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Synthesis of aromatic *C*-xylosides by position inversion of glucose

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Abstract—Two formally C-xylosylated analogs to 2-naphthyl β-D-xylopyranoside, which is known to initiate priming of glucosaminoglycan chains, were synthesized by a position inversion of glucose (i.e., position 1 becomes position 5). The D-*C*-xyloside showed priming, while the L-*C*-xyloside did not initiate priming.

1. Introduction

Xylose is an unusual structural component in mammalian cells. So far, it has only been found in one unique position, that is, as the linker between protein and carbohydrate in proteoglycans. Proteoglycans are composed of glucosaminoglycan (GAG) chains covalently attached to a core protein (Fig. 1). The first step in GAG assembly is xylosylation of a serine residue. 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. It is still unclear what determines whether heparan sulfate (HS), with a HexA–GlcNAc repeating motif, or chondroitin sulfate/dermatan sulfate (CS/DS), with a HexA–GalNAc repeating motif, is attached to the core protein but repetitive Ser-Gly sequences and a high proportion of Phe, Tyr, or Trp promote the formation of HS.[1] The resultant HS precursor polymers are subsequently modified through N-deacetylation/N-sulfation, epimerization, and O-sulfation.

Biosynthesis of GAG chains can also take place independently of core protein synthesis by using xylosides as primers. Xylosides with hydrophobic aglycon can penetrate cell membranes and initiate GAG synthesis by serving as acceptors in the first galactosylation step. The composition of the GAG assembled on the xyloside primer depends on the structure of the aglycon, which may reflect selective partitioning of primers into different intracellular compartments or into different branches of biosynthetic pathways. In most cases, priming of CS dominates and synthesis of free HS chains is low or undetectable. Increased yields of HS can be obtained when the aglycon of the 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 and possibly also re-internalized. β-D-Xyloside-primed HS chains have interesting biological properties, such as activation of basic fibroblast growth factor,[2] antithrombotic effects,[3] and growth inhibition of transformed cells.[4–6]

We have previously reported that the HS-priming glycoside 2-(6-hydroxynaphthyl)-β-D-xylopyranoside selectively inhibits growth of transformed or tumor-derived cells in vitro as well as in vivo.[5] Treatment with this xyloside at a pharmacologically relevant dose reduced the average tumor load by 70–97% in SCID mice. Attempts to determine the mechanism for the selective growth-inhibition suggest that the effect on transformed cells is not caused by the xyloside itself but by products derived from priming of HS.[5,7] Furthermore, the bioactivity is dependent on the hydroxyl substitution pattern in the naphthalene rings of the xyloside and on nuclear targeting of the xyloside-derived products. However, the knowledge of structure recognition of the proteoglycan-synthesizing enzymes is still scarce.

To further investigate these effects, we decided to synthesize the *C*-xyloside **2** as well as the enantiomeric

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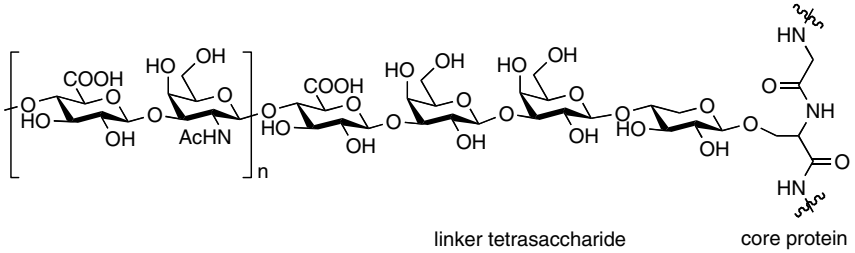


Figure 1. Glucosaminoglycan chains consist of a linker tetrasaccharide unit (GlcA(β1–3)Gal(β1–3)Gal(β1–4)Xylβ) coupled to serine residues of the protein chain.

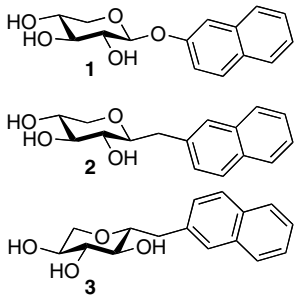


Figure 2. Xylosides used in this study.

