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Antiproliferative effects of peracetylated naphthoxylosides

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ABSTRACT

The antiproliferative activity, and the capability of priming of glycosaminoglycan chains, of two series of peracetylated mono- and bis-xylosylated dihydroxynaphthalenes have been investigated for normal HFL-1 cells, as well as transformed T24 cells, and compared to the unprotected analogs. Our data show increased antiproliferative activity upon peracetylation, but a loss of selectivity towards T24 cells.

Glycosaminoglycans (GAG) are linear polymers of repeating disaccharide units containing hexosamine and hexuronic acid linked to a core protein forming proteoglycans (PG). The first step in GAG assembly is xylosylation of a serine residue of the core protein, a specific linker tetrasaccharide, GlcA(β1-3)Gal(β1-3)Gal(β1-4)Xylβ, is assembled and serves as an acceptor for elongation of GAG chains (Fig. 1). This is followed by polymerization and addition of repeating disaccharides, that is (-4)GlcNAc(α1-4)GlcA(β1-) for heparan sulfate (HS) or (-3)GalNAc(β1-4)GlcA(β1-) for chondroitin sulfate/dermatan sulfate (CS/DS), which undergo concomitant modifications by serial epimerization and sulfation reactions resulting in enormous structural diversity of these macromolecules.

β-D-Xylosides with hydrophobic aglycons can penetrate plasma membranes and act as artificial primers for GAG formation independently of core protein synthesis.[1,2] The xylosides initiate GAG synthesis by serving as acceptors in the first galactosylation step. The composition of GAG assembled on the xyloside primers depend 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/DS dominates, and synthesis of free HS chains is low or undetectable. Increased yields of HS can be obtained when the aglycon of the xylosides comprises aromatic, polycyclic structures, such as naphthol-derivatives. 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,[3] antithrombotic effects,[4] and growth inhibition of transformed cells.[5–7]

We have previously reported that 2-(6-hydroxynaphthyl) β-D-xylopyranoside (XylNapOH, compound **5d**, Chart 1), which primes both HS and CS/DS synthesis, selectively inhibits growth of tumor cells in vitro as well as in vivo.[5,6] Treatment with this xyloside at a pharmacologically relevant dose reduced the average tumor load in SCID mice by 70–97%.[6] Attempts to determine the mechanism for the selective growth inhibition have also been made.[5,6] These results suggest that (i) the priming of HS synthesis is required for selective growth inhibition and (ii) the effect on transformed cells is not caused by the xyloside itself but by products derived from the priming of short GAG chains on the xyloside. Furthermore, the bioactivity is dependent on (iii) the hydroxyl substitution pattern in the naphthalene ring of the xyloside and (iv) nuclear targeting of the xyloside-derived products.

We have earlier synthesized series of mono- and bis-xylosylated dihydroxynaphthalenes and studied these compounds for polarity, antiproliferative effects and their ability of priming glycosaminoglycan chains[8,9] Our results showed that the antiproliferative effects of mono-xylosylated compounds versus normal human fetal lung fibroblasts (HFL-1) were correlated with the polarity of the compounds, that is less polar compounds showed a stronger antiproliferative effect as compared to the more polar ones. This indicates that the uptake of the xylosides is dependent on the polarity. However, in the case of human bladder carcinoma cells (T24), some compounds showed a clearly different behavior resulting in a selective antiproliferative effect, as shown in Figure 2a. The bis-xylosylated compounds were generally more stable,

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compared to the mono-xylosylated ones, but they initiated priming of glycosaminoglycan chains to less extent. Interestingly, the priming proceeded in two directions. In contrary to the mono-xylosylated analogs, the tested compounds did not show any antiproliferative properties (i.e. $ED_{50} > 400$ μM).

In a recent publication, we reported that a peracetylated analog of XylNapOH is more potent compared to the original compound. The acetylation probably makes the uptake faster and non-specific esterases will cleave off the acetates and liberate the active compound (i.e. it acts as a prodrug).[10] It is reasonable to assume that acetylation makes the uptake of all structural analogs similar, which gives us the opportunity to investigate the antiproliferative effects separated from the uptake. Therefore we have synthesized a collection of peracetylated mono- and bis-xylosylated dihydroxynaphthalenes and tested these for antiproliferative effects and priming (Chart 1). The synthesis of the acetylated and unprotected bis-xylosylated dihydroxynaphthalenes (compounds **1**–**10a,b**),[9] the unprotected mono-xylosylated dihydroxynaphthalenes (compounds **1**–**10d**, **7**–**10f**),[11] and the peracetylated compound **5c**,[12] have been published before. The other peracetylated mono-xylosylated dihydroxynaphthalenes (**1**–**10c**) were synthesized from the unprotected compounds by acetylation using acetic anhydride and pyridine. For determination of antiproliferative activity, normal HFL-1 cells and T24 cells were used. The peracetylated mono- and bis-xylosides were added to the growth medium at increasing concentrations, and cell proliferation was recorded using the crystal violet method.[5,6] The inhibitory effect of the compounds is expressed as ED_{50} (μM) scored after 96 h of exposure (Table 1).

