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4-Deoxy-4-fluoro-xyloside derivatives as inhibitors of glycosaminoglycan biosynthesis

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ABSTRACT

Various 4-deoxy-4-fluoro-xylosides were prepared using click chemistry for evaluating their potential utility as inhibitors of glycosaminoglycan biosynthesis. 2,3-Di-*O*-benzoyl-4-deoxy-4-fluoro-β-D-xylopyranosylazide, obtained from L-arabinopyranose by six steps, was treated with a wide variety of azide-reactive triple bond-containing hydrophobic agents in the presence of Cu^{2+} salt/ascorbic acid, a step known as click chemistry. After click chemistry, benzoylated derivatives were deprotected under Zemplén conditions to obtain 4-deoxy-4-fluoro-xyloside derivatives. A mixture of α:β-isomers of twelve derivatives were then separated on a reverse phase C18 column using HPLC and the resulting twenty four 4-deoxy-4-fluoro-xylosides were evaluated for their ability to inhibit glycosaminoglycan biosynthesis in endothelial cells. We identified two xyloside derivatives that selectively inhibit heparan sulfate and chondroitin sulfate/derman sulfate biosynthesis without affecting cell viability. These novel derivatives can potentially be used to define the biological actions of proteoglycans in model organisms and also as therapeutic agents to combat various human diseases in which glycosaminoglycans participate.

Proteoglycans are composed of a core protein and glycosaminoglycan (GAG) side chains such as heparan sulfate (HS) and chondroitin sulfate (CS). The GAG side chains are attached to core protein at certain serine residues through a common linkage tetrasaccharide, GlcAβ(1→3)Galβ(1→3)Galβ(1→4)Xylβ(1-*O*-Ser).[1,2] Interactions of the sulfated GAG side chains with a wide array of proteins, including proteases, growth factors, morphogens, cytokines, and extracellular matrix structural proteins regulate numerous biological functions.[3–6] Assembly of GAG chain involves the following events: xylosylation of certain serine amino acids located on the core proteins, formation of the linkage tetrasaccharide, elongation by alternate addition of glucuronyl residues and hexosamine residues (GlcNAc for HS and GalNAc for CS), and maturation of GAG side chains with various sulfation/epimerization events along the GAG chains.[7,8]

Various xylosides carrying hydrophobic aglycone groups have been shown to induce free GAG chains in several cellular systems and model organisms for nearly four decades.[9–12] We have earlier shown that a number of 4-deoxy-4-fluoroxylosides perturb GAG biosynthesis as they do not have an acceptor hydroxyl group at C-4 position for subsequent sugar attachment and elongation.[13] Furthermore, these modified xylosides could affect galactosyltransferase-1, an enzyme involved in assembly of the linkage region, and

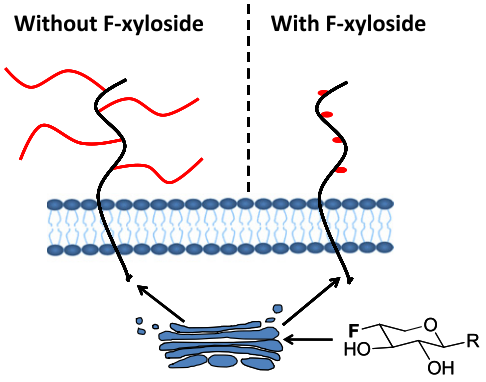

Figure 1. 4-Deoxy-4-fluoroxylosides are inhibitors of glycosaminoglycan/proteoglycan biosynthesis.

