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Synthesis and biology of bis-xylosylated dihydroxynaphthalenes

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Abstract—The 10 analogous bis-xylosylated dihydroxynaphthalenes have been synthesized and their chemical and biological properties investigated. The yield of the xylosylation reactions can be correlated to the electrostatic potential, and thus to the nucleophilicity, for the oxygen atoms of the dihydroxynaphthalenes. The bis-xylosylated compounds were more stable compared to the mono-xylosylated ones. They initiate priming of glycosaminoglycan chains to less extent but the priming proceeds in two directions. Contrary to the mono-xylosylated analogs, the tested compounds did not show any antiproliferative properties.

1. Introduction

Proteoglycans are composed of glycosaminoglycan (GAG) chains covalently attached to a core protein (Fig. 1). The first step in GAG assembly is xylosylation of a serine residue and 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. The resultant GAG precursor polymers are subsequently modified, presumably during chain elongation, through a series of reactions such as N-deacetylation/N-sulfation, epimerization of some GlcA residues to IdoA units, and also O-sulfation at various positions.

By using xylosides with hydrophobic aglycon, that can penetrate cell membranes, as primers, the biosynthesis of GAG chains can be artificially initiated independently of core protein synthesis. The xylosides initiate GAG synthesis by serving as acceptors in the first galactosylation step. The composition of the GAG chains assembled on the xyloside primers 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 chondroitin sulfate/dermatan sulfate (CS/DS) dominates and synthesis of free heparan sulfate (HS) chains is low or undetectable. It has recently been shown that in polarized cells xyloside based GAG chains secreted basolaterally are more intensely sulfated than their apical counterparts.[1] 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]

The HS-priming 2-(6-hydroxynaphthyl)-β-D-xylopyranoside (**5d**) has previously been reported to selectively inhibit 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 have also been made.[4,5] 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 HS on the xyloside. Furthermore, the bioactivity is dependent on (iii) the hydroxyl substitution pattern in the naphthalene rings of the xyloside and (iv) nuclear targeting of the xyloside-derived products. However, the knowledge of structure recognition of the proteoglycan synthesizing enzymes is still scarce.

Keywords: Carbohydrates; Dihydroxynaphthalene; Glycosylation; Xylose.
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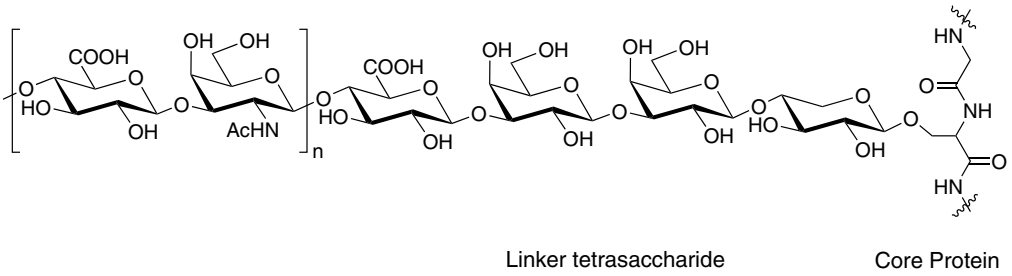

Figure 1. Glycosaminoglycan chains consist of a linker tetrasaccharide unit (GlcA(β1-3)Gal(β1-3)Gal(β1-4)Xylβ) coupled to serine residues of the protein chain. The general structure is then modified through N-deacetylation/N-sulfation, epimerization, and O-sulfation.

A series of mono-xylosylated dihydroxynaphthalenes (**1**–**10d**, **e**) which gave insight into the mechanisms of priming and antiproliferative properties of these compounds have been reported earlier.[7] In that study a correlation between the antiproliferative effect toward some cell lines and the polarity of the compounds was found. However, some compounds (e.g., 2-(6-hydroxynaphthyl)-β-D-xylopyranoside) were clearly different and gave a selective antiproliferative effect toward transformed cells (e.g., T24). It was also found that some compounds, especially the 1-(4-hydroxynaphthyl)-β-D-xylopyranoside (**1d**), were further hydroxylated in the Ham's F-12 medium used for cell experiments. In the present study, we investigate the stability, the GAG priming capability, and the antiproliferative effects of a series of bis-xylosylated compounds. The bis-xylosylated compounds expand the variation in properties, such as polarity, and also give the possibility for priming at two different positions in the same compound.

