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Xylose as a carrier for boron containing compounds

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Abstract—A xylosylated carborane was synthesized by standard carbohydrate methodology and tested on normal HFL-1 cells as well as transformed T24 cells. The xylosylated carborane initiated glycosaminoglycan (GAG) synthesis in both cell lines and treatment with the carborane gave a pronounced translocation of proteoglycans to the nuclei of T24 cells. However, most of the boron-containing compounds were secreted to the medium. We conclude that xylosides carrying carboranes are not suitable for boron neutron capture therapy (BNCT) for T24 cells. However, the uptake of boron-containing xyloside, the GAG priming capacity, and the nuclear translocation of glypican-1 make this xyloside a candidate for further investigation for selectivity toward other tumor cell lines.

The main difficulty in cancer therapy is to achieve high selectivity between healthy cells and tumor cells. Boron neutron capture therapy (BNCT) is a binary therapeutic method utilizing the ability of boron-10 to capture a thermal neutron and thereby creating an excited form of boron-11 which rapidly decays emitting an alpha particle and a lithium ion.[1] The released species can only travel a distance shorter than the diameter of most cells, and therefore the damage they cause in biological systems is confined to a small volume. If a sufficient concentration of boron-10 (approx. 10^9 atoms per cell) has been achieved in the tumor, and the neutrons are targeted to the tumor cells, a high selectivity and efficiency should be obtained. The measured biological effectiveness of BNCT-agents has been termed compound biological effectiveness (CBE) factor and is dependent on the drug administered and the distribution of boron within the cells, tumor and normal tissue.[2]

The agents used to deliver boron to the tumor cells need to have low toxicity as well as a pronounced uptake into tumor cells, compared to normal cells, to give high tumor to tissue ratio. The uptake into the tumor needs to be efficient to reach the necessary 10^9 atoms of boron-10, but the agent should also be rapidly cleared from normal tissue and blood. At present no single BNCT-agent possesses all these properties. The carboranes are interesting building blocks for drugs developed for BNCT due to their high boron content. Several carborane-containing compounds, including nucleosides and carbohydrates, have been investigated.[3–6]

We have earlier shown that 2-(6-hydroxynaphthyl) β-D-xylopyranoside **1** (Fig. 1) selectively inhibits the growth of tumor cells in vitro as well as in vivo and treatment with this xyloside reduced the average tumor load by 70–97% in a SCID mice model.[7]

Xylose is an unusual structural component in mammalian cells that has, so far, only been found in one unique position, that is, as the linker between protein and carbohydrate in proteoglycans. Proteoglycans are macromolecules composed of glycosaminoglycan (GAG) chains covalently attached by the xylose residue to a core protein. 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.[8–10]

The first step of the GAG biosynthesis is xylosylation of the serine residue and a specific linker tetrasaccharide,

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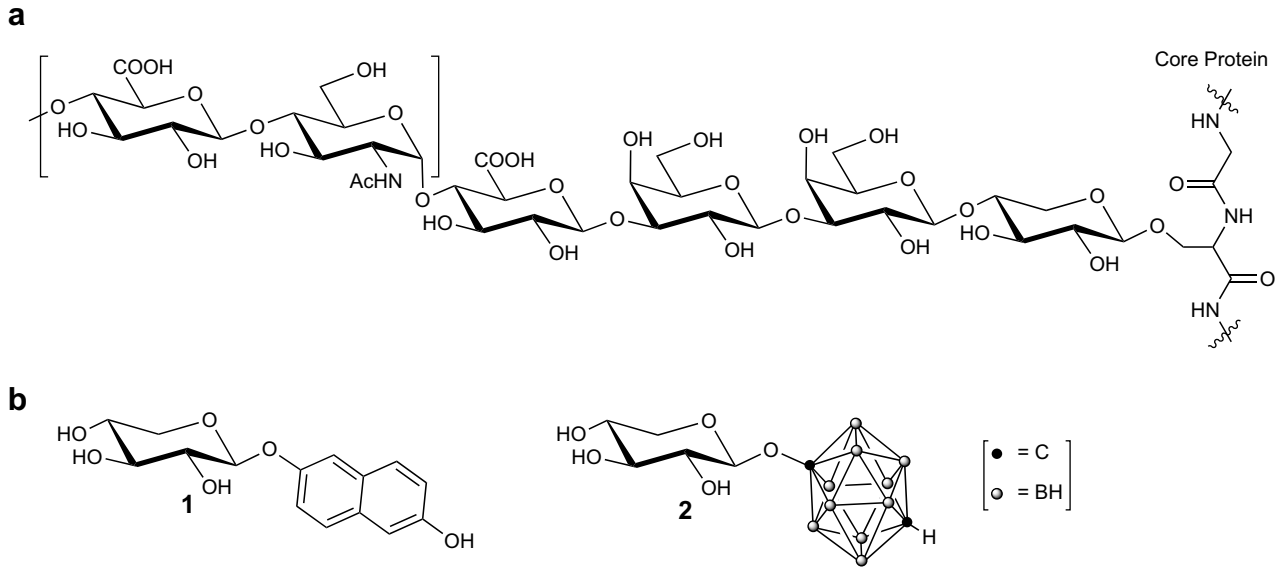