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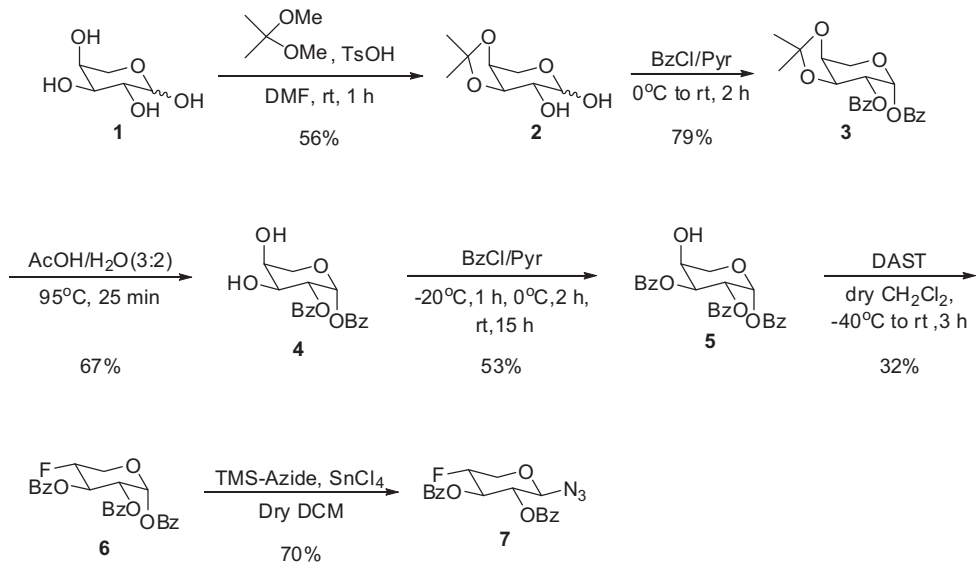
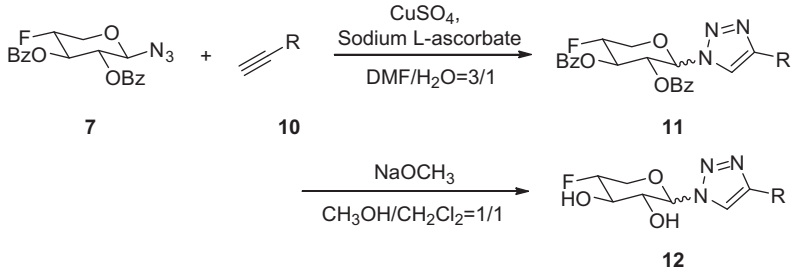


Scheme 1. Synthesis of 4-deoxy-4-fluoroxylopyranosyl azide derivative **7**.

Table 1
Synthesis of various 1-(4-deoxy-4-fluoro-D-xylopyranosyl)-1,2,3-triazoles



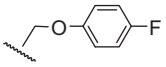
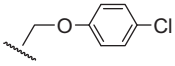
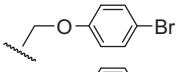
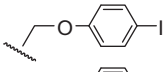
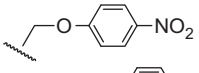
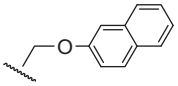
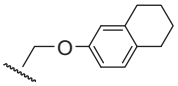
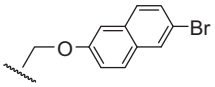
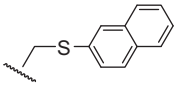
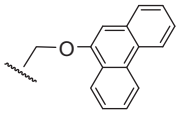
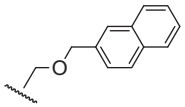
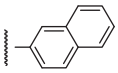
Entry	R	11			12		
		Reaction time	Yield (%)	Ratio (α:β)	Reaction time (h)	Yield (%)	Ratio (α:β)
1		16 h	93 (**11a**)	7:10	3	70 (**12a**)	3:5
2		18 h	87 (**11b**)	3:5	3	75 (**12b**)	7:10
3		18 h	91 (**11c**)	3:5	3	68 (**12c**)	3:5
4		17 h	95(**11d**)	7:10	3	71 (**12d**)	3:5
5		2 days	80 (**11e**)	4:5	3	81 (**12e**)	7:10
6		2 days	54 (**11f**)	2:1	3	58 (**12f**)	5:2
7		4 days	82 (**11g**)	4:5	8	60 (**12g**)	3:5
8		4 days	68 (**11h**)	5:2	3	56 (**12h**)	5:2
9		1 day	89 (**11i**)	1:2	3	63 (**12i**)	3:5

