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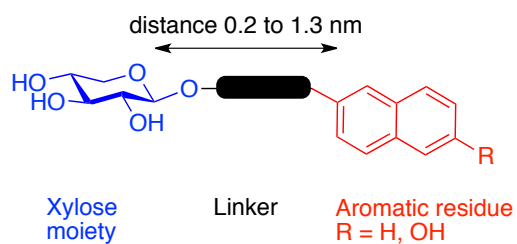
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## Graphical Abstract

### Synthesis and biology of oligoethylene glycol linked naphthoxyosides

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Karin Holmqvist, Andrea Persson, Richard Johnsson, Johanna Löfgren, Katrin Mani, Ulf Ellervik



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# Synthesis and biology of oligoethylene glycol linked naphthoxylosides

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## ABSTRACT

Proteoglycans (PGs) are important macromolecules in mammalian cells, consisting of a core protein substituted with carbohydrate chains, known as glycosaminoglycans (GAG). Simple xylosides carrying hydrophobic aglycons can enter cells and act as primers for GAG chain synthesis, independent of the core protein. Previously it has been shown that aromatic aglycons can be separated from the sugar residue by short linkers without affecting the GAG priming ability. To further investigate the effects of the xylose-aglycon distance on the GAG priming ability, we have synthesized xyloside derivatives with 2-naphthyl and 2-(6-hydroxynaphthyl) moieties connected to xylose, directly, via a methylene bridge, or with oligoethylene glycol linkers of three different lengths. The GAG priming ability and the antiproliferative activity of the xylosides, as well as the composition of the xyloside-primed GAG chains were investigated in a matched pair of human breast fibroblasts and human breast carcinoma cells. An increase of the xylose-aglycon distance from 0.24 nm to 0.37 nm resulted in an increased GAG priming ability in both cell lines. Further increase of the xylose-aglycon distance did not result in any pronounced effects. We speculate that by increasing the xylose-aglycon distance, and thereby the surface area of the xyloside, to a certain level would make it more accessible for enzymes involved in the GAG synthesis. The compositions of the primed GAG chains varied with different xylosides, independent of the xylose-aglycon distance, probably due to various affinities for enzymes and/or different cellular uptake. Furthermore, no correlations between the antiproliferative activities, the xylose-aglycon distances and the amounts or compositions of the GAG chains were detected suggesting involvement of other factors such as fine structure of the GAG chains, effects on endogenous PG synthesis, or other unknown factors for the antiproliferative activity.

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## 1. Introduction

Proteoglycans (PGs) are macromolecules, usually located in extracellular matrices or associated to cell-surfaces in mammalian cells. They are involved in a diversity of cellular processes such as cell development, differentiation, migration, adhesion, and proliferation. PGs consist of a core protein covalently substituted with one or more linear polyanionic carbohydrate chains, known as glycosaminoglycans (GAG). The GAG chains are assembled on the core protein by xylosylation of a serine residue, followed by attachment of two Gal residues and one GlcA residue, which together constitute a linker tetrasaccharide. Alternating disaccharides are then added to the linker tetrasaccharide, resulting in chondroitin sulfate/dermatan sulfate (CS/DS) or heparin sulfate (HS) GAG chains consisting of repeating -4GlcA $\beta$ 1-3GalNAc $\beta$ 1- or -4GlcA $\beta$ 1-4GlcNAc $\alpha$ 1-residues, respectively. Subsequent modifications of the

carbohydrate backbone, such as epimerization, *N*-deacetylation, and *O*- and *N*-sulfation result in a wide structural diversity, and thus, a considerable amount of biological functions of the GAG chains.<sup>1-3</sup>

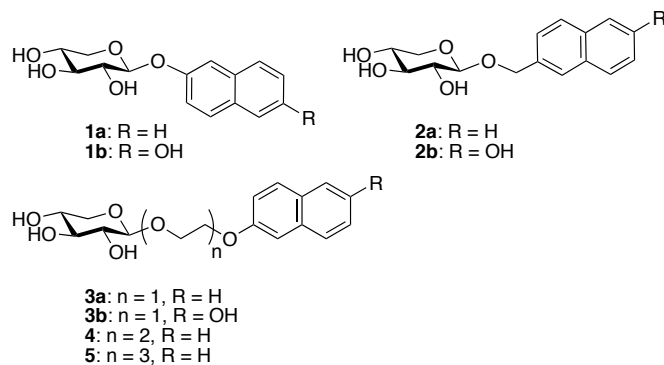
Xylose is an unusual carbohydrate in mammalian cells, hitherto only known to serve as an initiator of HS and CS/DS GAG synthesis on the PG core protein. Almost 40 years ago, it was shown that  $\beta$ -D-xylosides exogenously added to cells could act as primers for GAG formation. Xylose, when attached to various hydrophobic aglycons, is able to penetrate plasma membranes and compete with the xylosylated core protein for enzymes and substrates for GAG synthesis.<sup>4-5</sup> Xyloside-induced GAG priming occurs in a concentration dependent manner, but the efficacy varies widely among different cell types. These variations may be related to the structure of the aglycon, relative abundance of endogenous substrates, enzyme concentrations, the solubility of the xylosides, xyloside-uptake across the plasma

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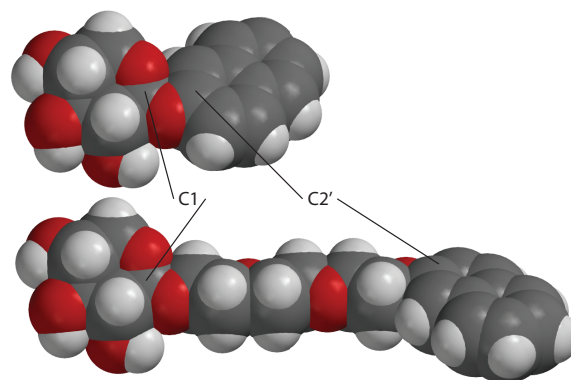
membrane and into the Golgi apparatus, and the relative affinity of the xylosides for the enzymes involved in the GAG synthesis. The biological effects of different xylosides have been investigated in various experimental systems. For example, inhibition of morphogenesis in various tissues, such as salivary glands,<sup>6</sup> embryonic mouse molars,<sup>7</sup> fetal mouse kidney,<sup>8</sup> and rat nervous tissue,<sup>9</sup> have been observed due to perturbed PG/GAG expression by *p*-nitrophenyl  $\beta$ -D-xyloside or 4-methylumbelliferyl  $\beta$ -D-xyloside treatment.

The spectrum of possible applications for xylosides is broad. For instance, they have been shown to exhibit (i) antiprion activities,<sup>10</sup> (ii) antithrombotic properties,<sup>11-12</sup> (iii) inhibiting effects on tumor invasion, (iv) beneficial effects on skin aging,<sup>13-14</sup> (v) enhancing spinal cord repair after injury,<sup>15</sup> (vi) antiangiogenesis effects,<sup>16</sup> and (vii) tumor selective antiproliferative activities<sup>17-19</sup>. Recently, we showed that 2-(6-hydroxynaphthyl)  $\beta$ -D-xylopyranoside, XylNapOH (**1b**, Figure 1), in contrast to 2-naphthyl  $\beta$ -D-xylopyranoside, XylNap (**1a**, Figure 1), selectively inhibited tumor growth both *in vitro* and *in vivo*.<sup>20</sup> XylNapOH-primed GAG chains from cancer cells were internalized both by cancer cells and healthy cells, resulting in apoptotic cells and thereby an antiproliferative effect. In contrast, XylNapOH-primed GAG chains from healthy cells were not internalized and lacked antiproliferative activity.



**Figure 1.** Structures of XylNap (**1a**), XylNapOH (**1b**), and analogs.

Earlier investigations on the biological effects of xylosides with aromatic aglycons indicate that the sugar residue can be separated from the aromatic aglycon by short linkers, without diminishing the GAG priming ability.<sup>21</sup> To systematically investigate the effects of the xylose-aglycon distance on the GAG priming ability, we have synthesized xylosides with the naphthyl moiety connected to the xylose directly, via a methylene bridge, or with oligoethylene glycol (OEG) linkers of different lengths (Figure 1). This increases the linker surface area of the xylosides and thereby making them more accessible for the enzymes involved in GAG biosynthesis. In addition, we have included 6-hydroxynaphthyl analogs, since the presence of a hydroxyl group in position 6 of the naphthol has been shown to result in significant anti-tumor effects.<sup>20</sup> Interestingly, we have previously shown that hydroxylation of only some of the positions in the aromatic system, e.g. position 6, leads to selective tumor toxicity.<sup>22</sup> Thus, we present a model system where the xylose residue is separated from the aromatic moiety, i.e. 2-naphthyl or 2-(6-hydroxynaphthyl), with linkers of different lengths, thus increasing the xylose-aglycon distance from approx. 0.2 up to 1.3 nm. OEG linkers were chosen since they are available in different lengths, they induce water solubility, and they are inexpensive. The model system, visualized by compounds **1a** and **5**, is shown in Figure 2.