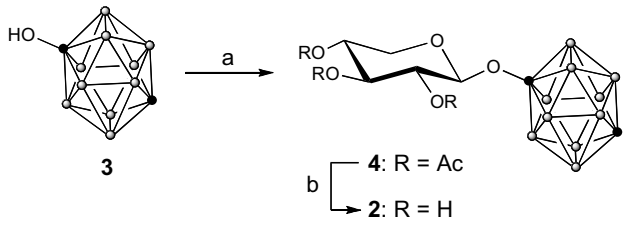
Figure 1. (a) Example of a proteoglycan. The glycosaminoglycan chains consist of a linker tetrasaccharide unit (GlcA(β1–3)Gal(β1–3)Gal(β1–4)Xylβ) coupled to serine residues of the protein. (b) 2-(6-hydroxynaphthyl) β-D-xylopyranoside (**1**) and 1-(1,12-dicarba-*closo*-dodecaboranyl) β-D-xylopyranoside (**2**).

GlcA(β1–3)Gal(β1–3)Gal(β1–4)Xylβ, is assembled and serves as an acceptor for elongation of GAG chains (Fig. 1).

The biosynthesis of GAG chains can also take place independently of the core protein by using xylopyranosides as primers. Xylosides with hydrophobic aglycon can thus penetrate cell membranes and initiate GAG synthesis by serving as acceptors in the first galactosylation step.[11–14] We reason that some of these GAG chains are transported into the cell nuclei and that the effects are pronounced in cancer cells. The xyloside thus gives a selective transportation of cell toxic compounds to tumor cells.

Since the volume of a carborane group roughly is the same as that of a three-dimensional sweep of a phenyl group,[6] carboranes carrying xylosides may show high selectivity toward transformed cells. We therefore decided to investigate the biological properties of the carborane xyloside **2** (Fig. 1).

1-Hydroxy-*p*-carborane **3**[15] has been reported earlier and Lewis acid-promoted xylosylation using peracetylated trichloroacetimidate xylosyl donor[16] gave the protected carborane xyloside **4** in 90% yield (Scheme 1). Deprotection under standard Zemplén conditions gave xyloside **2** in 91% yield.[17]



Scheme 1. Reagents and conditions: (a) $Xyl(OAc)_3OC(NH)CCl_3$, CH_2Cl_2, $BF_3{\cdot}OEt_2$, 0 °C, 30 min. (b) NaOMe/MeOH–CH_2Cl_2, 0.2 M, rt, 1 h.

To test the GAG priming ability of the xylosides, normal HFL-1 cells (human fetal lung fibroblasts) and T24 cells (human bladder carcinoma cells) were incubated with 100 μM xyloside and [^{35}S]sulfate. GAG chains were then isolated from the extracellular space, from cell extracts and from the nuclei, and subsequently analyzed by size separation chromatography. Both cell lines secreted alkali sensitive proteoglycans to the extracellular space (Fig. 2, Pool I). However, treatment with the xylosides also initiated synthesis of free GAG chains

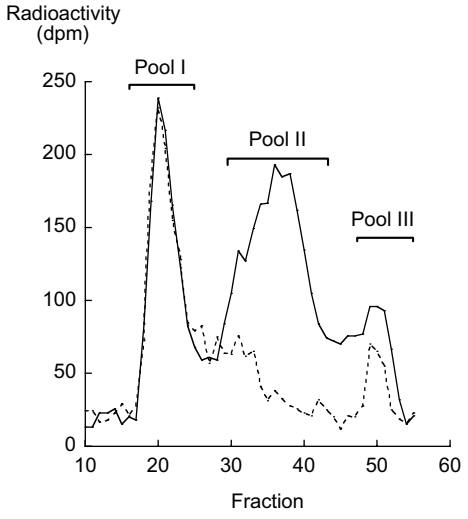


Figure 2. Priming of GAG chains in T24 cells incubated with compound **2**. Pool II and III contain xyloside-primed GAG chains of different lengths (solid line). The dashed line shows the results for untreated cells.