The antiproliferative activity of the peracetylated mono-xylosylated dihydroxynaphthalenes for HFL-1 cells was plotted versus the effect towards T24 cells (Fig. 2b). With few exceptions, the peracetylated compounds were more potent, compared to the unprotected analogs. However, with the exception for compounds **5c** and **9e**, the peracetylated mono-xylosylated compounds did not show selective growth inhibitory effect for transformed cells over HFL-1 cells.

We have earlier reported that bis-xylosylated dihydroxynaphthalenes initiated GAG synthesis but showed no antiproliferative activity. The 10 bis-xylosylated compounds were more polar compared to the mono-xylosylated analogs, and it is reasonable to assume that the lack of antiproliferative activity of these compounds could be due to either too high polarity or too big size, that makes

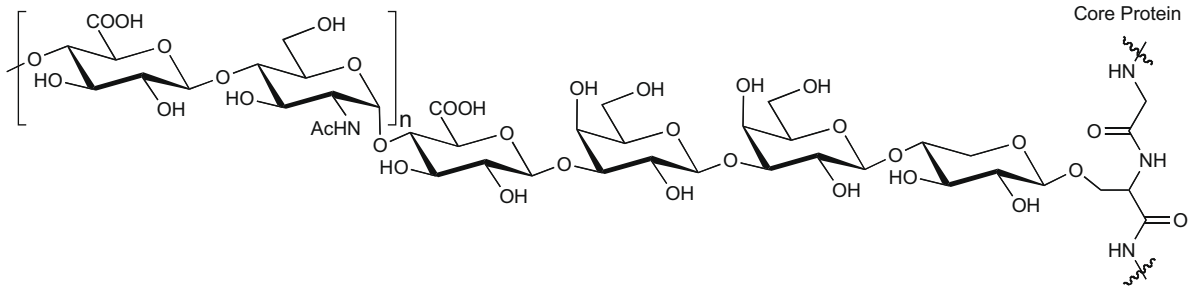


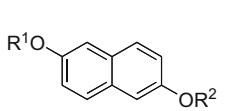
Figure 1. Example of a proteoglycan. The glycosaminoglycan chains consist of a linker tetrasaccharide unit coupled to serine residues of the protein.

1a: $R^1 = R^2 = Xyl(OAc)_3$
1b: $R^1 = R^2 = Xyl$
1c: $R^1 = Xyl(OAc)_3$, $R^2 = OAc$
1d: $R^1 = Xyl$, $R^2 = H$

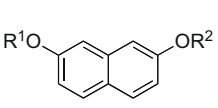
2a: $R^1 = R^2 = Xyl(OAc)_3$
2b: $R^1 = R^2 = Xyl$
2c: $R^1 = Xyl(OAc)_3$, $R^2 = OAc$
2d: $R^1 = Xyl$, $R^2 = H$

3a: $R^1 = R^2 = Xyl(OAc)_3$
3b: $R^1 = R^2 = Xyl$
3c: $R^1 = Xyl(OAc)_3$, $R^2 = OAc$
3d: $R^1 = Xyl$, $R^2 = H$

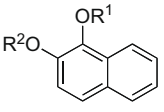
4a: $R^1 = R^2 = Xyl(OAc)_3$
4b: $R^1 = R^2 = Xyl$
4c: $R^1 = Xyl(OAc)_3$, $R^2 = OAc$
4d: $R^1 = Xyl$, $R^2 = H$



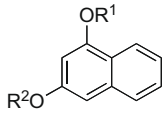
5a: $R^1 = R^2 = Xyl(OAc)_3$
5b: $R^1 = R^2 = Xyl$
5c: $R^1 = Xyl(OAc)_3$, $R^2 = OAc$
5d: $R^1 = Xyl$, $R^2 = H$



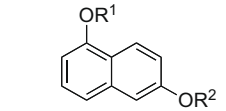
6a: $R^1 = R^2 = Xyl(OAc)_3$
6b: $R^1 = R^2 = Xyl$
6c: $R^1 = Xyl(OAc)_3$, $R^2 = OAc$
6d: $R^1 = Xyl$, $R^2 = H$



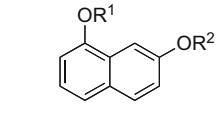
7a: $R^1 = R^2 = Xyl(OAc)_3$
7b: $R^1 = R^2 = Xyl$
7c: $R^1 = Xyl(OAc)_3$, $R^2 = OAc$
7d: $R^1 = Xyl$, $R^2 = H$
7e: $R^1 = OAc$, $R^2 = Xyl(OAc)_3$
7f: $R^1 = H$, $R^2 = Xyl$