**Figure 2.** Molecular models of compounds **1a** and **5**.

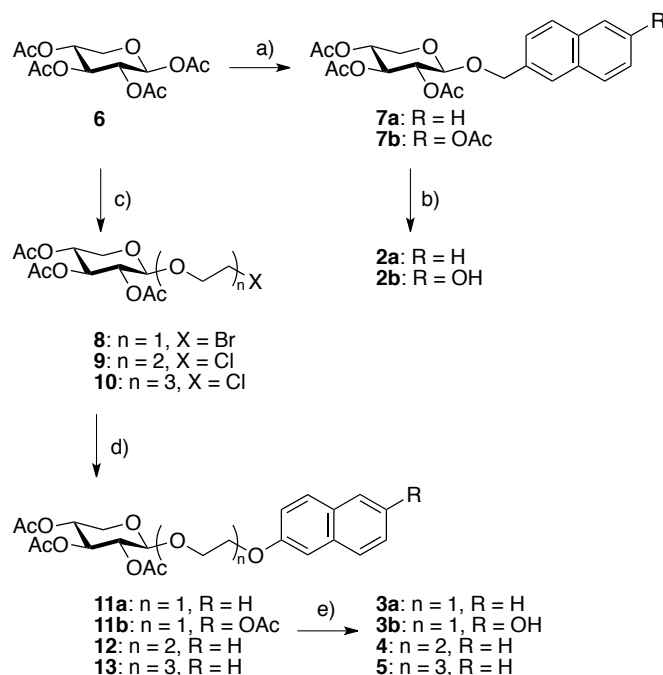
We have investigated the biological effects of the xylosides in terms of GAG priming ability and antiproliferative activity using human breast fibroblasts, CCD-1095Sk cells, and human breast carcinoma cells, HCC70 cells.

## 2. Results and discussion

### 2.1. Synthesis

Compounds **2a**<sup>23</sup>, **3a**<sup>21</sup>, and **8**<sup>24</sup> have been published before, however, full physical characterization has not been reported, and hence syntheses and analyses are described herein.

2-Naphthalenemethyl xylosides **2a-b** were synthesized from peracetylated xylose **6** via the acetate protected xylosides **7a-b** (Scheme 1). Synthesis of **7a** using 2-naphthalenemethanol was problematic, generating impurities inseparable from the desired product. Therefore, deacetylation using standard Zemplén conditions was performed yielding xyloside **2a** without isolation of compound **7a**. Formation of **7b** on the other hand was straightforward, using 6-acetoxy-2-naphthalenemethanol to obtain compound **7b** in 79% yield. Deacetylation of **7b** generated xyloside **2b** in good yield.



**Scheme 1.** Reagents and conditions: i) **6** (1.2 eq), 2-naphthalenemethanol (1 eq),  $\text{BF}_3 \cdot \text{Et}_2\text{O}$  (1.5 eq),  $\text{CH}_2\text{Cl}_2$ , 0 °C, **7a** (not isolated); **6** (1.2 eq), 6-acetoxy-2-naphthalenemethanol (1.5 eq),  $\text{BF}_3 \cdot \text{Et}_2\text{O}$  (1.5 eq),  $\text{CH}_2\text{Cl}_2$ , 0 °C,

**7b** (79%); ii) NaOMe (0.05 M), MeOH, **2a** (11% over two steps), **2b** (68%); iii) **6** (1.2 eq), 2-bromoethanol (1 eq), BF<sub>3</sub>·Et<sub>2</sub>O (1.5 eq), CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, **8** (69%); **6** (1.2 eq), 2-(2-chloroethoxy)-ethanol (1 eq), BF<sub>3</sub>·Et<sub>2</sub>O (1.5 eq), CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, **9** (43%); **6** (1.2 eq), 2-[2-(2-chloroethoxy)ethoxy]-ethanol (1 eq), BF<sub>3</sub>·Et<sub>2</sub>O (1.5 eq), CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, **10** (61%); iv) 2-Naphthol (2 eq), K<sub>2</sub>CO<sub>3</sub> (1.2 eq), DMF, 80 °C, **11a** (63%); 6-acetoxy-2-naphthol (2 eq), K<sub>2</sub>CO<sub>3</sub> (1.2 eq), DMF, 80 °C, **11b** (not isolated); 2-Naphthol (2 eq), K<sub>2</sub>CO<sub>3</sub> (1.2 eq), 18-crown-6 (10 eq), DMF, 80 °C, **12** (22%), **13** (29%); v) NaOMe (0.05 M), MeOH, **3a** (90%), **3b** (39% over two steps), **4** (73%), **5** (66%).

The syntheses of xylosides **3-5** started from peracetylated xylose **6**, which was reacted with OEG linkers of three different lengths to form compounds **8-10**. For the shortest linker, 2-bromoethanol was used. For the linkers containing diethylene glycol and triethylene glycol units, the bromide analogs were not commercially available, hence the chloride analogs were used. The coupling of 2-naphthol to **9** and **10**, respectively, to generate compounds **12** and **13** caused some difficulties, whereas the reaction to form **11a** was straightforward. This might have been a result of the weaker leaving group property of chloride compared to bromide. In addition, the longer OEG linker could theoretically coordinate a potassium ion to form crown ether-like complex. To avoid this, 18-crown-6 was added to the reaction mixtures, resulting in an increase in yield of **13** from trace amounts to approximately 30%. Furthermore, the reaction times for both **12** and **13** were considerably decreased, from 48 hours to 6 hours.

The coupling of 6-acetoxy-2-naphthol to **8** proceeded smoothly, but **11b** could not be isolated, since the aromatic acetate was cleaved off during the reaction and the mixture was thus deacetylated to obtain xyloside **3b**. Deacetylation of **11a**, **12**, and **13** generated xylosides **3a**, **4**, and **5** in good yields.

The distances between the anomeric carbon and the aromatic carbon connected to the linker were calculated using density functional theory at the B3LYP/6-31G\*\* level and default settings in Spartan '10 for Macintosh (Table 1).<sup>25</sup>

Ethylene glycol linkers are often preferred over aliphatic linkers due to the oxygen atoms that counterbalance the unpolar hydrocarbon parts of the linker. The gradient HPLC retention times, which can be used to substitute log *P* values,<sup>26</sup> were measured for the xylosides using a C-18 column and a mobile phase of water (0.1% TFA) with a gradient of MeCN from 1 minute increasing by 1.2% per minute (Table 1). The retention times increased with increasing xylose-aglycon distances, which probably is a combined effect of a slightly decreased polarity and increased surface area of the xylosides. The two series, naphthyl xylosides **1a**, **2a**, **3a**, **4**, and **5**, and hydroxynaphthyl xylosides **1b**, **2b**, and **3b**, show considerable differences in retention times, indicating the importance of the aromatic hydroxyl group on polarity.

**Table 1.** Retention times and xylose-aglycon distances. The retention times are the mean values of three separate measurements per compound. The xylose-aglycon distances are measured in nm from the anomeric carbon to the aromatic carbon coupled to the linker.