C-xyloside **3** (Fig. 2) and compare these with 2-naphthyl β-D-xylopyranoside (**1**) which is known to prime GAG synthesis.[8]

2. Results and discussion

C-Glycosides are generally difficult to synthesize in good yields and defined stereochemistry. Instead for a C-glycosylation strategy we decided to synthesize formal *C*-xylosides by cleavage of the anomeric position of glucose and subsequent coupling of the naphthalene residue to position 6. This position inversion strategy (i.e., position 1 becomes position 5) was first introduced by Guo et al. for simple compounds (i.e., *C*-(β-pentopyranosyl)-methanols).[9]

The commercially available peracetylated D- or L-glucose was converted into the anomeric bromides that were subsequently reduced using Bu_3SnH. The addition of the radical initiator AIBN gave stable yields (97% over two steps). The acetyl-protective groups were then exchanged for benzyl ethers and the primary benzyl group was selectively deprotected using DIBAL-H by the method recently introduced by Sinaÿ and co-workers.[10] The conversion of the primary alcohol to an aldehyde was first carried out using Dess–Martin periodinane which gave 70% and several byproducts. Unfortunately, purification on SiO_2 resulted in epimerization. However, Swern oxidation gave clean quantitative conversion and the product could be used in the next step without further purification.

We tried several reagent combinations for the coupling of the naphthalene unit to the aldehyde but only reaction of bromo-naphthalene with *n*-BuLi gave good results. The 6*R*- and 6*S*-diastereomers were formed in equal amounts. Reduction of the benzylic hydroxyl group proved to be troublesome, but Et_3SiH in combination with BF_3OEt_2 gave acceptable results. The optimized conditions turned out to be high concentration of BF_3OEt_2 and short reaction times (0.4 mM, 10 min, rt) which gave 71% yield. Deprotection of the benzyl groups using hydrogenation resulted in an inseparable mix of **2** and the analogous tetrahydronaphthalene derivative. Instead the benzyl groups were exchanged for acetyl groups,[11] which were subsequently removed under Zemplén conditions. The lower yields in some of the reactions starting from **4a**, compared to **4b**, are due to the smaller scale.

There are several observations that the biological effects of xylosides are dependent on the polarity (i.e., log *P*) of the compounds.[7,12] The gradient HPLC retention times, in contrary to isocratic retention times, can be treated as linear free-energy-related parameters,[13] that is, gradient HPLC retention times can be used to substitute log *P* values in biological evaluations. The gradient HPLC retention times for the naphthoxylosides were measured using a C-18 column and a mobile phase of water (0.1% trifluoroacetic acid) with a gradient of acetonitrile from one minute increasing by 1.2% per minute up to 30 min. The retention times were measured for three separate runs per compound and the calculated mean retention times are presented in Table 1. The *C*-xylosides **2** and **3** are slightly less polar compared to the *O*-xyloside **1**.

To test the xyloside's ability to prime GAG synthesis, mouse 3T3 fibroblasts were incubated with 0.1 mM xyloside and [^{35}S]sulfate followed by isolation and size separation of free GAG chains. All cells secreted alkali-sensitive proteoglycans (Fig. 3, Pool I). However, treatment with some xylosides also initiated synthesis of free GAG chains (Fig. 3, Pool II). The total amount of GAG-priming was determined by integration of fractions in Pool II (Table 1).

Table 1. Data for compounds **1**–**3**

Compound	Retention time (min)	Priming (dpm)
1	19.0 ± 0.2	8749
2	20.9 ± 0.3	735
3	20.9 ± 0.3	10

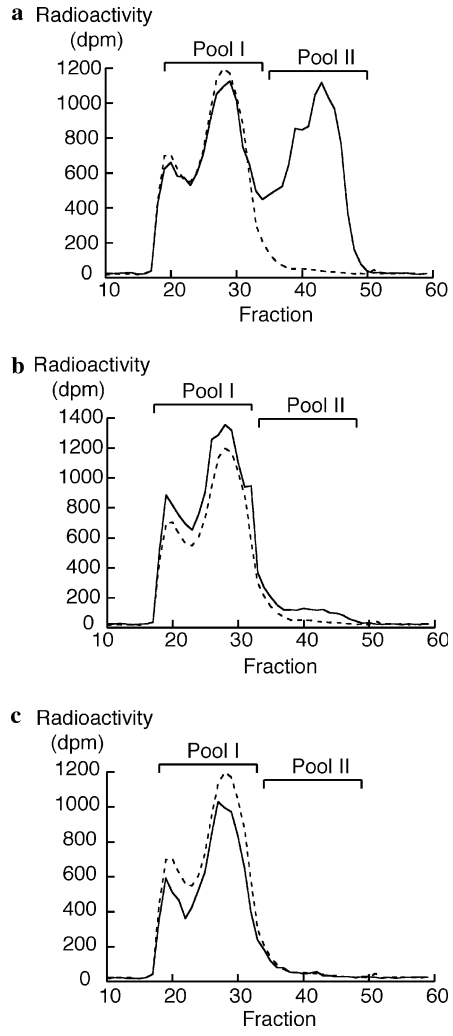


Figure 3. Priming of GAG-chains in mouse 3T3 fibroblasts incubated with **1** (a), **2** (b) or **3** (c). Pool II contains xyloside-primed GAG chains. Dashed lines show the result for untreated cells.