Table 1. GAG priming capability and antiproliferative activity (ED50, μM) of compound **2** toward HFL-1 cells and T24 cells

Cell line	GAG-priming (medium)	GAG-priming (intracellular)	GAG/PG (nuclei)	ED_{50} (μM)
HFL-1	3.7	n.d.	1.1	130
T24	2.9	0.6	1.9	330

The GAG-priming is given as the integrated value of [^{35}S]sulfate detected per minute for fractions containing GAG chains, divided by the integrated value for fractions of untreated cells.

of intermediate (Pool II) and short lengths (Pool III). The proportion of GAG-priming is given as the integrated value of [^{35}S]sulfate detected per minute for fractions in Pool II and III divided by the integrated value for fractions of untreated cells (Table 1). As a comparison, the GAG-priming capability (medium) of compound **1**, in T24 cells, was 7.2.[18]

Depending on the structure of the aglycon, different GAG chains, such as heparan sulfate (HS) or chondroitin sulfate/dermatan sulfate (CS/DS), are formed. Our earlier results indicate that priming of HS is necessary for the selective antiproliferative activity of xylosides.[7] Furthermore, the HS primed by antiproliferative xyloside appeared in the nuclei of growth inhibited cells.

The amount of HS chains primed by the cells treated with compound **2** was determined by digestion of GAG pool with HNO_2 at pH 1.5, which cleaves HS chains. No HS was detected and the secreted chains thus mainly consisted of CS/DS. Treatment with compound **2** did not increase the amount of intracellular, soluble GAG chains in T24 cells. However, the amount of GAG/PG in the nuclei of T24-cells treated with compound **2** was almost doubled (Table 1). In comparison, treatment of HFL-1 cells with compound **2** did not raise the levels of GAG/PG in the nuclei. The GAG/PG containing fractions isolated from the nuclei of T24 cells treated with compound **2** were hydrolyzed by alkali and the shown degradation indicated the presence of PG and not soluble GAG chains (Fig. 3).

Finally, the antiproliferative activity of the carborane carrying xyloside **2** was determined for HFL-1 cells and T24 cells. Xyloside **2** was added to the growth medium at various concentrations and cell proliferation was recorded using the crystal violet method.[3] The inhibitory effect of the compound is expressed as ED_{50} (μM) scored after 96 h of exposure relative to untreated cells (Table 1). The HFL-1 cells were more sensitive to xyloside **2**, compared to the effect on T24 cells.

In summary, we have synthesized a xylosylated carborane by standard carbohydrate methodology and tested this compound on normal HFL-1 cells as well as transformed T24 cells. The xylosylated carborane initiated GAG synthesis in both cell lines and also induced a pronounced translocation of proteoglycans into the nuclei of T24 cells. T24 cells predominantly express the HSPG glypican-1.[19] The amino acid sequence of glypican-1 contains a nuclear localization

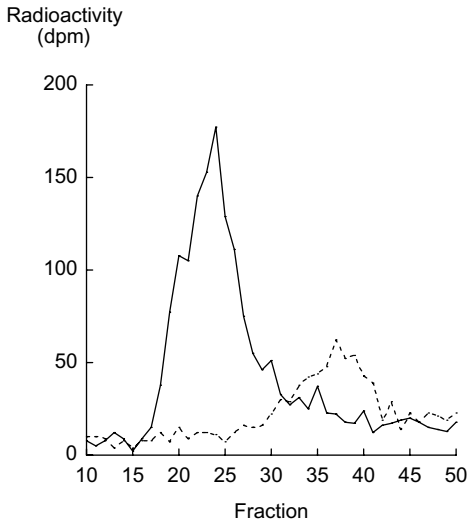


Figure 3. Isolation of PG/GAG from nuclei of T24 cells treated with compound **2** (solid line). Alkali hydrolysis indicate that the isolated material mainly consist of PG (dashed line).

signal KRRR(G/A)K, situated upstream from the HS attachment region, and transport of the full length glypican to the nuclei of COS cells, C6 glioma cells and T24 cells has been demonstrated.[20,21] Interestingly, the translocation of glypican-1 to the nuclei was shown to be a dynamic process and correlated with different phases of cell cycle, suggesting the involvement of glypican-1 in the regulation of cell division and survival.[19] It is not clear how glypican is transported across the membranes. However, it has been speculated that this is a cell specific process requiring interaction with other proteins expressed by certain cell types.[20] Treatment with carborane carrying xyloside may have arrested the T24 cells in the G1 phase acquiring prominent nuclear glypican-1 localization. We have earlier shown that the formation of HS is a necessary requirement for selective effect toward tumor cells and the exclusive formation of CS/DS may be one reason for this unselective targeting shown by carborane xylosides. We conclude that xylosides carrying carboranes are not suitable for BNCT therapy for T24 cells. However, the priming capability indicates uptake of carborane carrying xyloside by cells, and therefore it might be possible to induce HS priming in different cells. The uptake of boron containing xyloside, the GAG priming component, and the nuclear translocation of glypican-1 make this xyloside a candidate for further investigation for selective antiproliferative activity towards other tumor cell lines.

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Supplementary data

Supplementary data (experimental details, ^{1}H-NMR spectra) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.02.048.

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