2. Results and discussion

Glycosylation of dihydroxynaphthalenes is a difficult task due to the strongly activated aromatic system.[8] Depending on the substitution pattern, electrophilic aromatic substitution competes with glycosylation of the hydroxyl groups. Another problem is steric hindrance as well as intramolecular hydrogen bonds which lower the activity toward glycosylation in the 1,8-, 2,3-, and 1,2-dihydroxynaphthalenes (**3a**, **4a**, **7a**). The synthesis of the acetylated bis-xylosides was therefore first performed using peracetylated xylose activated by BF_3OEt_2, with addition of Et_3N to minimize anomerization, in CH_2Cl_2.[9] This gave excellent yields (>80%) for 2,6-dihydroxynaphthalene bis(2,3,4-tri-*O*-acetyl-β-D-xylopyranoside) (**5b**), 2,7-dihydroxynaphthalene bis(2,3,4-tri-*O*-acetyl-β-D-xylopyranoside) (**6b**), 1,6-dihydroxynaphthalene bis(2,3,4-tri-*O*-acetyl-β-D-xylopyranoside) (**9b**), and 1,7-dihydroxynaphthalene bis(2,3,4-tri-*O*-acetyl-β-D-xylopyranoside) (**10b**). However, 1,4-dihydroxynaphthalene bis(2,3,4-tri-*O*-acetyl-β-D-xylopyranoside) (**1b**), 1,5-dihydroxynaphthalene bis(2,3,4-tri-*O*-acetyl-β-D-xylopyranoside) (**2b**), and 1,3-dihydroxynaphthalene bis(2,3,4-tri-*O*-acetyl-β-D-xylopyranoside) (**8b**) were synthesized in low to moderate yields (22–64%), whereas 1,8-dihydroxynaphthalene bis(2,3,4-tri-*O*-acetyl-β-D-xylopyranoside) (**3b**), 2,3-dihydroxynaphthalene bis(2,3,4-tri-*O*-acetyl-β-D-xylopyranoside) (**4b**), and 1,2-dihydroxynaphthalene bis(2,3,4-tri-*O*-acetyl-β-D-xylopyranoside) (**7b**) could not be synthesized by this method. In the syntheses of compounds **1b**, **2b**, **7b**, and **8b** we observed mono-xylosylated material, whereas the attempted syntheses of compounds **3b** and **4b** gave complete degradation of the starting material (Chart 1).

To further investigate xylosylation of dihydroxynaphthalenes, the electrostatic potentials and the distances between the phenolic oxygen atoms for the ten dihydroxynaphthalenes were calculated using density functional theory at the B3LYP/6-31G* level and default settings in Spartan '02 for Macintosh (Table 1).[10] As expected, the average electrostatic potential was correlated to the yield in the glycosylation reactions, that is, higher electrostatic potential, which correlates to the nucleophilicity of the oxygen atoms, gave better yields in the reaction (Fig. 2). However, the isolation of mono-xylosylated material in some cases also indicates steric effects.

To xylosylate the unreactive compounds **3a**, **4a**, and **7a**, a trichloroacetimidate donor, activated with TMSOTf, was used.[11,12] Trichloroacetimidate donors are well known to be more active compared to the analogous acetylated compounds and thus better suited for the less reactive phenols.[8] This gave **4b** in 58% and **7b** in 33%, but still **3b** could not be synthesized. The problem is probably the strong hydrogen bond between the two hydroxyl groups of the 1,8-dihydroxynaphthalene which renders this compound less nucleophilic. Since compound **3a** is highly acidic (pK_a 6.71),[13] the synthesis was instead performed under basic conditions. **3b** was then synthesized using the corresponding bromosugar and phase transfer conditions, with NaOH as base, to give a yield of 13%.[14–16] The synthesis of **7b** was also performed under these conditions giving 14% yield. This indicates that **3a** is the substance most affected by the acidity of the hydroxyl groups. The deprotection was performed using methanolic NaOMe (0.05 M). Problems with high crystallinity affected the purification of some compounds. **1c**, **2c**, and **5c** could thus not be purified using normal phase column chromatography since they were virtually insoluble in most used solvents (with the exception of DMSO), and therefore they were purified using reverse phase HPLC. All compounds were additionally purified on reverse phase HPLC prior to biological evaluation.