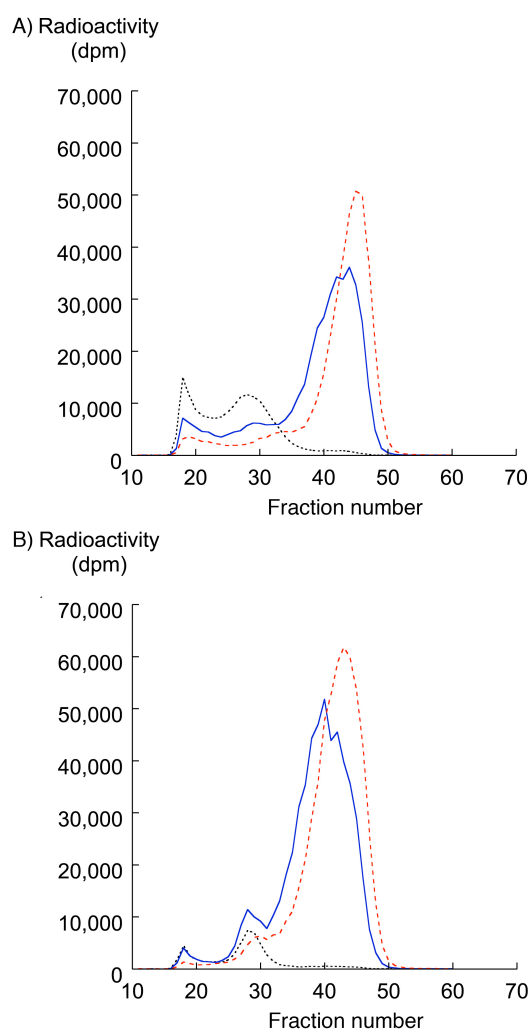
Compound	Retention times (min)	Xylose-aglycon distances (nm)
<b>1a</b>	32.25 ± 0.012	0.24
<b>2a</b>	33.39 ± 0.010	0.37
<b>3a</b>	35.61 ± 0.005	0.60
<b>4</b>	36.90 ± 0.004	0.95
<b>5</b>	37.70 ± 0.003	1.31
<b>1b</b>	22.26 ± 0.034	0.24
<b>2b</b>	25.97 ± 0.014	0.37
<b>3b</b>	27.22 ± 0.008	0.60

## 2.2. Biology

To investigate the ability of the xylosides to induce GAG priming, human breast fibroblasts (CCD-1095Sk cells) and human breast carcinoma cells (HCC70 cells) were treated with 100 μM of the xylosides in low-sulfate medium supplemented with [<sup>35</sup>S]sulfate for 24 h. [<sup>35</sup>S]Sulfate-labeled polyanionic material from culture media was then isolated by ion exchange chromatography and hydrophobic interaction chromatography. The isolated material was further separated based on size by gel permeation FPLC on a Superose 6 column.

**Table 2.** GAG priming and peak maximum. GAG priming is given as the integrated value of valid fractions from cells treated with xyloside, divided by the integrated value of valid fractions from untreated cells. Valid fractions are fractions 34-51 for CCD-1095Sk cells, and fractions 35-51 for HCC70 cells.

Compound	GAG priming		Peak maximum	
	CCD-1095Sk	HCC70	CCD-1095Sk	HCC70
<b>1a</b>	24	16	40	43
<b>2a</b>	55	31	40	43
<b>3a</b>	79	26	40	44
<b>4</b>	80	29	40	44
<b>5</b>	74	21	40	43
<b>1b</b>	74	37	42	44
<b>2b</b>	98	42	42	45
<b>3b</b>	90	29	43	45



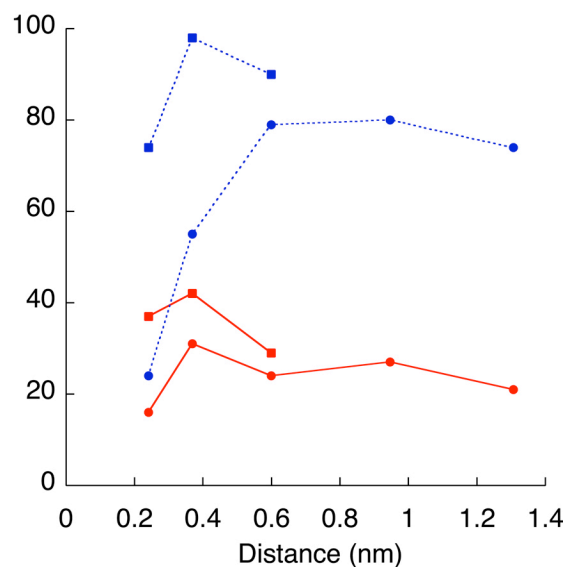
**Figure 3.** Example of size separation on Superose 6 column of secreted radiolabeled polyanionic material from (A) HCC70 cells and (B) CCD-1095Sk cells incubated with 100  $\mu$ M of **3a** (blue solid line), and **3b** (red dashed line) for 24 h. The black dotted line shows the result from untreated cells.

As shown in Table 2 and Figure 3, treatment with the xylosides resulted in GAG priming in both CCD-1095Sk cells and HCC70 cells (data can be found as supplementary data). The level and the Superose 6 profile of GAG priming for compounds **1a** and **1b** were in agreement with previously published data.<sup>18</sup> In general, the level of [<sup>35</sup>S]sulfate-incorporation was lower in untreated CCD-1095Sk cells than in untreated HCC70 cells. However, when treated with xylosides the level of [<sup>35</sup>S]sulfate-incorporation was higher in CCD-1095Sk cells than in HCC70 cells, indicating a higher level of GAG priming, or a higher level of sulfation of GAG chains in the CCD-1095Sk cells. In addition, the xyloside-primed GAG chains synthesized in CCD-1095Sk cells were, on average, somewhat longer than those synthesized in HCC70 cells (Table 2, see peak maximum). Furthermore, the primed GAG chains induced by the hydroxynaphthyl xylosides **1b**, **2b** and **3b**, were, on average, slightly shorter than those from the corresponding naphthyl xylosides **1a**, **2a**, **3a**, **4** and **5**, in both cell lines (Table 2, see peak maximum).

An increase in xylose-aglycon distance from 0.24 nm to 0.37 nm resulted in an increased GAG priming ability in both cell lines (Figure 4), probably due to an increased surface area of the xylosides, making them more accessible for the enzymes involved in the GAG synthesis. Further increase in the xylose-

aglycon distance did not enhance the level of primed GAG chains, except for the naphthyl xylosides in CCD-1095Sk cells where the level of GAG priming increased with increasing xylose-aglycon distance up to 0.60 nm and then reached a plateau. Such a trend was not found in HCC70 cells for naphthyl xylosides and these differences can be explained either by different GAG synthesis machinery in the two cell types, or by different uptake and/or internal distribution of the xylosides. In both cell lines the hydroxynaphthyl xylosides show a somewhat higher degree of priming, compared to the compounds without a 6-hydroxyl group.

#### Priming



**Figure 4.** GAG priming plotted against xylose-aglycon distance. The proportion of GAG priming is given as the integrated value of fractions from cells treated with xyloside, divided by the integrated values for fractions from untreated cells. The xylose-aglycon distances are measured in nm from the anomeric carbon to the aromatic carbon coupled to the linker. Circles indicate naphthyl xylosides, while squares indicate hydroxynaphthyl xylosides. Blue dotted lines are results from CCD-1095Sk cells, and red solid lines indicate results from HCC70 cells.

To investigate the composition of the xyloside-primed material, Superose 6 fractions 35-51 for HCC70 cells, as well as fractions 34-51 for CCD-1095Sk cells, were pooled and subjected to HNO<sub>2</sub> pH 1.5 degradation, followed by size separation on Superose 6 column. Remaining undigested material was either pooled and subjected to chondroitin ABC lyase digestion, followed by size separation on Superose 6, or presumed to contain only CS/DS (data not shown). As shown in Table 3, treatment with HNO<sub>2</sub> pH 1.5 resulted in partial degradation of the subjected material. Digestions with chondroitin ABC lyase resulted in complete degradation of the subjected material (data not shown). Together, this indicates that secreted xyloside-primed materials were composed of both HS and CS/DS GAG chains. The proportion of xyloside-primed HS was higher in HCC70 cells than in CCD-1095Sk cells, except for compound **5**. Furthermore, the hydroxynaphthyl xylosides resulted in higher proportions of HS priming compared to the naphthyl xylosides (Table 3). As determined from Tables 2 and 3, no correlation between the proportion of HS and the total amount of primed material could be observed. In addition, there was no obvious correlation between the xylose-aglycon distance and the proportion of HS.

**Table 3.** Proportion of HS. The proportion of HS is given as the integrated value of fractions with HS containing material, divided by the integrated value of fractions with the total material.

Compound	Proportion of HS of total GAGs (%)	
	CCD-1095Sk	HCC70
<b>1a</b>	3	9
<b>2a</b>	7	14
<b>3a</b>	3	9
<b>4</b>	2	5
<b>5</b>	20	15
Average for naphthyl xylosides	7	<b>10</b>
<b>1b</b>	6	28
<b>2b</b>	7	14
<b>3b</b>	26	34
Average for hydroxynaphthyl xylosides	<b>13</b>	<b>25</b>

Fritz and co-workers have previously shown that both compounds **1a** and **3a** induce priming of HS to the same extent in CHO cells.<sup>21</sup> They also showed that an increase of the distance between the aglycon and the xylose with an unpolar linker, i.e. 4-(2-naphthoxy)-1-butyl  $\beta$ -D-xyloside, resulted in decreased proportion of HS synthesis. This is in agreement with our data, since compound **4**, which similarly to their hydroxybutyl-linked xyloside also had a lower amount of HS compared to compounds **1a** and **3a**. In addition, they found no correlation between partitioning of xylosides in octanol-water and HS priming ability, which is also verified by our data.