8a: $R^1 = R^2 = Xyl(OAc)_3$
8b: $R^1 = R^2 = Xyl$
8c: $R^1 = Xyl(OAc)_3$, $R^2 = OAc$
8d: $R^1 = Xyl$, $R^2 = H$
8e: $R^1 = OAc$, $R^2 = Xyl(OAc)_3$
8f: $R^1 = H$, $R^2 = Xyl$



9a: $R^1 = R^2 = Xyl(OAc)_3$
9b: $R^1 = R^2 = Xyl$
9c: $R^1 = Xyl(OAc)_3$, $R^2 = OAc$
9d: $R^1 = Xyl$, $R^2 = H$
9e: $R^1 = OAc$, $R^2 = Xyl(OAc)_3$
9f: $R^1 = H$, $R^2 = Xyl$



10a: $R^1 = R^2 = Xyl(OAc)_3$
10b: $R^1 = R^2 = Xyl$
10c: $R^1 = Xyl(OAc)_3$, $R^2 = OAc$
10d: $R^1 = Xyl$, $R^2 = H$
10e: $R^1 = OAc$, $R^2 = Xyl(OAc)_3$
10f: $R^1 = H$, $R^2 = Xyl$

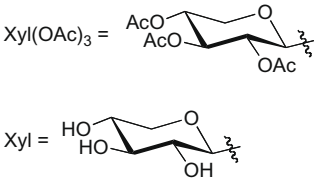


Chart 1. Peracetylated and unprotected bis-xylosylated compounds (**1**–**10a**,**b**) as well as the peracetylated and unprotected mono-xylosylated compounds (**1**–**10c**–**f**) described in this paper.

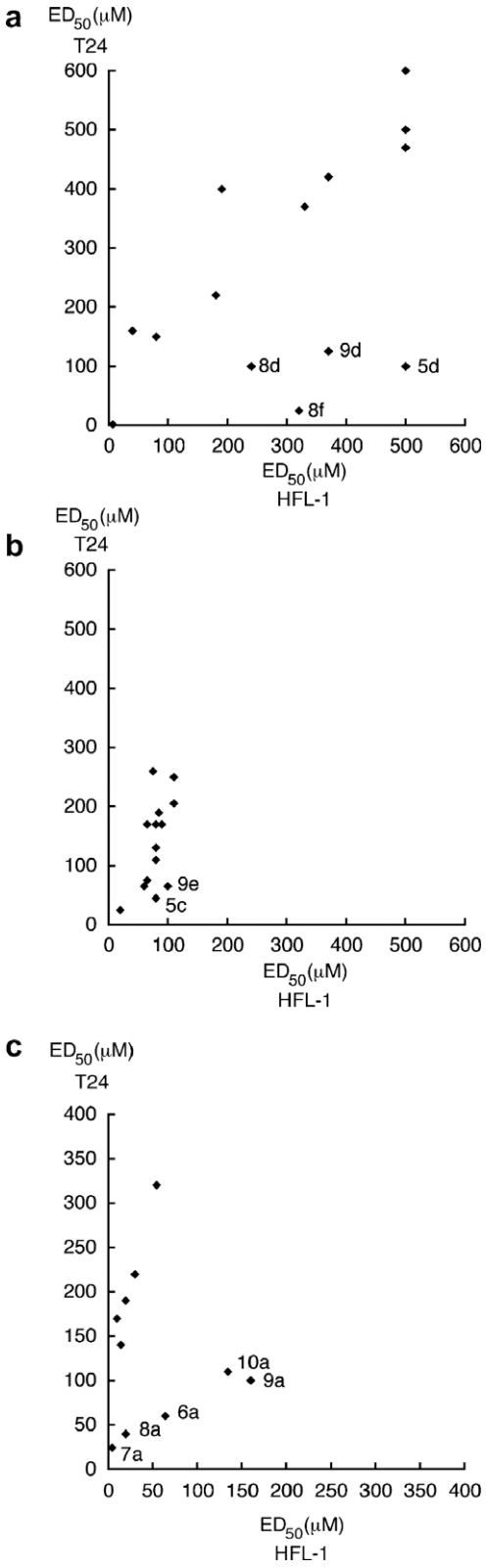


Figure 2. (a) Antiproliferative activity (ED_{50}, μM) of unprotected mono-xylosylated dihydroxynaphthalenes (**1**–**10d**, **7**–**10f**) for HFL-1 cells versus T24 cells. Biological data for the unprotected mono-xylosylated dihydroxynaphthalenes have been published before.[8] (b) Antiproliferative activity (ED_{50}, μM) of peracetylated mono-xylosylated dihydroxynaphthalenes (**1**–**10c**, **7**–**10e**) for HFL-1 cells versus T24 cells. (c) Antiproliferative activity (ED_{50}, μM) of peracetylated bis-xylosylated dihydroxynaphthalenes (**1**–**10a**) for HFL-1 cells versus T24 cells.

