced the average tumor load by 70–97% in a SCID mice model.[13]

Keywords: Carbohydrates; Xylose; Fluorescent; Glycosaminoglycan; Proteoglycan.
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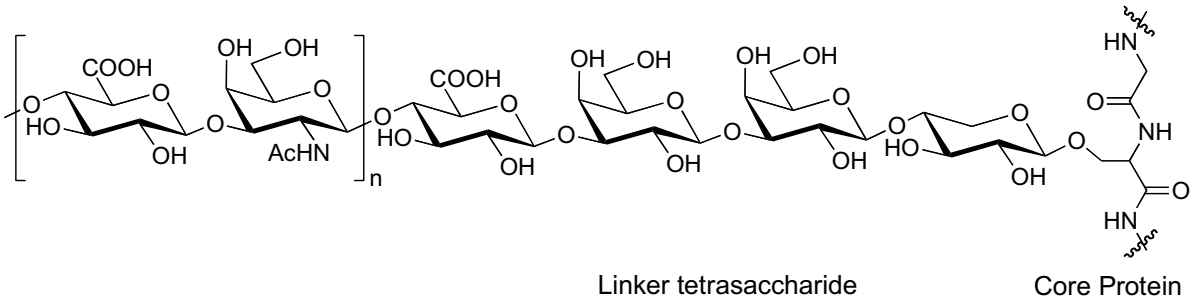


Figure 1. Glycosaminoglycan chains consist of a linker tetrasaccharide unit (GlcA(β1–3)Gal(β1–3)Gal(β1–4)Xylβ) coupled to serine residues of the protein chain.

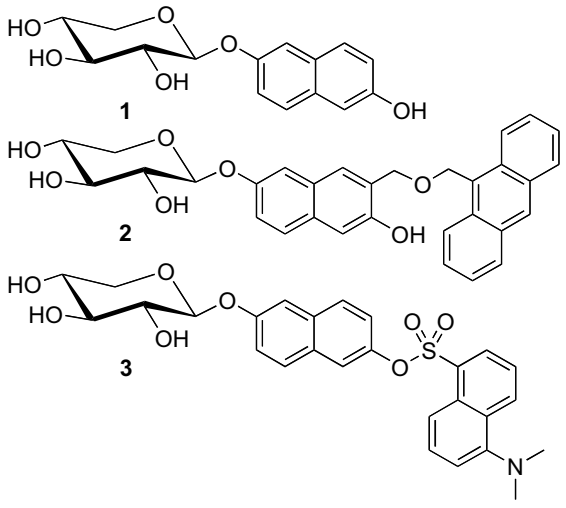


Figure 2. Compound **3** was synthesized as a fluorescent analog to **1**, and a more soluble analog compared to the recently described **2**.

A fluorescent analog (**2**) was previously synthesized and evaluated.[16] This molecule was highly lipophilic and was taken up by all cell lines tested but did not initiate GAG synthesis. Fluorescent probes are usually bulky molecules that alter the physical properties of the labeled compounds.[17] Dansyl (5-dimethylaminonaphthalene-1-sulfonyl) is a small and relatively polar fluorescent probe, which will alter the labeled compound to a less extent compared to other probes.

6-Acetoxy-2-naphthol[18] (**4**) was xylosylated with 1,2,3,4-tetra-*O*-acetyl-β-D-xylopyranoside in CH_2Cl_2 and NEt_3 with $BF_3{\cdot}OEt_2$ as promoter to give 2-(6-acetoxy-naphthyl)-2,3,4-tetra-*O*-acetyl-β-D-xylopyranoside (**5**) in 81% yield.[19] The addition of sub-equimolar amount of base has been shown to minimize anomerization of both starting material and product.[20] To selectively deprotect the more easily cleaved aryl-acetate it was anticipated to use a stoichiometric amount of strong base. Unfortunately NaOH in dioxane/H_2O gave low selectivity, that is, deacetylation of the carbohydrate residue. Other bases, such as LiOH and K_2CO_3, were tested with similar results. The deprotected product is probably more soluble in the solvent system compared to the starting material which therefore promote complete deacetylation. Deprotection using a catalytic amount of KCN in MeOH is a slow method where partly deacetylated carbohydrates have been isolated.[21] Reasoning that the aryl-acetate is more easily cleaved, this method was tested and gave 2-(6-hydroxynaphthyl)-2,3,4-tetra-*O*-acetyl-β-D-xylopyranoside (**6**) in a reasonable 45% yield. The free hydroxyl group was then coupled with dansyl chloride in CH_2Cl_2 with NEt_3 as base to give 2-(6-(5-dimethylamino-naphthalene-1-sulfonyloxy)-naphthyl)-2,3,4-tetra- *O*-acetyl-β-D-xylopyranoside (**7**) in 87% yield.[22] The product was finally deacetylated using standard Zemplén conditions (0.05 M NaOMe/MeOH) to give 2-(6-(5-dimethylamino-naphthalene-1-sulfonyloxy)-naphthyl)-β-D-xylopyranoside (**3**) in 95% yield.