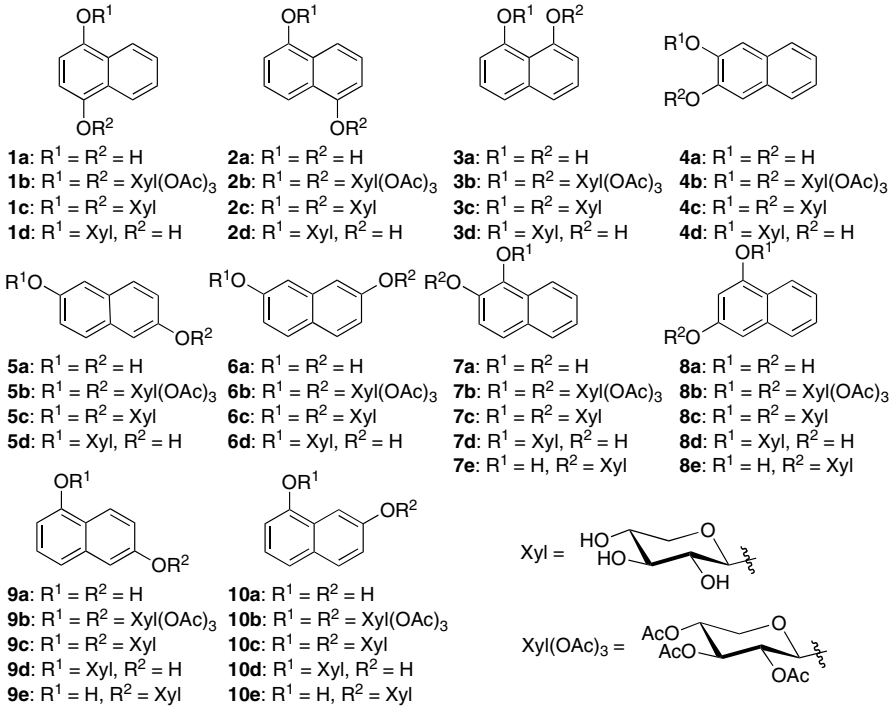

Chart 1. Dihydroxynaphthalenes (**1**–**10a**) and the analogous bis-xylosylated compounds (**1**–**10b**, **c**) as well as the mono-xylosylated compounds (**1**–**10d**, **e)** described in this paper.

Table 1. Yield of bis-xylosylation of dihydroxynaphthalenes, average electrostatic potential and distance between the two oxygen atoms of the naphthalene unit

Compound	Yield of bis-xylosylation (%)	Average electrostatic potential (kcal/mol)	Distance (nm)
1a	64	−30.3	0.56
2a	55	−28.0	0.60
3a	0	−23.6	0.26
4a	0	−23.1	0.27
5a	82	−32.0	0.78
6a	81	−30.9	0.73
7a	0	−23.7	0.27
8a	22	−27.5	0.48
9a	95	−29.9	0.63
10a	96	−28.4	0.51

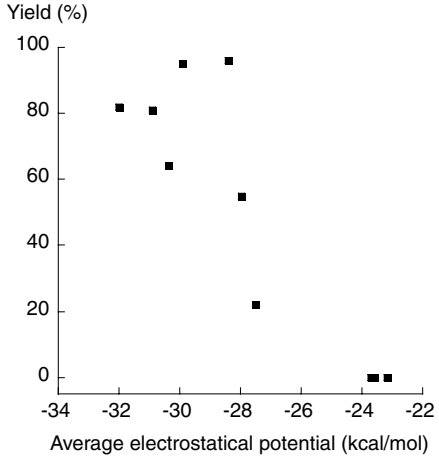

Figure 2. Yield of the bis-xylosylation reactions versus average electrostatic potential (kcal/mol) of the two oxygen atoms of the naphthalene unit.