Table 1
Antiproliferative activity (ED_{50}, μM) of naphthoxylosides toward HFL-1 cells and T24 cells

Compound	HFL-1	T24	Compound[a]	HFL-1	T24
1c	80	130	**1d**	6	1
2c	90	170	**2d**	500	470
3c	110	205	**3d**	180	220
4c	75	260	**4d**	190	400
5c	80	45	**5d**	500	100
6c	85	190	**6d**	500	500
7c	65	170	**7d**	80	150
7e	110	250	**7f**	40	160
8c	20	25	**8d**	240	100
8e	60	65	**8f**	320	25
9c	80	110	**9d**	370	125
9e	100	65	**9f**	500	600
10c	80	170	**10d**	330	370
10e	65	75	**10f**	370	420

[a] Biological data for the unprotected mono-xylosylated dihydroxynaphthalenes have been published before.[8]

transport to the nuclei impossible. Another possibility is that the bis-xylosylated analogs, due to the lack of a free phenolic hydroxyl are less growth inhibiting. It is well known that polyhydroxylated naphthalenes are toxic due to redox cycling between semiquinones and quinones resulting in superoxide radicals and apoptosis.[13–15]

To shed further light on the low toxicity shown by the unprotected bis-xylosides, we investigated the antiproliferative activity of the corresponding peracetylated compounds **1**–**10a** towards HFL-1 cells and T24 cells (Table 2). Interestingly, all ten analogs showed strong antiproliferative activity. The compounds could be divided into two different groups; some compounds showed similar activity for HFL-1 and T24 cells, while the other compounds showed a significantly stronger effect towards HFL-1 cells (Fig. 2c). No compounds showed a significant selectivity towards transformed cells.

To test the xyloside's ability to prime GAG synthesis, T24 and HFL-1 cells were incubated with 100 μM xyloside and [^{35}S] sulfate followed by isolation and size separation of free GAG chains. The proportion of GAG-priming is given as the integrated value of fractions containing free GAG chains in cells treated with the xyloside, divided by the integrated values for the fractions of untreated cells (Table 3). The amount of GAG priming is comparable to the priming shown by the unprotected analogs, and no obvious trends can be seen between toxicity and priming.

To summarize, we have evaluated two series of peracetylated mono- or bis-xylosylated naphthoxylosides and compared the antiproliferative effects with unprotected analogs. The peracetylated compounds generally showed stronger antiproliferative effects

Table 2
Antiproliferative activity (ED_{50}, μM) of bis-xylosylated dihydroxynaphthalenes towards HFL-1 cells and T24 cells

Compound	HFL-1	T24	Compound[a]	T24
1a	15	140	**1b**	400
2a	10	170	**2b**	400
3a	20	190	**3b**	
4a	30	220	**4b**	
5a	55	320	**5b**	>>400
6a	65	60	**6b**	450
7a	5	25	**7b**	
8a	20	40	**8b**	>>400
9a	160	100	**9b**	
10a	135	110	**10b**	

[a] Biological data for the unprotected bis-xylosylated dihydroxynaphthalenes have been published before.[9]

Table 3
GAG Priming of peracetylated mono and bis-xylosylated compounds in HFL-1 and T24 cells. The proportion of GAG priming is given as the integrated value of fractions containing free GAG chains in cells treated with the xyloside, divided by the integrated values for the fractions of untreated cells

Compound	HFL-1	T24	Compound	HFL-1	T24
1c	4.8	9.0	**1a**	13.6	8.0
2c	2.7	6.1	**2a**	5.4	6.9
3c	0.8	2.4	**3a**	2.4	1.7
4c	2.2	4.6	**4a**	2.8	4.2
5c	2.5	5.4	**5a**	15.5	8.2
6c	2.2	6.5	**6a**	6.4	5.4
7c	0.6	0.5	**7a**	0.8	1.4
7e	1.8	7.1			
8c	2.2	4.5	**8a**	4.7	3.4
8e	1.0	3.7			
9c	1.8	6.4	**9a**	12.7	8.8
9e	1.3	6.9			
10c	6.2	8.2	**10a**	11.1	0.5
10e	2.1	4.8			

but, with few exceptions, did not show selectivity for transformed T24 cells. Interestingly, the peracetylated bis-xylosides showed strong antiproliferative effects, which indicate that the low effects shown by the unprotected analogs, probably originates in difficulties in the uptake rather than low inherent toxicity. All compounds initiated priming of GAG chains, which show that they are capable of entering the cells.

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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.2009.01.065.

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