Table 1 (*continued*)

Entry	R	11			12		
		Reaction time	Yield (%)	Ratio (α:β)	Reaction time (h)	Yield (%)	Ratio (α:β)
10		3 days	45 (**11j**)	10:3	3	80 (**12j**)	5:2
11		2 days	87 (**11k**)	1:2	3	40 (**12k**)	7:10
12		7 days	75 (**111**)	1:2	3	70 (**121**)	2:5

Ratio [azide **7**:alkyne **10** = 1:1.5].

thereby inhibit proteoglycan (PG) biosynthesis (Fig. 1). In our efforts to develop new approaches to selectively block PG biosynthesis, we synthesized additional library of 4-deoxy-4-fluoroxylosides using click chemistry and examined them for their ability to modulate GAG biosynthesis in endothelial cells. We identified a number of 4-deoxy-4-fluoroxylosides that efficiently inhibit PG biosynthesis in a selective manner. We predict that these GAG biosynthetic inhibitors are valuable chemical biology agents to define the biological actions of GAG chains in model organisms. We describe here the syntheses of various 1-(4-deoxy-4-fluoro-β-D-xylopyranosyl)-1,2,3-triazole derivatives and their biological evaluation as GAG biosynthetic inhibitors in endothelial cells.

The synthesis of GAG biosynthetic inhibitors was begun with L-arabinopyranose **1**. We employed protection of C-3 and C-4 hydroxy groups as isopropylidene acetal of L-arabinopyranose **1** in the first step to obtain 3,4-*O*-isopropylidene-L-arabinopyranose **2**. Compound **2** was then subjected to 1,2-di-*O*-benzylation, removal of the 3,4-*O*-isopropylidene acetal and 3-*O*-benzylation to give compound **5** as shown in Scheme 1.[14,15] Deoxyfluorocarbohydrate analogs, which contain a fluorine atom instead of a hydroxyl group, are extensively used as mechanistic probes or inhibitors of various enzymes involved in glycosylation. Therefore, we predicted that replacement of the C-4 hydroxyl group with a fluorine atom in xylose might block the transfer of the galactose residue from Gal-UDP catalyzed by galactosyltransferase-1. The treatment of compound **5** carrying unprotected hydroxyl group at C-4 with diethylaminosulfur trifluoride should lead to nucleophilic displacement of the hydroxyl group by fluoride with concomitant inversion of configuration.[16] Thus, the decisive step in our synthetic strategy is the inversion of the axial hydroxyl group at C-4 of the partially benzoylated L-arabinose derivative **5** with diethylaminosulfur trifluoride (DAST) to obtain the 4-deoxy-4-fluoro-xylopyranoside derivative **6**.[17] Compound **6** was then appended with various hydrophobic groups (aglycone) at the anomeric carbon. We and other groups have shown previously that the nature of the hydrophobic aglycone dramatically influences the priming ability of xylosides, as different hydrophobic aglycones are differentially recognized by the biosynthetic enzymes involved in the assembly of GAG chains.[18,19] We identified a number of hydrophobic groups that were important for optimal priming of GAG chains by click-xylosides in our previous studies.[19,20] On the basis of those findings, we have selectively chosen a set of promising hydrophobic groups to be appended at the anomeric carbon of 4-deoxy-4-fluoroxylopyranosyl azide. We utilized the robust click chemical methodology in the present study, as we successfully employed such an approach in our earlier studies, to prepare various 4-deoxy-4-fluoroxylosides as potential biosynthetic inhibitors. We synthesized the xylopyranosyl azide derivative **7** by treating compound **6** with tetramethylsilyl azide and $SnCl_4$ under dry solvent conditions as shown in Scheme 1 (see Supplementary data for more detail).