3. Conclusions

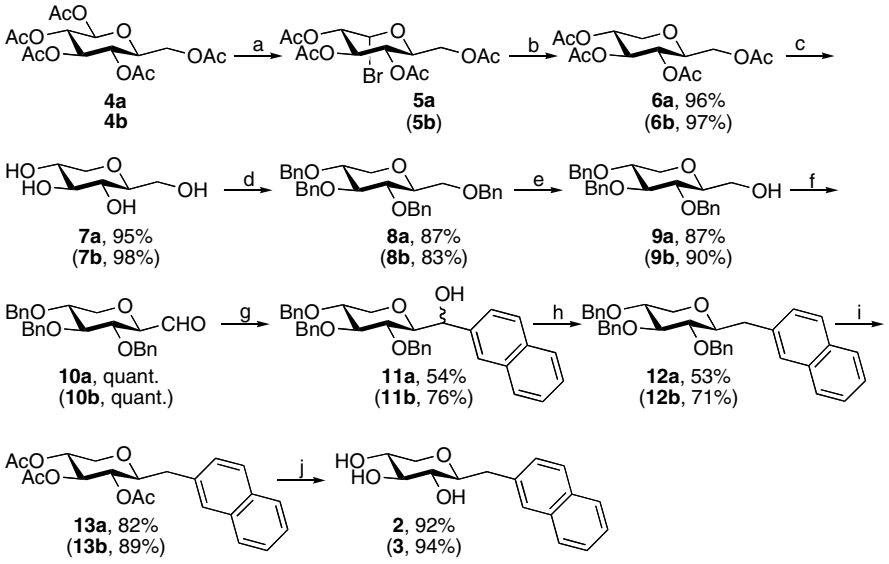
We have synthesized two *C*-xylosides (**2** and **3**) by a position inversion-strategy starting with glucose. This strategy opens up for the synthesis of other xylosides, as well as lyxose, arabinose, and ribose derivatives (see Scheme 1).

Our results show that the ability of GAG priming is much lower for the *C*-xyloside **2** compared to the *O*-xyloside **1** despite the lower polarity of **2**. However, the lower GAG-priming ability of **2** is in accordance with an earlier study where the corresponding phenyl glycosides were tested.[14] The L-*C*-xyloside **3** was not able to initiate any GAG priming, which correlates well with earlier results on L-*O*-xylosides.[4] All together these results demonstrate the importance of the sugar structure (D-xylose) as well as the glycosidic bond (O vs C) for GAG priming.

4. Experimental

4.1. General synthetic methods

NMR spectra were recorded at 300, 400 or 500 MHz. ^{1}H NMR spectra were assigned using 2D-methods (COSY, HETCOR, and long range HETCOR). Chemical shifts are given in parts per million downfield from the signal for Me_4Si, with reference to residual undeuterated solvents. Reactions were monitored by TLC using alumina plates coated with silica gel and visualized using either UV light or by charring with *p*-anisaldehyde (dip solution). Preparative chromatography was performed with silica gel (35–70 μm, 60 Å). Known (**4a**,[15] **5a**,[16] **5b**,[17] **7a**,[18] **7b**,[17] **8b**,[9] and **9b**[10]) and commercially available compounds (**4b** and **6b**) were in agreement with previously published data.



Scheme 1. Reagents and conditions: (a) Ac_2O, HBr/AcOH, 90 min; (b) Bu_3SnH, AIBN, KF, 20 min; (c) NaOMe, 45 min; (d) NaH, BnBr, DMF, 16 h; (e) DIBAL-H, toluene, 50 °C, 2 h; (f) $(COCl)_2$, DMSO, CH_2Cl_2, Et_3N, −61 °C, 45 min; (g) 2-bromonaphthalene, *n*-BuLi, THF, −78 °C, 2.5 h; (h) Et_3SiH, BF_3OEt_2, CH_2Cl_2, 10 min; (i) Ac_2O, BF_3OEt_2, 0 °C, 17 h; (j) NaOMe, 1 h. Parenthesized yields are for the synthesis starting with D-Glc (**4b**).

4.2. Tetra-*O*-acetyl-α-L-glucopyranosyl bromide (5a)

Compound **4a** (1.03 g, 2.64 mmol) was dissolved in AcOH (3.5 mL). Ac_2O (0.5 mL) was added followed by fresh HBr/AcOH 33% (3.5 mL) and stirred for 90 min at rt. Argon was flushed through the solution for 15 min. The solution was then lyophilized to give crude material that was used in the next step without further purification.