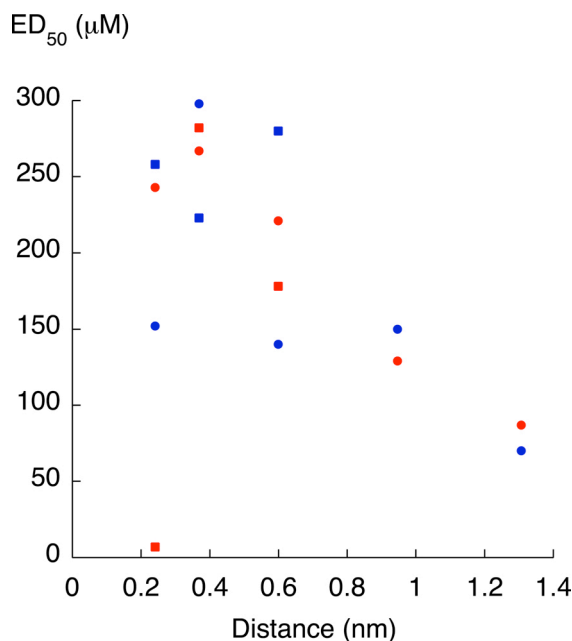
To determine the antiproliferative activity, CCD-1095Sk cells and HCC70 cells were incubated with increasing concentrations of the xylosides (5-500  $\mu$ M) for 96 h. Cell proliferation was recorded using the crystal violet method, and the antiproliferative effect expressed as ED<sub>50</sub> ( $\mu$ M) (Table 4).

**Table 4.** Antiproliferative activity. The antiproliferative activity was determined in CCD-1095Sk cells and HCC70 cells and expressed as ED<sub>50</sub> ( $\mu$ M).

Compound	Antiproliferative activity (ED <sub>50</sub> , $\mu$ M)	
	CCD-1095Sk	HCC70
<b>1a</b>	152	243
<b>2a</b>	298	267
<b>3a</b>	140	221
<b>4</b>	150	129
<b>5</b>	70	87
<b>1b</b>	258	<b>7</b>
<b>2b</b>	223	282
<b>3b</b>	280	178

Only compound **3b** and the previously published compound **1b** were selectively toxic towards the HCC70 cells. However, in these experiments the HCC70 cells were much less sensitive towards **3b** compared to **1b**. We have earlier shown **1b** to be tumor selective both *in vivo* and *in vitro* and in several different cell lines. Interestingly, in HCC70 cells the antiproliferative effects of most compounds were correlated to the xylose-aglycon distances, and thus the polarity (Figure 5). With the exception of

the tumor-selective compound **1b**, longer linkers and thus less polar compounds showed a stronger effect as compared to the shorter and more polar ones. In CCD-1095Sk cells, a correlation between the xylose-aglycon distance and the antiproliferative activity was less pronounced but the general trend was similar. Furthermore, in none of the cell lines, correlations between the antiproliferative activity and the aglycon structure, the GAG priming ability, or the HS proportion could be deduced.



**Figure 5.** ED<sub>50</sub> values ( $\mu$ M) plotted against the xylose-aglycon distance. The xylose-aglycon distances are measured in nm from the anomeric carbon to the aromatic carbon coupled to the linker. Circles indicate naphthyl xylosides, while squares indicate hydroxynaphthyl xylosides. Blue markers are results from CCD-1095Sk cells, and red markers indicate results from HCC70 cells. The outlier is compound **1b**.

### 3. Conclusions

We have synthesized xylosides separated from a 2-naphthyl aglycon by distances from 0.24 to 1.31 nm. In addition, we have synthesized analogs to the naphthyl xylosides with 2-(6-hydroxynaphthyl) aglycons. By increasing the xylose-aglycon distance from 0.24 nm to 0.37 nm, enhancement of the GAG priming ability was observed. It is reasonable that an increase in the xylose-aglycon distance, and thereby the accessibility of the xylose, to a certain level would facilitate binding of xylose by enzymes involved in the GAG synthesis. Additionally, xylosides with even longer xylose-aglycon distances resulted in an increase of the GAG priming ability in normal cells, whereas the effect was omitted in tumor cells. Furthermore, upon xyloside-treatment CCD-1095Sk cells expressed higher total levels of GAG chains, or higher degree of sulfations, compared to HCC70 cells. HCC70 cells, on the other hand, expressed higher levels of HS GAGs in the presence of the xylosides than CCD-1095Sk cells, indicating that the cell type and relative abundance of endogenous substrates and enzymes are relevant for the GAG composition. According to our results, the hydroxynaphthyl xylosides induced a somewhat higher degree of GAG priming, compared to the naphthyl xylosides. Furthermore, treatment with the hydroxynaphthyl xylosides resulted in higher proportions of HS priming compared to the naphthyl ones. Taken together, these results suggest that the biosynthesis of xyloside-primed GAG chains is dependent on the cell type and the aglycon structure. No correlations between the antiproliferative activity and the



amounts or compositions of the GAG chains were detected. Compound **1b** was unique in that it showed a tumor-selective toxicity, suggesting involvement of other factors such as fine structure of the GAG chains, effects on endogenous PG synthesis or other unknown factors for the antiproliferative activity. Future investigations will focus on the origin of this selectivity.

## 4. Experimental section

### 4.1. Synthesis

All solvents were dried using MBRAUN SPS-800 Solvent purification system prior to use, unless otherwise stated. The purchased reagents were used without further purification. NMR-spectra were recorded on a Bruker Avance II at 400 MHz ( $^1\text{H}$ ) and 100 MHz ( $^{13}\text{C}$ ) and assigned using 2D-methods (COSY, HMQC) with a gradient selection. Chemical shifts are reported in ppm downfield from  $\text{Me}_4\text{Si}$ , with reference to residual solvent peaks ( $\delta\text{H CDCl}_3 = 7.26$  ppm,  $\text{MeOH-}d_4 = 3.31$  ppm,  $\text{D}_2\text{O} = 4.79$  ppm,  $\text{C}_6\text{D}_6 = 7.16$  ppm) and solvent signals ( $\delta\text{C CDCl}_3 = 77.0$  ppm,  $\text{MeOH-}d_4 = 49.0$  ppm,  $\text{C}_6\text{D}_6 = 128.06$  ppm). Mass spectra were recorded on a Micromass Q-ToF (ESI). Optical rotations were measured on Perkin Elmer instruments, Model 341 polarimeter. Agilent 1100 Series HPLC with Agilent ZORBAX SB-C<sub>18</sub> column, 3.5  $\mu\text{m}$ , 2.1 $\times$ 150 mm was used to record retention times. Waters 600 Series HPLC with Waters Symmetry C<sub>18</sub> column, 5  $\mu\text{m}$ , 19 $\times$ 100 mm, gradient of MeCN in H<sub>2</sub>O (0.1% TFA) was used for purification. Reactions were monitored by TLC using alumina plates coated with silica gel and visualized using UV light or by charring with an ethanolic anisaldehyde solution. Preparative chromatography was performed with silica gel (35-70  $\mu\text{m}$ , 60  $\text{\AA}$ ) or performed on a Biotage Isolera One flash purification system using Biotage SNAP KP-Sil silica cartridges.

Synthesis and physical characterization of compound **1a** has been described.<sup>21</sup> A simplified synthetic route was used that comprised treatment of a mixture of 1,2,3,4-tetra-*O*-acetyl- $\beta$ -D-xylopyranose and 2-naphthol in  $\text{CH}_2\text{Cl}_2$  with  $\text{BF}_3\cdot\text{OEt}_2$  followed by de-*O*-acetylation using standard Zemplén conditions. Synthesis of **1b** has been described in detail previously.<sup>27</sup> The analyses of the products were in accordance with published data.

#### 4.1.2 (2-Naphthyl)-methyl $\beta$ -D-xylopyranoside (**2a**)

Peracetylated xylose **6** (319 mg, 1.00 mmol) and 2-naphthalenemethanol (273 mg, 1.50 mmol) were dissolved in  $\text{CH}_2\text{Cl}_2$  (3 mL) and  $\text{BF}_3\cdot\text{Et}_2\text{O}$  (190  $\mu\text{L}$ , 1.50 mmol) was added dropwise to the solution at 0  $^\circ\text{C}$ . The mixture was allowed to reach rt and stirred for 1.5 hour.  $\text{CH}_2\text{Cl}_2$  was added and the mixture was washed with water (20 mL) and  $\text{NaHCO}_3$  (sat. aq.) (20 mL). The organic layer was dried over  $\text{Na}_2\text{SO}_4$ , filtered and concentrated to yield a white solid. The crude was filtered through a pile of silica, concentrated, and used in the next step without further purification.