Gradient HPLC retention times can be used to substitute log *P* values in biological evaluations.[17] The gradient HPLC retention times for the compounds were measured using a C-18 column and a mobile phase of water (0.1% TFA) with a gradient of MeCN from 1 min increasing by 1.2% per minute. The retention times were measured for three separate runs per compound, and the calculated mean retention times are presented in Table 2. As a comparison retention times for the mono-xylosylated compounds **1**–**10d**, **e** are shown. As expected, the bis-xylosylated compounds, carrying two polar carbohydrate residues, show shorter retention times. A substantial variation of the polarity due to the substitution pattern can also be seen. For example, the compounds with 1,2-, 1,3- or 1,8-substitution are more unpolar, while 1,4- and 2,6-substitution gives polar compounds. Obviously, the polarity is connected to the distance between the polar groups, that is, the carbohydrate residues. When the distance between the two phenolic oxygen atoms of the naphthalene systems was plotted against the HPLC retention times a correlation was found as indicated in Figure 3.

To evaluate the stability of the compounds, the xylosides were dissolved in DMSO/water (1:1, 20 mM), diluted with cell growth medium (Ham's F-12 medium) to 0.2 mM, and incubated at 37 °C. Samples were taken at 0, 1, 3, 24, 48, 72, and 96 h, and the amounts of xyloside, as compared to 4,4′-dihydroxybiphenyl used as an

Table 2. HPLC retention times (min) for bis- and mono-xylosylated dihydroxynaphthalenes

Bis-xylosylated compound	Retention time (min)	Mono-xylosylated compound	Retention time (min)
1c	15.93 ± 0.00	**1d**	19.60 ± 0.01
2c	17.03 ± 0.04	**2d**	18.70 ± 0.01
3c	24.17 ± 0.03	**3d**	28.54 ± 0.02
4c	23.54 ± 0.07	**4d**	25.73 ± 0.03
5c	15.86 ± 0.01	**5d**	17.94 ± 0.09
6c	18.09 ± 0.01	**6d**	20.25 ± 0.01
7c	24.52 ± 0.02	**7d**	27.93 ± 0.02
		7e	27.97 ± 0.03
8c	20.56 ± 0.00	**8d**	21.99 ± 0.02
		8e	24.33 ± 0.01
9c	18.02 ± 0.01	**9d**	20.12 ± 0.06
		9e	19.03 ± 0.04
10c	22.33 ± 0.01	**10d**	24.01 ± 0.03
		10e	22.44 ± 0.01

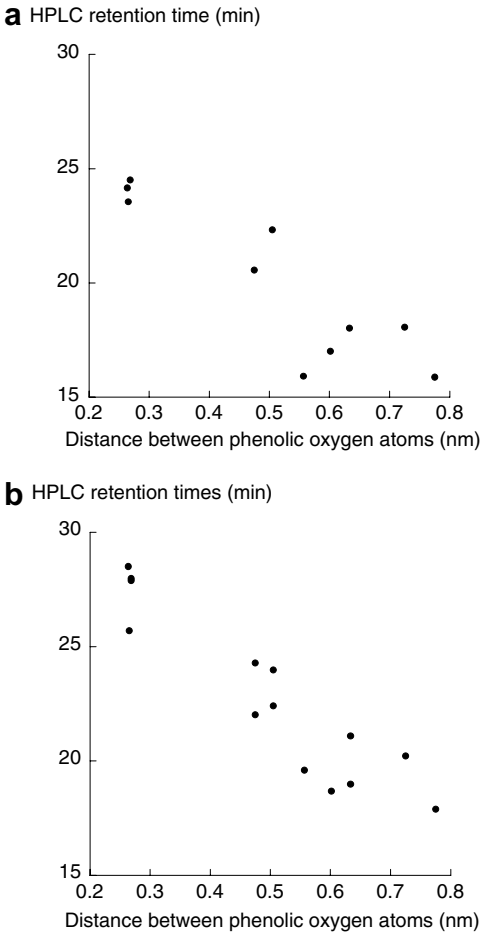

Figure 3. HPLC retention time correlated to the distance between the phenolic oxygen atoms for (a) bis-xylosylated compounds and (b) mono-xylosylated compounds.