4.3. Tetra-*O*-acetyl-α-D-glucopyranosyl bromide (5b)

Synthesized as compound **5a**.

4.4. 2,3,4,6-Tetra-*O*-acetyl-1,5-anhydro-L-glucitol (6a)

The crude compound **5a** was dissolved in dry Et_2O (4.6 mL) under Ar. Bu_3SnH (0.780 mL, 2.90 mmol) was added to the solution followed by a catalytic amount of AIBN. The solution was stirred in rt for 30 min. KF (0.520 g) dissolved in H_2O (2 mL) was added and the mixture was stirred rapidly for 20 min. The mixture was filtered through Celite and the filter cake was washed with Et_2O. The H_2O phase was extracted with Et_2O. The organic phases were washed with H_2O, concentrated, and recrystallized from Et_2O and pentane to give **6a** (0.844 g, 96%) as white crystals; mp 70–71 °C; $[\alpha]_D^{21}$ −40.0 (*c* 0.7 in $CDCl_3$); ^1H NMR ($CDCl_3$): δ 5.21 (t, 1H, $J = 9.5$ Hz; H-3), 4.99–5.06 (m, 2H, H-2, H-4), 4.12–4.24 (m, 3H; H-1_e, 2× H-6), 3.58–3.63 (m, 1H; H-5), 3.31 (t, 1H, $J = 11.0$ Hz; H-1_a), 2.10, 2.04, 2.04, 2.02, (s, 3H each; OAc); ^{13}C NMR data ($CDCl_3$): δ 170.9, 170.6, 170.0, 169.7, 76.7, 73.9, 69.2, 68.6, 67.1, 62.4, 21.0, 20.95, 20.92, 20.8; HRMS calcd for $C_{14}H_{21}O_9$ (M+H): 333.1186, found: 333.1193.

4.5. 2,3,4,6-Tetra-*O*-acetyl-1,5-anhydro-D-glucitol (6b)

Synthesized as compound **6a** (3.30 g, 97%). $[\alpha]_D^{21}$ +42.3 (*c* 1.0 in $CHCl_3$); [lit.[19] $[\alpha]_D$ +42.6 (*c* 1.4 in $CHCl_3$)].

4.6. 1,5-Anhydro-L-glucitol (7a)

Compound **6a** (0.813 g, 2.45 mmol) was dissolved in NaOMe/MeOH (20 mL, 0.05 M) and stirred for 45 min at rt. Amberlite IR-120 H^+ was added and stirred until the solution turned neutral. The Amberlite was filtered off and washed with MeOH. The organic phase was concentrated to give **7a** (382 mg, 95%) as a white amorphous solid; $[\alpha]_D^{21}$ −39.0 (*c* 0.5 in MeOD); ^1H NMR (MeOD): δ 3.89 (dd, 1H, $J_1 = 11.0$ Hz, $J_2 = 5.4$ Hz, H-1), 3.83 (dd, 1H, $J_1 = 11.8$ Hz, $J_2 = 2.1$ Hz; H-6), 3.60 (dd, 1H, $J_1 = 11.8$ Hz, $J_2 = 5.8$ Hz, H-6), 3.19–3.49 (m, 5H; H-1, H-2, H-3, H-4, H-5); ^{13}C NMR data (MeOD): δ 82.6, 80.1, 72.0, 71.6, 71.1, 63.2; HRMS calcd for $C_6H_{12}O_5Na$ (M+Na): 187.0582, found: 187.0578.

4.7. 1,5-Anhydro-D-glucitol (7b)

Synthesized as compound **7a** (1.6 g, 98%). $[\alpha]_D^{21}$ +41.9 (*c* 0.7 in MeOH); [lit.[9] $[\alpha]_D^{25}$ +42 (*c* 0.5 in H_2O)].