Gradient HPLC retention times can be used to substitute log *P* values in biological evaluations.[23] The gradient HPLC retention times for compounds **1**, **2**, and **3** were measured using a C-18 column and a mobile phase of water (0.1% TFA) with a gradient of MeCN from 1 min increasing by 1.2% per min. The retention times were measured for three separate runs per compound, and the calculated mean retention times are presented in Table 1. Unfortunately, the dansyl moiety, despite the polar sulfonyl and dimethylaminogroups, makes compound **3** highly unpolar as reflected by the retention time. The absorbance spectrum of compound **3** (in MeCN) and fluorescence spectrum (in MeCN, λ_{Ex} = 353 nm) are presented in Figure 3.[24,25]

To investigate if compound **3** could be used as a probe for the study of proteoglycan biosynthesis the uptake as well as the GAG-priming capability were tested on transformed T24 cells. In order to study the uptake of the xyloside, T24 cells were treated with 0.1 mM of **3** for 6 h, then fixed, and internalization and localization was analyzed using fluorescence microscopy. As shown in Figure 4 compound **3** was located in intracellular compartments mainly to the para- and perinuclear regions indicating an efficient internalization of this compound (Scheme 1).

Table 1. Gradient retention times for compounds **1**, **2**, and **3**

Compound	Retention time (min)
1	17.73 ± 0.03
2	43.40 ± 0.02
3	38.48 ± 0.08

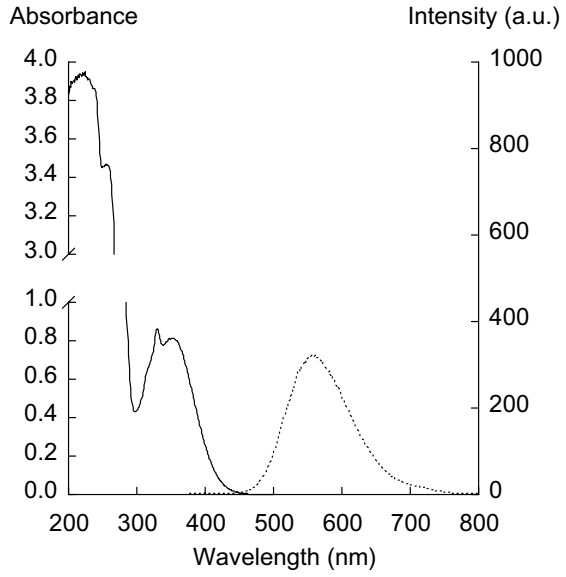


Figure 3. Absorbance spectrum of compound **3** (0.25 mM in MeCN, solid line) and fluorescence spectrum of compound **3** (0.25 mM in MeCN, λ_{Ex} = 353 nm, dotted line).