The xylopyranosyl azide derivative **7** was then treated with a wide variety of triple bond-containing hydrophobic agents **10** in the presence of Cu^{2+} salt/ascorbic acid. The products of click reaction, 1,2,3-triazole derivatives **11**, were obtained in moderate to high yields as a mixture of α:β-isomers (Table 1). After click chemistry, benzoylated derivatives **11** were deprotected under Zemplén conditions to obtain the 4-deoxy-4-fluoroxyloside derivatives **12** (Table 1) (see Supplementary data for more detail). The α:β-isomers of compound **12** could not be separated by conventional silica gel chromatography. A mixture of the α:β-isomers of **12** *** were completely separated by reverse phase chromatography of HPLC system (see Supplementary data). The structures of obtained products, **12aα**–**12lα** and **12aβ**–**12lβ** were confirmed by ^{1}H, ^{13}C, ^{19}F NMR, and MS spectra.

Bovine lung microvascular endothelial cells (BLMVEC) were examined for viability in the presence of 4-deoxy-4-fluoroxylosides (**12aα**/**β**–**12lα**/**β**) at various concentrations using CellTiter-Blue® reagent.[21] Cell viability assay identified seven 4-deoxy-4-fluoroxylosides, **12aα**, **12aβ**, **12eα**, **12eβ**, **12kα**, **12kβ**, and **12lα**, which were not toxic to the cells at 300 μM or lower concentrations. Based on these results and our earlier findings that most 4-deoxy-4-fluoroxylsoides inhibit cellular GAG biosynthesis efficiently at 300 μM

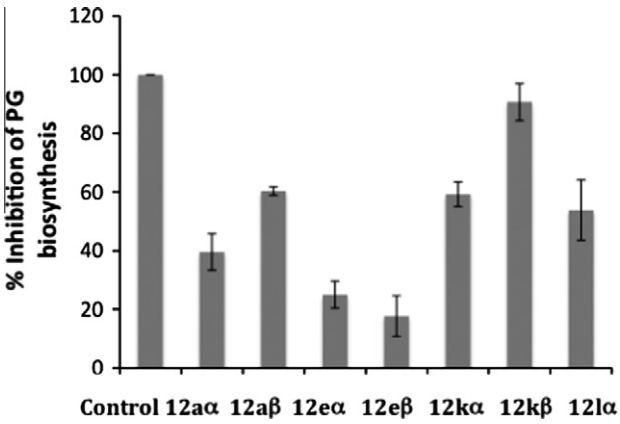


Figure 2. Inhibitory effect of 4-deoxy-4-fluoroxylosides on GAG biosynthesis in BLMVEC. Cells were incubated with 300 μM of 4-deoxy-4-fluoroxylosides for 24 h in the presence of [^{3}H]-glucosamine. Control cells were grown in the absence of compounds under identical conditions for comparison. GAG chains were then purified and radioactivity was measured using a liquid scintillation counter as described in 'References and Notes'.