4.8. 1,5-Anhydro-2,3,4,6-tetra-*O*-benzyl-L-glucitol (8a)

Compound **7a** (374 mg, 2.28 mmol) was dissolved in freshly distilled DMF (9.5 mL) and NaH (1.2 g) was added under Ar. The mixture was cooled to 10 °C and BnBr (2.0 mL, 17 mmol) was added dropwise. The mixture was stirred at rt for 16 h. MeOH (10 mL) was slowly added, followed by H_2O (11 mL). HCl (4 M) was added until the solution becomes neutral. The crude product was extracted with CH_2Cl_2 and the organic phase was washed with H_2O, dried ($MgSO_4$), and concentrated. The residue was chromatographed (SiO_2, 3:1 heptane/EtOAc) to give **8a** (1.05 g, 87%) as a colorless syrup. $[\alpha]_D^{21}$ −9.8 (*c* 1.0 in $CDCl_3$). ^1H NMR ($CDCl_3$): δ 7.19–7.32 (m, 18H; ArH), 7.07–7.11 (m, 2H; ArH), 4.80, 4.93 (ABq, 1H each, $J = 11.0$ Hz; OBn), 4.45, 4.78 (ABq, 1H each, $J = 10.7$ Hz; OBn), 4.59, 4.67 (ABq, 1H each, $J = 11.6$ Hz; OBn), 4.46, 4.54 (ABq, 1H each, $J = 12.2$ Hz; OBn), 3.99 (dd, 1H, $J_1 = 11.1$ Hz, $J_2 = 4.7$ Hz; H-1_e), 3.48–3.66 (m, 5H; H-2, H-3, H-6 × 2, H-4), 3.33 (ddd, 1H, $J_1 = 9.5$ Hz, $J_2 = 4.3$ Hz, $J_3 = 2.1$ Hz ; H-5), 3.12–3.21 (m, 1H; H-1_a); ^{13}C NMR data ($CDCl_3$): δ 139.0, 138.38, 138.36, 138.1, 128.7, 128.6, 128.2, 128.15, 128.11, 128.08, 127.93, 127.91, 127.8, 86.6, 79.5, 78.7, 78.0, 75.8, 75.3, 73.8, 73.5, 69.2, 68.4; HRMS calcd for $C_{34}H_{37}O_5$ (M+H): 525.2641, found: 525.2639.

4.9. 1,5-Anhydro-2,3,4,6-tetra-*O*-benzyl-D-glucitol (8b)

Synthesized as compound **8a** (6.90 g, 83%). $[\alpha]_D^{21}$ +10.6 (*c* 0.9 in $CHCl_3$), $[\alpha]_D^{21}$ +27.1 (*c* 0.8 in CH_2Cl_2); [lit.[9] $[\alpha]_D^{20}$ +27 (*c* 0.5 in $CHCl_3$)].

4.10. 1,5-Anhydro-2,3,4-tri-*O*-benzyl-L-glucitol (9a)

Compound **8a** (1.02 g, 1.95 mmol) was dissolved in distilled toluene (27 mL). DIBAL (20 mL, 1 M in CH_2Cl_2), was added dropwise under Ar. The temperature was increased to 50 °C and the mixture was stirred for 2 h. The solution was poured on ice, HCl (26 mL, 1 M) was added and the mixture was stirred rapidly for 10 min. The mixture was diluted with EtOAc (100 mL) and the H_2O phase was extracted with EtOAc. The combined organic phases were washed with NaCl (satd aq), dried ($MgSO_4$), and concentrated. The residue was chromatographed (SiO_2, 1:1 heptane/EtOAc) to give **9a** (733 mg, 87%) as a white amorphous solid. $[\alpha]_D^{20}$ −17.0 (*c* 0.6 in $CHCl_3$) ^1H NMR ($CDCl_3$): δ 7.28–7.38 (m, 15H; ArH), 4.88, 5.00 (ABq, 1H each, $J = 11.0$ Hz; OBn), 4.65, 4.90 (ABq, 1H each, $J = 11.0$ Hz; OBn), 4.65, 4.74 (ABq, 1H each, $J = 11.6$ Hz; OBn), 4.01 (dd, 1H, $J_1 = 11.0$ Hz, $J_2 = 4.9$ Hz; H-1_e), 3.81–3.87 (m, 1H; H-1_e), 3.58–3.70 (m, 3H; H-2, H-3, H-6), 3.50 (t, 1H, $J = 9.1$ Hz; H-4), 3.27–3.32 (m, 2H; H-5, H-6), 3.24 (t, 1H, $J = 10.7$ Hz; H-1_a), 1.76 (t, 1H, $J = 6.45$ Hz; OH); ^{13}C NMR data ($CDCl_3$): δ 138.9, 138.34, 138.27, 128.7, 128.6, 128.3, 128.12, 128.06, 127.9, 86.4, 79.9, 78.8, 77.8, 75.8, 75.4, 73.6, 68.2, 62.5; HRMS calcd for $C_{27}H_{31}O_5$ (M+H): 435.2171, found: 435.2182.

4.11. 1,5-Anhydro-2,3,4-tri-*O*-benzyl-D-glucitol (9b)

Synthesized as compound **9a** (170 mg, 90%). $[\alpha]_D^{20}$ +16.5 (*c* 0.9 in $CHCl_3$); [lit.[10] $[\alpha]_D^{20}$ +18 (*c* 1.0 in $CHCl_3$)].