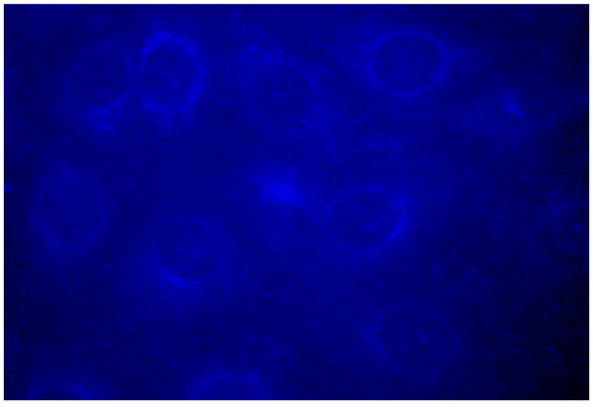
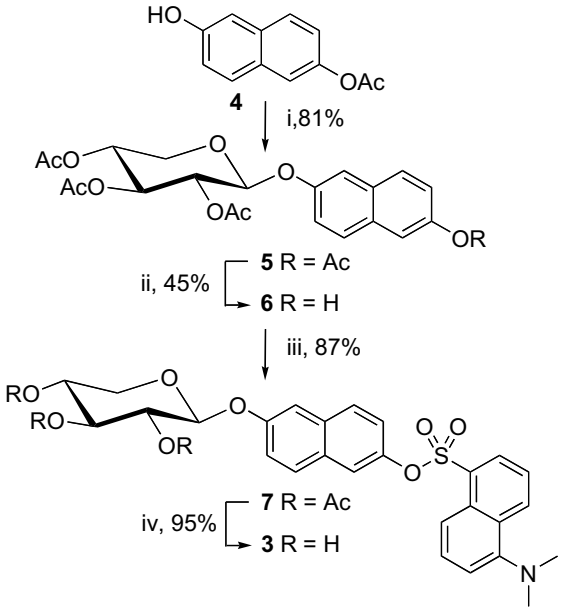


Figure 4. Uptake of compound **3** studied by fluorescence microscopy of T24 cells treated with 0.1 mM of compound **3** for 6 h.

In order to analyze the GAG-priming ability of compound **3**, T24 cells were left untreated or were treated with 0.1 mM xyloside and [^{35}S]sulfate over night and free GAG chains in the medium were isolated. Untreated cells secreted radiolabeled polyanionic material into the culture medium to some extent (Fig. 5). However treatment with compound **3** suppressed endogenous proteoglycan (pool I) and GAG (pool II) production and no xyloside primed GAG chains were recovered from the medium. There are several possible reasons why this compound did not initiate GAG chain synthesis, The structure of the aglycone and the structure of glycosidic linkage between aglycone and xyloside have been shown to play an important role for the priming ability and for the structure of the GAG chains synthesized by the xylopyranosides.[6,26] The compound used in this study may be internalized but not distributed to correct intracellular compartments to take part in GAG biosynthesis or may not be recognized by the GAG-priming enzymes. The results are similar to the earlier studied fluorescent analog **2**, and it may be assumed that



Scheme 1. Synthesis of compound **3**. Reagents and conditions: (i) 1,2,3,4-tetra-*O*-acetyl-β-D-xylopyranoside, NEt_3, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 135 min; (ii) KCN, MeOH, 0 °C, rt, 100 min; (iii) dansyl chloride, NEt_3, CH_2Cl_2, 90 min; (iv) MeOH/NaOMe, 30 min.

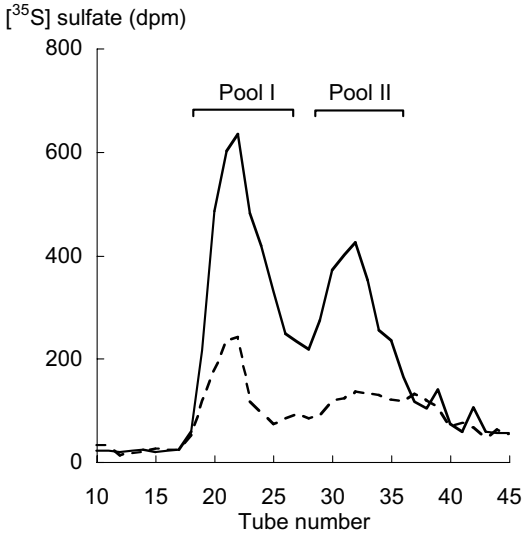


Figure 5. Secreted proteoglycan from T24 cells treated with compound **3**. Cells grown to confluence were labeled with [^{35}S]sulfate for 24 h in the absence (solid line) or presence (dashed line) of 0.1 mM of compound **3**.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.01.063.

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