The crude residue (344 mg) was dissolved in  $\text{MeOH}/\text{CH}_2\text{Cl}_2$  (1:1, 6 mL) and NaOMe (1 M, 0.3 mL) was added. After 1.5 h of stirring at rt, AcOH (6 mL) was added and the reaction mixture was concentrated to yield a white solid. HPLC gave **2a** after lyophilization as a white solid (33 mg, 11%).  $[\alpha]_{\text{D}}^{20} -58$  (*c* 0.3, MeOH);  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  7.81-7.87 (m, 4H, ArH), 7.46-7.52 (m, 3H, ArH), 5.05 (d, 1H, *J* 11.7 Hz, CH<sub>2</sub>), 4.76 (d, 1H, *J* 11.7 Hz, CH<sub>2</sub>), 4.55 (d, 1H, *J* 5.8 Hz, H-1), 4.13 (dd, 1H, *J* 11.9, 4.3 Hz, H-5e), 3.77-3.82 (m, 1H, H-4), 3.62 (t, 1H, *J* 7.2 Hz, H-3), 3.53-3.56 (m, 1H, H-2), 3.43 (dd, 1H, *J* 12.0, 7.9 Hz, H-5a), 2.91,

2.70, 2.38 (bs, 1H each, OH);  $^{13}\text{C NMR}$  ( $\text{MeOH-}d_4$ ):  $\delta$  136.6, 134.8, 134.6, 129.0, 128.9, 128.7, 127.7, 127.1, 127.0, 104.2, 77.9, 75.0, 72.0, 71.3, 67.0; HRMS calcd for  $\text{C}_{16}\text{H}_{18}\text{O}_5\text{Na}^+$   $[\text{M}+\text{Na}]$ : 313.1052; found: 313.1067.

#### 4.1.3 (2-(6-Hydroxy-naphthyl))-methyl $\beta$ -D-xylopyranoside (**2b**)

Acetoxynaphthyl xyloside **7b** (87 mg, 0.18 mmol) was dissolved in MeOH (8 mL) and NaOMe (1 M, 0.4 mL) was added. After 1.5 h of stirring at rt, AcOH (8 mL) was added and the reaction mixture was concentrated to yield a white solid. HPLC gave **2b** after lyophilization as a white solid (39 mg, 68%).  $[\alpha]_{\text{D}}^{20} -57$  (*c* 0.3, MeOH);  $^1\text{H NMR}$  ( $\text{MeOH-}d_4$ ):  $\delta$  7.74 (s, 1H, ArH-1), 7.70 (d, 1H, *J* 8.9 Hz, ArH-7), 7.62 (d, 1H, *J* 8.5 Hz, ArH-4), 7.43 (dd, 1H, *J* 8.5, 1.7 Hz, ArH-3), 7.09 (d, 1H, *J* 2.3 Hz, ArH-5), 7.06 (dd, 1H, *J* 8.8, 2.4 Hz, ArH-6), 4.95, 4.73 (ABq, 1H each, *J* 11.4 Hz, CH<sub>2</sub>), 4.34 (d, 1H, *J* 7.3 Hz, H-1), 3.91 (dd, 1H, *J* 11.4, 5.4 Hz, H-5e), 3.48-3.54 (m, 1H, H-4), 3.24-3.33 (m, 2H, H-2, H-3), 3.21 (dd, 1H, *J* 11.4, 10.2 Hz, H-5a);  $^{13}\text{C NMR}$  ( $\text{MeOH-}d_4$ ):  $\delta$  156.7, 136.1, 133.3, 130.5, 129.6, 128.0, 127.7, 127.4, 119.4, 109.8, 104.0, 77.9, 75.0, 72.1, 71.3, 67.0; HRMS calcd for  $\text{C}_{16}\text{H}_{18}\text{O}_6\text{Na}^+$   $[\text{M}+\text{Na}]$ : 329.1001, found: 329.1022.

#### 4.1.4 2-(2-Naphthoxy)-1-ethyl $\beta$ -D-xylopyranoside (**3a**)

Synthesized using similar conditions as for **2b**. Naphthyl xyloside **11a** (66 mg, 0.15 mmol) gave **3a** after HPLC and lyophilization as a white solid (43 mg, 90%).  $[\alpha]_{\text{D}}^{20} -29$  (*c* 0.4, MeOH);  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  7.71-7.78 (m, 3H, ArH), 7.42-7.46 (m, 1H, ArH), 7.33-7.37 (m, 1H, ArH), 7.15-7.18 (m, 2H, ArH), 4.53 (d, 1H, *J* 6.1 Hz, H-1), 4.23-4.33 (m, 3H, OCH<sub>2</sub>), 4.11 (dd, 1H, *J* 11.9, 4.4 Hz, H-5e), 3.97-4.02 (m, 1H, OCH<sub>2</sub>), 3.76-3.81 (m, 1H, H-4), 3.63 (t, 1H, *J* 7.6 Hz, H-3), 3.49-3.53 (m, 1H, H-2), 3.41 (dd, 1H, *J* 11.9, 8.1 Hz, H-5a), 2.91 (bs, 2H, OH), 2.38 (bs, 1H, OH);  $^{13}\text{C NMR}$  ( $\text{MeOH-}d_4$ ):  $\delta$  158.1, 136.1, 130.6, 130.4, 128.6, 127.8, 127.3, 124.7, 119.8, 107.8, 105.4, 77.8, 74.9, 71.2, 69.1, 68.5, 67.0; HRMS calcd for  $\text{C}_{17}\text{H}_{20}\text{O}_6\text{Na}^+$   $[\text{M}+\text{Na}]$ : 343.1158; found: 343.1187.

#### 4.1.5 2-(6-Hydroxy-2-naphthoxy)-1-ethyl $\beta$ -D-xylopyranoside (**3b**)

A suspension of 6-acetoxy-2-naphthol (97 mg, 0.48 mmol) and  $\text{K}_2\text{CO}_3$  (40 mg, 0.29 mmol) in DMF (2 mL) was stirred for 1 h before xyloside **8** (100 mg, 0.261 mmol) was added. The reaction mixture was heated to 80  $^\circ\text{C}$  for 1 hour and worked-up as for **11a**. The crude was filtered through a pile of silica, concentrated, and used in the next step without further purification.

The crude residue (76 mg) was dissolved in  $\text{MeOH}/\text{CH}_2\text{Cl}_2$  (1:1, 4 mL) and NaOMe (1 M, 0.2 mL) was added. After 1.5 h of stirring at rt, AcOH (4 mL) was added and the reaction mixture was concentrated to yield a white solid. HPLC gave **3b** after lyophilization as a white solid (34 mg, 39%).  $[\alpha]_{\text{D}}^{20} -36$  (*c* 0.2, MeOH);  $^1\text{H NMR}$  ( $\text{D}_2\text{O}$ ):  $\delta$  7.76 (d, 1H, *J* 9.0 Hz, ArH), 7.72 (d, 1H, *J* 9.0 Hz, ArH), 7.33 (d, 1H, *J* 2.6 Hz, ArH), 7.14-7.23 (m, 3H, ArH), 4.49 (d, 1H, *J* 7.8 Hz, H-1), 4.32 (t, 2H, *J* 4.5 Hz, OCH<sub>2</sub>), 4.19-4.24 (m, 1H, OCH<sub>2</sub>), 4.04-4.09 (m, 1H, OCH<sub>2</sub>), 3.93 (dd, 1H, *J* 11.5, 5.5 Hz, H-5e), 3.61 (ddd, 1H, *J* 10.5, 9.1, 5.4 Hz, H-4), 3.43 (1H, t, *J* 9.2 Hz, H-3), 3.26-3.34 (m, 2H, H-2, H-5a);  $^{13}\text{C NMR}$  ( $\text{MeOH-}d_4$ ):  $\delta$  156.0, 154.7, 131.8, 130.5, 129.2, 128.6, 120.0, 119.5, 110.1, 108.2, 105.3, 77.7, 74.8, 71.1, 69.2, 68.5, 66.9; HRMS calcd for  $\text{C}_{17}\text{H}_{20}\text{O}_7\text{Na}^+$   $[\text{M}+\text{Na}]$ : 359.1107;



found: 359.1126.