4.12. 2,6-Anhydro-3,4,5-tri-*O*-benzyl-D-glucose (10a)

Oxalyl chloride (0.015 mL, 0.175 mmol) was diluted with dry CH_2Cl_2 (0.28 mL) and cooled to −61 °C. DMSO (0.022 mL, 0.310 mmol) in dry CH_2Cl_2 (0.140 mL) was added and the mixture was stirred for 5 min. Compound **9a** (50 mg, 0.115 mmol) in dry CH_2Cl_2 (0.140 mL) was added dropwise and stirred for 30 min at −61 °C. Et_3N (0.081 mL) was added and the stirring continued for 15 min at −61°C. The mixture was allowed to attain rt and H_2O (1 mL) was added. The mixture was diluted with CH_2Cl_2 and the organic phase was washed with H_2O, concentrated, and dried under vacuum overnight to give crude **10a** as a yellow syrup that was used in the next step without further purification.

4.13. 2,6-Anhydro-3,4,5-tri-*O*-benzyl-L-glucose (10b)

Synthesized as compound **10a**.

4.14. (6*R* and 6*S*)-1,5-Anhydro-2,3,4-tri-*O*-benzyl-6-*C*-naphthyl-L-glucitol (11a)

2-Bromonaphthalene (95.3 mg, 0.460 mmol) was dissolved in distilled THF (1.0 mL) and cooled to −78 °C. *n*-BuLi (1.6 M in hexane, 0.287 mL, 0.460 mmol) was added dropwise and stirred at −78 °C for 45 min. Compound **10a** was dissolved in distilled THF (0.5 mL) and added dropwise (10 min) and stirred for 2.5 h at −78 °C. The mixture was allowed to attain rt and NH_4Cl (2 mL, satd aq) was added. The mixture was diluted with Et_2O and the organic phase was washed with H_2O, concentrated, and chromatographed (SiO_2, 2:1 heptane/EtOAc) to give a 6*R*/6*S*-mixture of **11a** (35.0 mg, 54%) as a white amorphous solid used in the next step without separation of diastereomers.

4.15. (6*S* and 6*R*)-1,5-Anhydro-2,3,4-tri-*O*-benzyl-6-*C*-naphthyl-D-glucitol (11b)

Synthesized as compound **11a** (195 mg, 76%).

4.16. 2-((2,3,4-Tri-*O*-benzyl-β-D-xylopyranosyl) methyl)-naphthalene (12a)

Compound **11a** (35.0 mg, 0.0624 mmol) was dissolved in dry CH_2Cl_2 (3.5 mL) and Et_3SiH (0.140 mL) was added under Ar. BF_3·OEt (0.170 mL) was added and the mixture was stirred for 10 min. $NaHCO_3$(4 mL, satd aq) was added, the H_2O phase was extracted with CH_2Cl_2, and the organic phases were washed with H_2O, dried ($MgSO_4$) and concentrated. The residue was chromatographed (SiO_2, 4:1 heptane/EtOAc) to give **12a** (18.0 mg, 53%) as a clear syrup. $[\alpha]_D^{21}$ +11.0 (*c* 1.0 in $CDCl_3$). ^1H NMR ($CDCl_3$): δ 7.75–7.82 (m, 3H; ArH), 7.65 (s, 1H; ArH), 7.41–7.46 (m, 2H; ArH), 7.28–7.38 (m, 16H; ArH), 5.02, 4.87 (ABq, 1H each, J = 10.9 Hz; OBn), 5.00, 4.70 (ABq, 1H each, J = 11.0 Hz; OBn), 4.71, 4.63 (ABq, 1H each, J = 11.7 Hz; OBn), 3.94 (dd, 1H, J_1 = 11.2 Hz, J_2 = 4.9 Hz; H-5), 3.60–3.72 (m, 2H; H-3, H-4), 3.54 (dt, 1H, J_1 = 9.3 Hz, J_2 = 2.3 Hz; H-1), 3.29–3.38 (m, 2H; H-2, H-2, Nap-CH_2), 3.09 (t, 1H, J = 10.7 Hz; H-5), 2.77 (dd, 1H, J_1 = 14.3 Hz, J_2 = 9.1 Hz; Nap-CH_2); ^{13}C NMR data ($CDCl_3$): δ 138.9, 138.5, 138.4, 136.4, 133.7, 132.4, 128.72, 128.68, 128.6, 128.1, 128.05, 128.03, 127.98, 127.89, 127.89, 127.87, 127.8, 127.7, 126.0, 125.5, 86.7, 81.8, 80.9, 79.1, 75.8, 75.5, 73.4, 68,2, 38.7 ; HRMS calcd for $C_{37}H_{36}O_4$ (M+H): 545.2692, found: 545.2714.