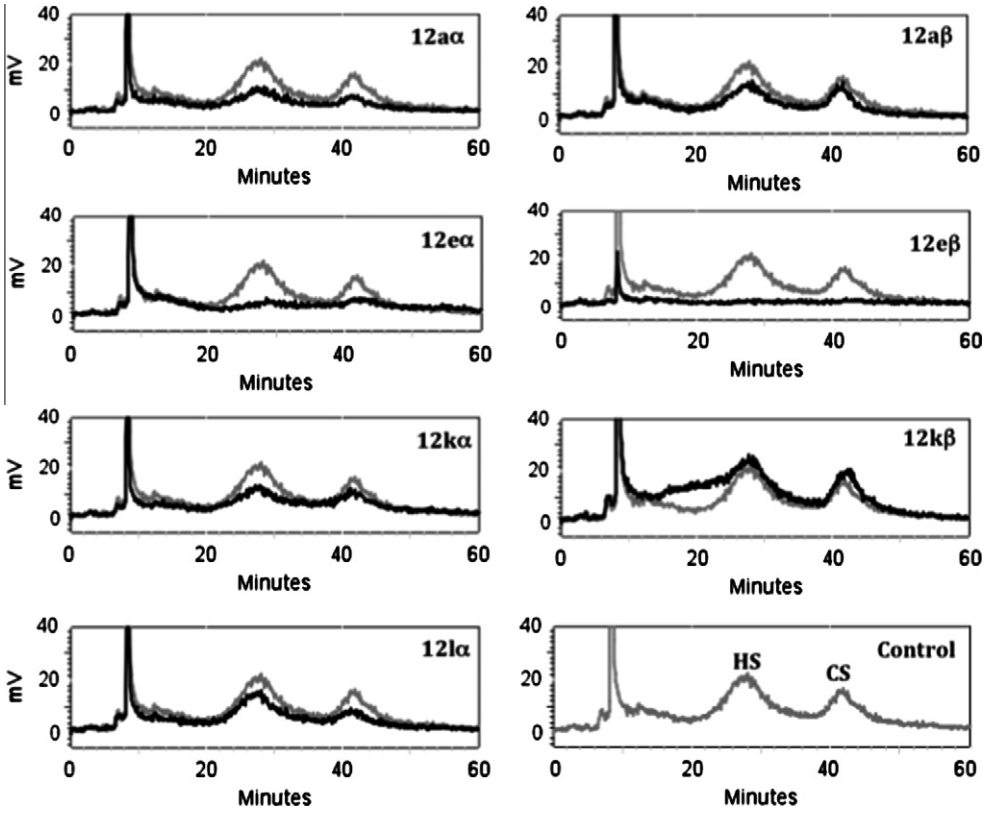


Figure 3. 4-Deoxy-4-fluoroxylosides affect GAG biosynthesis in BLMVEC. ^{3}H-labeled-GAG chains were isolated from control and 4-deoxy-4-fluoroxylosides treated cells as described in the experimental section and were loaded onto a DEAE HPLC column. Elution profiles of GAG chains were analyzed using in-line radiomatic detector coupled to HPLC. The bound GAG chains were eluted with a linear gradient of 1 M NaCl. The chromatographic profiles of GAG chains isolated from control cells that were treated with DMSO (gray trace) or cells that were treated (black trace) with 4-deoxy-4-fluoroxylosides (**12aα**, **12eα**, **12kα**, **12lα**, **12aβ**, **12eβ**, and **12kβ**) are shown here. HS: heparan sulfate, CS: chondroitin sulfate.

or higher concentrations, we decided to screen these promising seven xyloside derivatives at 300 μM for their ability to inhibit PG biosynthesis in BLMVEC cells. Cells were treated with 300 μM of 4-fluoroxylosides, **12aα**, **12aβ**, **12eα**, **12eβ**, **12kα**, **12kβ**, and **12lα**, in the presence of ^{3}H-glucosamine for 24 h. GAG chains were then purified and quantified by measuring radioactivity using a liquid scintillation counter as described in the experimental section. The results indicated that all of these 4-deoxy-4-fluoroxylosides, except **12kβ**, inhibit PG biosynthesis to various degrees in BLMVEC (Fig. 2).[22] Furthermore, we utilized anion-exchange HPLC to analyze the inhibitory activity of these seven xylosides on cellular GAG biosynthesis. HS and CS appeared in the anion-exchange chromatogram as two independent peaks between 20 and 50 min (Fig. 3). It is known that HS elutes earlier than CS in the linear salt gradient because HS carries overall less negative charge density.[20] Cells that are treated with DMSO alone have no effects on their GAG biosynthesis and thus used as control. Among the 4-deoxy-4-fluoroxylosides screened, **12eα** and **12eβ** were the best inhibitors of PG biosynthesis and these inhibitors were able to reduce GAG production by ~80% (Figs. 2 and 3) without affecting cell viability.[23]