#### 4.1.6 5-(2-Naphthoxy)-3-oxo-1-pentyl $\beta$ -D-xylopyranoside (4)

Synthesized using similar conditions as for **2b**. Naphthyl xyloside **12** (32 mg, 0.065 mmol) gave **4** after HPLC and lyophilization as a white solid (17 mg, 73%).  $[\alpha]_D^{20}$  -24 (*c* 1.1,  $\text{CDCl}_3$ );  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  7.67-7.73 (m, 3H, ArH), 7.38-7.42 (m, 1H, ArH), 7.28-7.32 (m, 1H, ArH), 7.14 (dd, 1H, *J* 8.9, 2.5 Hz, ArH), 7.08 (d, 1H, *J* 2.5 Hz, ArH), 4.87 (bs, 1H, OH), 4.80 (bs, 1H, OH), 4.36 (bs, 1H, OH), 4.23 (d, 1H, *J* 7.2 Hz, H-1), 4.14 (t, 2H, *J* 4.6 Hz,  $\text{OCH}_2$ ), 3.89-3.94 (m, 2H,  $\text{OCH}_2$ , H-5e), 3.80-3.83 (m, 2H,  $\text{OCH}_2$ ), 3.64-3.70 (m, 4H,  $\text{OCH}_2$ , H-4), 3.48 (t, 1H, *J* 8.3 Hz, H-3), 3.37-3.41 (m, 1H, H-2), 3.16-3.22 (m, 1H, H-5a);  $^{13}\text{C NMR}$  ( $\text{MeOH}-d_4$ ):  $\delta$  158.1, 136.1, 130.5, 130.4, 128.6, 127.9, 127.3, 124.7, 119.8, 107.7, 105.2, 77.7, 74.9, 71.7, 71.2, 70.8, 69.7, 68.5, 66.9; HRMS calcd for  $\text{C}_{19}\text{H}_{24}\text{O}_7\text{Na}^+$  [*M*+*Na*]: 387.1420; found: 387.1438.

#### 4.1.7 8-(2-Naphthoxy)-3,5-dioxo-1-octyl $\beta$ -D-xylopyranoside (5)

Synthesized using similar conditions as for **2b**. Naphthyl xyloside **13** (22 mg, 0.041 mmol) gave **5** after HPLC and lyophilization as a colorless oil (11 mg, 66%).  $[\alpha]_D^{20}$  -25 (*c* 0.6,  $\text{CHCl}_3$ );  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  7.70-7.76 (m, 3H, ArH), 7.40-7.44 (m, 1H, ArH), 7.30-7.34 (m, 1H, ArH), 7.13-7.18 (m, 2H, ArH), 4.51 (bs, 1H, OH), 4.28 (d, 1H, *J* 6.7 Hz, H-1), 4.22-4.24 (m, 2H,  $\text{OCH}_2$ ), 3.93-3.98 (m, 3H,  $\text{OCH}_2$ , H-5e, OH), 3.88-3.90 (m, 2H,  $\text{OCH}_2$ ), 3.62-3.74 (m, 8H,  $\text{OCH}_2$ , H-4), 3.46-3.51 (m, 2H, H-3, OH), 3.37-3.41 (m, 1H, H-2), 3.23 (dd, 1H, *J* 11.7, 9.2 Hz, H-5a);  $^{13}\text{C NMR}$  ( $\text{MeOH}-d_4$ ):  $\delta$  158.1, 136.1, 130.5, 130.4, 128.6, 127.9, 127.3, 124.6, 119.8, 107.8, 105.2, 77.7, 74.9, 71.7, 71.54, 71.47, 71.2, 70.8, 69.7, 68.5, 66.9; HRMS calcd for  $\text{C}_{21}\text{H}_{28}\text{O}_8\text{Na}^+$  [*M*+*Na*]: 431.1682; found: 431.1693.

#### 4.1.8 (2-(6-Acetoxy)-naphthyl)-methyl 2,3,4-tri-*O*-acetyl- $\beta$ -D-xylopyranoside (7b)

Peracetylated xylose **6** (119 mg, 0.374 mmol) and 6-acetoxy-2-naphthalenemethanol<sup>28</sup> (53 mg, 0.25 mmol) were dissolved in  $\text{CH}_2\text{Cl}_2$  (3 mL) and  $\text{BF}_3\cdot\text{Et}_2\text{O}$  (50  $\mu\text{L}$ , 0.39 mmol) was added dropwise to the solution at rt. After 1 hour of stirring, the mixture was neutralized with  $\text{NEt}_3$  and concentrated. Column chromatography ( $\text{SiO}_2$ , 1:1 EtOAc/heptane) gave **7b** as a white amorphous solid (91 mg, 79%).  $[\alpha]_D^{20}$  -54 (*c* 0.6, MeOH);  $^1\text{H NMR}$  ( $\text{C}_6\text{D}_6$ ):  $\delta$  7.50-7.57 (m, 4H, ArH-1, ArH-4, ArH-5, ArH-8), 7.31 (dd, 1H, *J* 8.5, 1.6 Hz, ArH-7), 7.19 (dd, 1H, *J* 8.9, 2.3 Hz, ArH-3), 5.37-5.42 (m, 2H, H-1, H-3), 5.06-5.11 (m, 1H, H-4), 4.81, 4.50 (ABq, 1H each, *J* 12.5 Hz,  $\text{CH}_2$ ), 4.45-4.47 (m, 1H, H-2), 3.95 (dd, 1H, *J* 11.7, 5.1 Hz, H-5e), 3.03 (dd, 1H, *J* 11.8, 8.6 Hz, H-5a), 1.82, 1.72, 1.70, 1.60 (s, 3H each, OAc);  $^{13}\text{C NMR}$  ( $\text{C}_6\text{D}_6$ ):  $\delta$  169.8, 169.3, 169.0, 168.5, 149.3, 135.0, 133.9, 131.6, 129.5, 126.6, 126.5, 122.0, 118.9, 99.9, 72.1, 71.4, 70.1, 69.5, 62.2, 20.6, 20.38, 20.35, 20.2. HRMS calcd for  $\text{C}_{24}\text{H}_{26}\text{O}_{10}\text{Na}^+$  [*M*+*Na*]: 497.1424; found: 497.1428.

#### 4.1.9 2-Bromoethyl 2,3,4-tri-*O*-acetyl- $\beta$ -D-xylopyranoside (8)<sup>24</sup>

Peracetylated xylose **6** (1.16 g, 3.64 mmol) was dissolved in  $\text{CH}_2\text{Cl}_2$  (10 mL) and 2-bromoethanol (215  $\mu\text{L}$ , 3.03 mmol) was added.  $\text{BF}_3\cdot\text{Et}_2\text{O}$  (570  $\mu\text{L}$ , 4.49 mmol) was added dropwise to the solution at 0 °C. The mixture was allowed to reach rt and stirred for 1.5 hour.  $\text{CH}_2\text{Cl}_2$  was added and the mixture was washed with water (20 mL) and  $\text{NaHCO}_3$  (sat. aq.) (20 mL). The organic layer was dried over  $\text{Na}_2\text{SO}_4$ , filtered and concentrated to yield an off-

white solid. Column chromatography ( $\text{SiO}_2$ , 1:25  $\rightarrow$  1:10 EtOAc/ $\text{CH}_2\text{Cl}_2$ ) gave **8** as a white solid (801 mg, 69%).  $[\alpha]_D^{20}$  -48 (*c* 1.2, MeOH);  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  5.17 (t, 1H, *J* 8.4 Hz, H-3), 4.92-4.97 (m, 2H, H-2, H-4), 4.55 (d, 1H, *J* 6.8 Hz, H-1), 4.07-4.17 (m, 2H, H-5e,  $\text{CH}_2$ ), 3.77-3.83 (m, 1H,  $\text{CH}_2$ ), 3.45-3.48 (m, 2H,  $\text{CH}_2$ ), 3.39 (dd, 1H, *J* 12, 8.4 Hz, H-5a), 2.08, 2.054, 2.047 (s, 3H each, OAc);  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  170.2, 170.0, 169.7, 100.9, 71.2, 70.5, 69.4, 68.9, 62.2, 30.2, 20.94, 20.91, 20.88; HRMS calcd for  $\text{C}_{13}\text{H}_{19}\text{BrO}_8\text{Na}^+$  [*M*+*Na*]: 405.0161; found: 405.0178.