4.17. 2-((2,3,4-Tri-*O*-benzyl-β-L-xylopyranosyl)methyl)-naphthalene (12b)

Synthesized as compound **12a** (27.6 mg, 71%). $[\alpha]_D^{22}$ −10.3° (*c* 1.0 in $CDCl_3$). HRMS calcd for $C_{37}H_{37}O_4$ (M+H): 545.2692, found: 545.2700.

4.18. 2-((2,3,4-Tri-*O*-acetyl-β-D-xylopyranosyl)methyl)-naphthalene (13a)

Compound **12a** (17.0 mg, 0.0312 mmol) was dissolved in Ac_2O (3.2 mL) and cooled to 0 °C. BF_3·OEt (0.050 mL) was added and the mixture was stirred for 17 h at 0 °C. The mixture was diluted with EtOAc and washed with $NaHCO_3$ (satd aq) and H_2O. The organic phase was dried (Na_2SO_4), and concentrated. The residue was chromatographed (SiO_2, 2:1 heptane/EtOAc) to give **13a** (10.3 mg, 82%) as a white amorphous solid. $[\alpha]_D^{21}$ −17.4 (*c* 0.8 in $CDCl_3$). ^1H NMR ($CDCl_3$): δ 7.75–7.83 (m, 3H; ArH), 7.63 (s, 1H; ArH), 7.49–7.40 (m, 2H; ArH), 7.33 (dd, 1H, J_1 = 8.4 Hz, J_2 = 1.6 Hz; ArH), 5.19 (t, 1H, J = 9.4 Hz; H-3), 5.03–4.92 (m, 2H; H-2, H-4), 4.06 (dd, 1H , J_1 = 11.2 Hz, J_2 = 5.6 Hz; H-5), 3.66–3.73 (m, 1H; H-1), 3.18 (t, 1H, J = 11.0 Hz; H-5), 3.00, 2.92 (ABq, 1H, J = 14.3 Hz; Nap-CH_2), 3.00, 2.90 (ABq, 1H, J = 14.4 Hz; Nap-CH_2); ^{13}C NMR data ($CDCl_3$): δ 170.7, 170.0, 134.9, 133.6, 132.5, 128.1, 127.9, 127.83, 127.78, 126.3, 125.7, 79.2, 74.2, 72.7, 69.5, 67.0 38.6, 21.0, 20.94, 20.92; HRMS calcd for $C_{22}H_{24}O_7Na$ (M+Na): 423.1420, found: 423.1431.

4.19. 2-((2,3,4-Tri-*O*-acetyl-β-L-xylopyranosyl)methyl)-naphthalene (13b)

Synthesized as compound **13a** (18 mg, 89%). $[\alpha]_D^{22}$ +18.5 (*c* 1.2 in $CDCl_3$); HRMS calcd for $C_{22}H_{24}O_7Na$ (M+Na): 423.1420, found: 423.1416.

4.20. 2-((β-D-Xylopyranosyl)methyl)-naphthalene (2)

Compound **13a** (10.3 mg, 0.0257 mmol) was dissolved in NaOMe/MeOH (25 mL, 0.05 M) and stirred for 60 min at rt. Amberlite IR-120 H^+ was added and stirred until the solution turned neutral. The Amberlite was filtered off and washed with MeOH. The organic phase was concentrated to give **2** (6.5 mg, 92%) as a white amorphous solid. $[\alpha]_D^{21}$ −21.1 (*c* 0.2 in MeOD). ^1H NMR (MeOD): δ 7.67–7.82 (m, 4H; ArH), 7.37–7.45 (m, 3H; ArH), 3.81

(dd, 1H, J_1 = 11.1 Hz, J_2 = 5.4 Hz; H-5$_e$), 3.27–3.47 (m, 4H; H-4, H-1, Nap-CH_2, H-3), 3.13 (t, 1H, J = 9.1 Hz; H-2), 3.05 (t, 1H, J = 10.9 Hz; H-5$_a$), 2.80 (dd, 1H, J_1 = 14.2 Hz, J_2 = 8.6 Hz; Nap-CH_2), ^{13}C NMR data (MeOD): δ 136.6, 133.8, 132.5, 128.1, 127.7, 127.3, 127.2, 125.6, 125.0, 81.5, 78.8, 73.9, 70.4, 69.8, 38.0; HRMS calcd for $C_{16}H_{19}O_4$ (M+H): 275.1283, found: 275.1290.