In conclusion, we have synthesized various 1-(4-deoxy-4-fluoro-β-D-xylopyranosyl)-1,2,3-triazole derivatives **12**, screened for their ability to inhibit PG biosynthesis and identified two derivatives, **12eα** and **12eβ**, as best inhibitors of GAG biosynthesis. Future studies will be directed toward investigating the utility of these biosynthetic inhibitors in defining the structural basis for the biological actions of glycosaminoglycan glycome and advancing our knowledge of structural and functional heparanomics. These derivatives can also be used as therapeutic agents in various diseases including cancer where glycosaminoglycans are known to participate at various stages of diseases progression through regulating GAG-extracellular matrix and cell–cell interactions.

Acknowledgments

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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.2010.10.085.

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21. *Cytotoxicity assay*: BLMVEC were examined for viability in the presence of 4-deoxy-4-fluoroxylosides derivatives using CellTiter-Blue® reagent (Promega). Cells were seeded into duplicate wells of a 96-well plate at a density of 2.5×10^4 cells/well in 125 μl of Ham's F-12 medium with 10% fetal bovine serum (Hyclone). Cells were treated with DMSO (control) or 4-fluoroxylosides at different concentrations (0.1 mM, 0.3 and 1.0 mM) for 24 h at 37 °C. Cells were also treated with 3% H_2O_2 as a negative control. CellTiter-Blue reagent (25 μl) was added to each well and incubated for 2 h. The reaction was then stopped by the addition of 50 μl of 3% SDS solution. The fluorescence was measured using a Spectra-Max M5 microplate reader (Molecular Devices, Sunnyvale, CA) at an excitation wavelength of 560 nm and an emission wavelength of 590 nm.
22. *Screening of 4-deoxy-4-fluoroxylosides in BLMVEC*: Inhibition of glycosaminoglycan biosynthesis by 4-deoxy-4-fluoroxylosides was examined using BLMVEC. Cells were seeded in a 12-well plate at a density of 2×10^5 cells/well in 1 ml of Ham's F-12 medium with 10% fetal bovine serum (Hyclone). Seeded cells were incubated for 24 h at 37 °C. Each well was subsequently washed and supplemented with 890 μl of DMEM medium with 1 mM glucose and 10% dialyzed fetal bovine serum before treated with 10 μl of DMSO (vehicle, control) or 10 μl of stock solution containing 4-fluoroxylosides (30 mM in DMSO) for 24 h. One hundred microliter of ^{3}H-glucosamine (Perkin–Elmer, 100 μCi in deionized water) was added to each well to radiolabel the GAG chains. After 24 h, pronase solution was added to each well and incubated at 37 °C overnight to harvest the GAG chains. The treated samples were centrifuged at 16,000*g* for 5 min and the supernatant was diluted with half-a-volume of 0.016% Triton X-100 and loaded on a 0.2 ml DEAE-sepharose column (Amersham Biosciences). The column was first pre-equilibrated with 2 ml of wash buffer (20 mM NaOAc, 0.1 M NaCl and 0.01% Triton X-100, pH 6.0) and then washed with 6 ml of wash buffer. The bound GAGs were eluted with 1.2 ml of elution buffer (20 mM NaOAc, 1 M NaCl, pH 6.0). Fifty microliter of eluate from each well were analyzed for radioactivity using a liquid scintillation counter.
23. *Anion exchange chromatography*: The extent of inhibition of GAG biosynthesis was determined by analyzing the elution profile of the GAG chains on an anion exchange column coupled with an in-line radiometric detector. Radiolabeled GAGs were loaded onto a DEAE-HPLC column and then eluted for 80 min with a linear gradient of 0.2–1 M NaCl.