#### 4.1.10 5-Chloro-3-oxo-1-pentyl 2,3,4-tri-*O*-acetyl- $\beta$ -D-xylopyranoside (9)

Peracetylated xylose **6** (383 mg, 1.20 mmol) was dissolved in  $\text{CH}_2\text{Cl}_2$  (3 mL) and 2-(2-chloroethoxy)-ethanol (106  $\mu\text{L}$ , 1.00 mmol) was added.  $\text{BF}_3\cdot\text{Et}_2\text{O}$  (190  $\mu\text{L}$ , 1.50 mmol) was added dropwise to the solution at 0 °C. The reaction was performed and worked-up as for **8**. Column chromatography ( $\text{SiO}_2$ , 1:25 EtOAc/ $\text{CH}_2\text{Cl}_2$ ) gave **9** as a white solid (163 mg, 43%).  $[\alpha]_D^{20}$  -49 (*c* 1.2,  $\text{CHCl}_3$ );  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  5.16 (t, 1H, *J* 8.4 Hz, H-3), 4.91-4.97 (m, 2H, H-2, H-4), 4.57 (d, 1H, *J* 6.8 Hz, H-1), 4.13 (dd, 1H, *J* 11.6, 5.2 Hz, H-5e), 3.89-3.94 (m, 1H,  $\text{CH}_2$ ), 3.72-3.75 (m, 3H,  $\text{CH}_2$ ), 3.66-3.70 (m, 2H,  $\text{CH}_2$ ), 3.60-3.62 (m, 2H,  $\text{CH}_2$ ), 3.37 (dd, 1H, *J* 11.8, 8.6 Hz, H-5a), 2.06, 2.05, 2.04 (s, 3H each, OAc);  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  170.2, 170.0, 169.6, 100.8, 71.6, 71.5, 70.9, 70.5, 69.0, 68.8, 62.2, 43.0, 20.91, 20.88; HRMS calcd for  $\text{C}_{15}\text{H}_{23}\text{ClO}_9\text{Na}^+$  [*M*+*Na*]: 405.0928; found: 405.0952.

#### 4.1.11 8-Chloro-3,5-dioxo-1-octyl 2,3,4-tri-*O*-acetyl- $\beta$ -D-xylopyranoside (10)

Peracetylated xylose **6** (383 mg, 1.20 mmol) was dissolved in  $\text{CH}_2\text{Cl}_2$  (3 mL) and 2-[2-(2-chloroethoxy)ethoxy]-ethanol (145  $\mu\text{L}$ , 1.00 mmol) was added.  $\text{BF}_3\cdot\text{Et}_2\text{O}$  (190  $\mu\text{L}$ , 1.50 mmol) was added dropwise to the solution at 0 °C. The reaction was performed and worked-up as for **8**. Column chromatography ( $\text{SiO}_2$ , 1:13  $\rightarrow$  1:5 EtOAc/ $\text{CH}_2\text{Cl}_2$ ) gave **10** as a white solid (260 mg, 61%).  $[\alpha]_D^{20}$  -46 (*c* 0.8,  $\text{CHCl}_3$ );  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  5.12 (t, 1H, *J* 8.6 Hz, H-3), 4.86-4.92 (m, 2H, H-2, H-4), 4.52 (d, 1H, *J* 6.8 Hz, H-1), 4.08 (dd, 1H, *J* 11.6, 5.2 Hz, H-5e), 3.84-3.89 (m, 1H,  $\text{CH}_2$ ), 3.58-3.73 (m, 11H,  $\text{CH}_2$ ), 3.33 (dd, 1H, *J* 12, 8.8 Hz, H-5a), 2.02, 2.01, 1.99 (s, 3H each, OAc);  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  170.1, 169.9, 169.5, 100.7, 71.42, 71.39, 70.8, 70.7, 70.4, 68.9, 68.7, 62.0, 42.8, 20.79, 20.76; HRMS calcd for  $\text{C}_{17}\text{H}_{27}\text{ClO}_{10}\text{Na}^+$  [*M*+*Na*]: 449.1190; found: 449.1215.

#### 4.1.12 2-(2-Naphthoxy)-1-ethyl 2,3,4-tri-*O*-acetyl- $\beta$ -D-xylopyranoside (11a)

A suspension of 2-naphthol (75 mg, 0.52 mmol) and  $\text{K}_2\text{CO}_3$  (41 mg, 0.30 mmol) in DMF (2 mL) was stirred for 1 h before xyloside **8** (100 mg, 0.263 mmol) was added. The reaction mixture was heated to 80 °C over night before it was allowed to reach rt and water (5 mL) was added. The water phase was extracted with EtOAc (3 x 10 mL), and the combined organic layers were washed with brine (40 mL), dried over  $\text{Na}_2\text{SO}_4$ , filtered and concentrated to yield a brown solid. Column chromatography ( $\text{SiO}_2$ , 1:2 EtOAc/heptane) gave **11a** as an off-white solid (75 mg, 63%).  $[\alpha]_D^{20}$  -49 (*c* 0.8,  $\text{CHCl}_3$ );  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  7.74 (q, 3H, *J* 8.5 Hz, ArH), 7.42-7.46 (m, 1H, ArH), 7.32-7.36 (m, 1H, ArH), 7.12-7.16 (m, 2H, ArH), 5.19 (t, 1H, *J* 8.4 Hz, H-3), 4.94-5.00 (m, 2H, H-2, H-4), 4.67 (d, 1H, *J* 6.8 Hz, H-1), 4.23-4.25 (m, 2H,  $\text{CH}_2\text{O}$ ), 4.14-4.20 (m, 2H,  $\text{CH}_2\text{O}$ , H-5e), 3.95-4.00 (m, 1H,  $\text{CH}_2\text{O}$ ), 3.41 (dd, 1H, *J* 12, 8.8 Hz, H-5a), 2.06, 2.01, 1.93 (s, 3H each, OAc);  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  170.2,

170.0, 169.7, 156.7, 134.6, 129.6, 129.2, 127.8, 126.9, 126.6, 123.9, 118.9, 106.9, 101.1, 71.5, 70.6, 69.0, 67.9, 67.3, 62.2, 20.9, 20.85, 20.75; HRMS calcd for C<sub>23</sub>H<sub>26</sub>O<sub>9</sub>Na<sup>+</sup> [M+Na]: 469.1475; found: 469.1501.

#### 4.1.13 5-(2-Naphthyloxy)-3-oxo-1-pentyl 2,3,4-tri-O-acetyl-β-D-xylopyranoside (12)

A suspension of 2-naphthol (76 mg, 0.53 mmol), K<sub>2</sub>CO<sub>3</sub> (41 mg, 0.29 mmol), and 18-crown-6 (695 mg, 2.63 mmol) in DMF (2 mL) was stirred for 1 h before xyloside **9** (100 mg, 0.262 mmol) was added. The reaction was performed and worked-up as for **11a**. Column chromatography (SiO<sub>2</sub>, 2:3 EtOAc/heptane) gave **12** as a slightly yellow oil (28 mg, 22%). [α]<sub>D</sub><sup>20</sup> -33 (*c* 1.1, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.71-7.77 (m, 3H, ArH), 7.41-7.45 (m, 1H, ArH), 7.31-7.35 (m, 1H, ArH), 7.14-7.19 (m, 2H, ArH), 5.16 (t, 1H, *J* 8.5 Hz, H-3), 4.91-4.96 (m, 2H, H-2, H-4), 4.57 (d, 1H, *J* 6.8 Hz, H-1), 4.22-4.24 (m, 2H, CH<sub>2</sub>O), 4.11 (dd, 1H, *J* 11.8, 5.1 Hz, H-5e), 3.89-3.96 (m, 3H, CH<sub>2</sub>O), 3.73-3.77 (m, 3H, OCH<sub>2</sub>), 3.34 (dd, 1H, *J* 11.8, 8.8 Hz, H-5a), 2.04 (s, 6H, OAc), 2.03 (s, 3H, OAc); <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 170.2, 170.0, 169.7, 156.8, 134.6, 129.6, 129.2, 127.8, 126.9, 126.5, 123.8, 119.1, 106.8, 100.9, 71.5, 70.9, 70.7, 70.0, 69.0, 68.9, 67.6, 62.1, 20.88, 20.85; HRMS calcd for C<sub>25</sub>H<sub>30</sub>O<sub>10</sub>Na<sup>+</sup> [M+Na]: 513.1737; found: 513.1742.

#### 4.1.14 8-(2-Naphthyloxy)-3,5-dioxo-1-octyl 2,3,4-tri-O-acetyl-β-D-xylopyranoside (13)

Synthesized using similar conditions as for **12**. Xyloside **10** (101 mg, 0.237 mmol) gave **13** after column chromatography (SiO<sub>2</sub>, 1:1 → 3:1 EtOAc/heptane) as a slightly yellow oil (37 mg, 29%). [α]<sub>D</sub><sup>20</sup> -37 (*c* 1.1, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.71-7.77 (m, 3H, ArH), 7.41-7.43 (m, 1H, ArH), 7.32-7.35 (m, 1H, ArH), 7.14-7.18 (m, 2H, ArH), 5.15 (t, 1H, *J* 8.6 Hz, H-3), 4.90-4.94 (m, 2H, H-2, H-4), 4.53 (d, 1H, *J* 6.8 Hz, H-1), 4.24-4.26 (m, 2H, CH<sub>2</sub>O), 4.10 (dd, 1H, *J* 11.8, 5.1 Hz, H-5e), 3.88-3.93 (m, 3H, CH<sub>2</sub>O), 3.63-3.75 (m, 7H, OCH<sub>2</sub>), 3.32 (dd, 1H, *J* 11.8, 8.8 Hz, H-5a), 2.04, 2.023, 2.018 (s, 3H each, OAc); <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 170.2, 170.0, 169.6, 156.8, 134.6, 129.5, 129.1, 127.7, 126.9, 126.4, 123.8, 119.1, 106.8, 100.8, 71.5, 71.0, 70.9, 70.8, 70.4, 69.9, 69.0, 68.8, 67.5, 62.1, 20.8; HRMS calcd for C<sub>27</sub>H<sub>34</sub>O<sub>11</sub>Na<sup>+</sup> [M+Na]: 557.1999; found: 557.2016.