4.21. 2-((β-L-Xylopyranosyl)methyl)-naphthalene (3)

Synthesized as compound **2** (18 mg, 94%). $[\alpha]_D^{22}$ +22.3 (*c* 0.2 in MeOD). HRMS calcd for $C_{16}H_{19}O_4Na$ (M+Na): 297.1103, found: 297.1111.

4.22. HPLC of naphthoxylosides

High-performance liquid chromatography was run on a Hewlett Packard Series II 1090 Liquid chromatograph and a Supelco™ LC-18-DB column (15 cm × 4.6 mm, 5 μm). The system was controlled by the Hewlett Packard ChemStation for LC software suite. Mobile phase consisted of H_2O + 0.1% trifluoroacetic acid (TFA) with a gradient of acetonitrile from one minute increasing by 1.2% per minute until 30 min. The mean retention times were calculated from three separate runs per compound.

4.23. General biological methods

The normal mouse 3T3 fibroblasts were obtained from ATCC, Rockville, MD. Regular cell culture media, L-glutamine, penicillin–streptomycin, trypsin, and donor calf serum were obtained from Life Technologies. Minimal essential medium (MEM) was purchased from Sigma. $Na_2{}^{35}SO_4$ (1310 Ci/mmol) and D-[1-^{3}H]galactose (20 Ci/mmol) were obtained from Amersham International, UK. The prepacked Superdex Peptide HR 10/30, Dextran T-500, and octyl-Sepharose CL-4B were from Pharmacia-LKB, Sweden, and DE-53 DEAE–cellulose was from Whatman. Water for HPLC-analysis was from a Millipore Milli-Q system.

4.24. Cell culture, radiolabeling, and extraction procedures

Cells were cultured as monolayers in MEM supplemented with fetal bovine serum (10%, v/v), L-glutamine (2 mM), penicillin (100 U/mL), and streptomycin (100 μg/mL) in an incubator with humidified atmosphere and 5% CO_2 at 37 °C. Confluent cells were incubated in low-sulfate, $MgCl_2$-labeling medium supplemented with glutamine (2 mM), [^{35}S]sulfate (50 mCi/mL), and different xylosides. Dilutions were made from 20 mM stock solutions in DMSO/H_2O (1:1, v/v). Incorporation of [^{3}H]galactose was performed in MEM containing 20 μCi/mL [^{3}H]galactose. After the incubation period, culture medium was collected and pooled with two washings of ice-cold PBS (0.137 M NaCl, 3 mM KCl, 8 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.5). Cells were extracted with 0.1–0.2 mL/cm^2 dish of a solution of 0.15 M NaCl, 10 mM EDTA, 2% (v/v)Triton X-100, 10 mM KH_2PO_4, pH 7.5, and 5 μg/mL ovalbumin containing 10 mM *N*-ethylmaleimide, and 1 mM diisopropylphosphoro-fluoridate on a slow shaker at 4 °C for 10 min.

4.25. Isolation of xyloside-primed radiolabeled GAG

The procedures have been described in detail previously.[4] [^{35}S]Sulfate-labeled polyanionic macromolecules were isolated from the culture medium by ion exchange-chromatography on DEAE–cellulose at 4 °C. Samples were mixed with 1.3 vol of urea (7 M), Tris (10 mM), pH 7.5, and Triton X-100 (0.1%), and passed over a 1 mL-column of DE-53 equilibrated with urea (6 M), NaOAc (0.5 M), pH 5.8, ovalbumin (5 μg/mL), and Triton X-100 (0.1%). After sample application, the columns were washed successively with 10 mL-portions of (a) equilibration buffer, (b) urea (6 M), Tris (10 mM), pH 8.0, ovalbumin (5 μg/mL), and Triton X-100 (0.1%), and (c) 50 mM Tris, pH 7.5. Bound material was eluted with 5× 1 mL guanidine–HCl (4 M), NaOAc (50 mM), pH 5.8, and ovalbumin (5 μg/mL). Radioactive fractions were pooled, precipitated with ethanol (95%, 5 vol) overnight at −20 °C using 100 μg of dextran as carrier. After centrifugation in a Beckman JS-7.5 at 4000 rpm and 4 °C for 45 min, the material was dissolved in guanidine–HCl (4 M), NaOAc (50 mM), pH 5.8, and free xyloside-primed GAG chains were separated from PG by hydrophobic interaction chromatography on octyl-Sepharose followed by gel permeation FPLC on Superose 6. Radioactivity was determined in a β-counter.

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Supplementary data

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