## 4.2 Computational chemistry

Based on our experience in computational chemistry on carbohydrates, no conformational search was performed. Instead, the methyl β-D-xylose was minimized using density functional theory at the B3LYP/6-31G\*\* (vacuum) in Spartan '10 for Macintosh<sup>25</sup>, from two different conformers corresponding to the two different hydrogen bonding patterns for H-2 to O-1. The lowest energy conformer was used in further calculations. For the naphthyl residue the same approach was made where the two lowest energy conformers were minimized and the conformation with the lowest energy was used in further calculations. The linker region was chosen as all staggered and the three components were connected and minimized using density functional theory at the B3LYP/6-31G\*\* (water) in Spartan '10 for Macintosh<sup>25</sup>.

## 4.3 Biology

### 4.3.1 Materials

Human breast cancer cell line HCC70 and human breast fibroblast cell line CCD-1095Sk were obtained from ATCC (Manassas, VA). Minimal essential medium (MEM) Earle's and RPMI-1640 were purchased from Biochrom AB (Germany) and ATCC, respectively. Ham's F-12 medium, insulin, and transferrin were purchased from Sigma-Aldrich (St. Louis, MO), and low sulfate MgCl<sub>2</sub>-medium from Life Technologies (Carlsbad, CA). L-glutamine, penicillin-streptomycin, trypsin and fetal calf serum (FBS) were obtained from Life Technologies. Epidermal growth factor (EGF) was purchased from BD Biosciences (San Jose, CA). [<sup>35</sup>S]Sulfate (25 Ci/L) was obtained from Perkin Elmer (Waltham, MA), and crystal violet was obtained from Merck (Whitehouse Station, NJ). Microculture plates were purchased from Nunc A/S, Denmark. The FPLC system, prepacked Superose 6 HR 10/30m, Dextran T-500 and octyl-Sepharose CL-4B were from Pharmacia-LKB (Gaithersburg, MD), and DE-53 DEAE-cellulose was from Whatman/GE Healthcare (Sweden). Water for HPLC-analysis was from a Millipore Milli-Q system (Billerica, MA). For centrifugations a Heraeus Multifuge 1 S-R (Germany) was used, and for β-radioactivity measurements a Wallac 1414 Liquid Scintillation Counter (Perkin Elmer), controlled by a WinSpectral software system, was used. Microplate readings were performed on a Labsystems Multiskan RC (Finland) through the software Genesis (Labsystems).

### 4.3.2 Cell culture, radiolabeling and extraction procedures

Cells were cultured as monolayers according to manufacturer's instructions. Confluent cells were incubated at 37 °C in a 5% CO<sub>2</sub> in air atmosphere in low-sulfate, MgCl<sub>2</sub>-medium supplemented with 10% (v/v) FBS, 1% (v/v) L-glutamine, penicillin (100 U/mL), streptomycin (100 µg/mL), 50 µCi/mL of [<sup>35</sup>S]sulfate, and different xylosides at a concentration of 100 µM. Controls without xylosides were included. Dilutions of xylosides were made from 20 mM stock solutions in Me<sub>2</sub>SO/H<sub>2</sub>O (1:1, v/v). After 24 h of incubation, culture media were collected. The cells were washed three times with PBS, and subsequently extracted with 0.2 mL/cm<sup>2</sup> dish of 0.15 M NaCl, 10 mM EDTA, 10 mM KH<sub>2</sub>PO<sub>4</sub>, pH 7.5, 5 µg/mL ovalbumin, 2% (v/v) Triton X-100 containing 0.1% (v/v) phenylmethylsulfonyl fluoride in EtOH (sat.) on a slow shaker at 4 °C for 15 min.

### 4.3.3 Isolation of xyloside primed radiolabeled polyanionic material

The procedures have been described in detail previously.<sup>18</sup> [<sup>35</sup>S]Sulfate labeled polyanionic macromolecules were isolated from the culture medium and cell extract by ion exchange chromatography, performed by allowing the samples to pass over a 0.2 mL column of DEAE equilibrated with 6 M urea, 0.5 M NaOAc, pH 5.8, 5 µg/mL ovalbumin, 0.1% (v/v) Triton X-100 (2.5 mL). After sample application, the columns were washed successively with a) equilibration buffer (5 mL), b) 6 M urea, 10 mM Tris, pH 8, 5 µg/mL ovalbumin, 0.1% (v/v) Triton X-100 (5 mL), and c) 50 mM Tris-HCl, pH 7.5 (5 x 2 mL). Bound material was eluted with 2 x 0.5 mL 4 M guanidine-HCl, 50 mM NaOAc, pH 5.8. The eluted [<sup>35</sup>S]sulfate labeled polyanionic material was separated from hydrophobic material by using a 0.2 mL octyl-Sepharose column equilibrated with 4 M guanidine-HCl, 50 mM NaOAc, pH 5.8 (2.5 mL). After equilibration, 300 µg of BSA was added to avoid unspecific binding, and subsequently the columns were washed again with 4 M guanidine-HCl, 50 mM

NaOAc, pH 5.8 (2 mL). After sample application the unbound [<sup>35</sup>S]sulfate labeled polyanionic material was washed with 3 x 0.2 mL 4 M guanidine-HCl, 50 mM NaOAc, pH 5.8. Precipitation of radioactive samples was performed with EtOH (95%, 5.6 vol) over night at -20 °C using 100 µg of dextran as a carrier. After centrifugation at 4400 rpm for 25 min, the remaining pellets were dissolved in 4 M guanidine-HCl, 50 mM NaOAc, pH 5.8, 0.2% (v/v) Triton X-100, followed by gel permeation FPLC on Superose 6. Radioactivity was determined in a β-counter and the values were adjusted to protein concentration in each cell line. For degradations [<sup>35</sup>S]sulfate labeled polyanionic material from Superose 6 separation was pooled and precipitated as described above, followed by gel permeation FPLC on Superdex Peptide. Radioactivity was determined in a β-counter.

#### 4.3.4 Degradation procedures

For CS/DS degradation samples were dissolved in 0.1 M Tris-NaOAc, 10 mM EDTA, pH 7.3 (300 µL), and incubated with chondroitin ABC lyase (7 µL, 5 U/mL) at 37 °C overnight. Degradation was monitored at A<sub>232 nm</sub>. HS degradation was performed using low pH nitrous acid catalyzed deaminative cleavage.<sup>29</sup>

#### 4.3.5 In vitro growth assay using crystal violet method

The procedures have been described in detail previously.<sup>18</sup> Cells were harvested by trypsinization and inoculated in 96-well microculture plates at plating densities of 5000 cells/well in MEM Earle's medium supplemented with 10% (v/v) FBS, 1% (v/v) L-glutamine, penicillin (100 U/mL), and streptomycin (100 µg/mL) or RPMI-1640 supplemented with 10% (v/v) FBS, penicillin (100 U/mL), and streptomycin (100 µg/mL). After 24 h of plating the cells were allowed to proliferate in Ham's F-12 medium supplemented with insulin (10 µg/mL), transferrin (25 µg/mL), 1% (v/v) L-glutamine, 10 ng/mL EGF, and xylosides of various concentrations (5 – 500 µM). Blanks only containing medium, and controls without xylosides were included. After 96 h the cells were fixed in 0.25% (v/v) glutaraldehyde in Hanks' balanced salt solution for 20 min, and then the cells nuclei were stained with 0.1% (v/v) crystal violet for 30 min. The cells were washed extensively and lysed in 1% (v/v) Triton X-100 for 24 h, followed by measurement of the amount of bound dye at A<sub>595 nm</sub> in a microplate reader. The inhibitory effect of the compounds is expressed as ED<sub>50</sub> after exposure relative to untreated cells.

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#### Supplementary data

Supplementary data associated to this article can be found, in the online version, at

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