internal standard, were measured by HPLC using a C-18 column and a mobile phase of water (0.1% TFA) with a gradient of MeCN from 1 min increasing by 1.2% per minute. No decomposition of any xyloside was observed, even after 96 h. This is to be compared to earlier data for the analogous mono-xylosylated compounds where compounds **8d** and **10e** showed slight decomposition and compounds **1d** and **7e** were totally decomposed after less than 80 h.[7] The addition of the second xylose moiety thus seems to give a shielding effect that prevents oxidation as compared to the mono-xylosylated analogs.

To test the xyloside's ability to prime GAG synthesis, T24 cells (human bladder carcinoma cells) were incubated with 100 μM xyloside and [^{35}S]sulfate followed by isolation and size separation of free GAG chains. All cells secreted alkali sensitive proteoglycans (example shown in Fig. 4, Pool I). However, treatment with some xylosides also initiated synthesis of free GAG chains (Fig. 4, Pool II). The proportion of GAG-priming is given as the integrated value of fractions in Pool II (Table 3) divided by the integrated value for fractions of untreated cells. In addition we have also tested selected mono-xylosylated compounds and the results are shown in Table 3. Apart from compounds **2c** and **5c**, the bis-xylosylated compounds seem to be less capable of GAG priming compared to the mono-xylosylated ones. No correlations between the GAG priming capability and the polarity of the compounds or the distance between the xylose residues have been found.

The separation procedure of the GAG chains also indicates the relative size of different GAG chains, that is, longer chains are eluted faster from the column. As indicated in Figure 5 and Table 3, the GAG chains from bis-xylosylated dihydroxynaphthalenes elute faster compared to the mono-xylosylated analogs, that is, the GAG chains are longer. This is probably due to priming in two directions, even if it is not possible from this experiment to exclude priming of longer chains in only one direction.

In order to verify bidirectional priming by dihydroxynaphthalenes, the xyloside-primed GAG chains synthesized by compounds **2c**, **5c**, and **9c** were subjected to periodate oxidation-alkaline elimination that would selectively cleave the xylose structure. The samples were then rechromatographed on Superose 6. As expected,

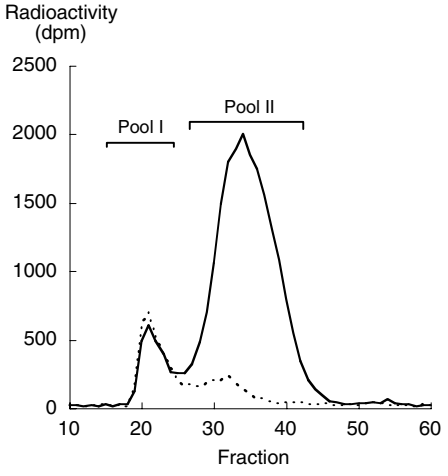

Figure 4. Priming of GAG chains in T24 cells incubated with **5c**. Pool II contains xyloside-primed GAG chains. The dashed line show the result for untreated cells.

Table 3. GAG-Priming of bis- and mono-xylosylated compounds and antiproliferative activity (ED_{50}, μM) of bis-xylosylated dihydroxynaphthalenes towards T24 cells

Bis-xylosylated compound	Priming	Peak maximum (fraction)	Peak maximum (periodate cleavage)	Antiproliferative activity (ED_{50}, μM)	Mono-xylosylated compound	Priming	Peak maximum (fraction)
1c	1.2	35		400	**1d**	7.5	42
2c	8.7	35	42	400	**2d**	7.7	44
3c	1.6	35					
4c	2.5	33					
5c	8.8	34	44	≫400	**5d**	7.2	43
6c	1.3	33		450	**6d**	5.7	42
7c	1.0	32					
8c	1.4	35		≫400	**8d**	2.7	38
					8e	4.3	40
9c	3.6	35	43				
10c	2.7	35					

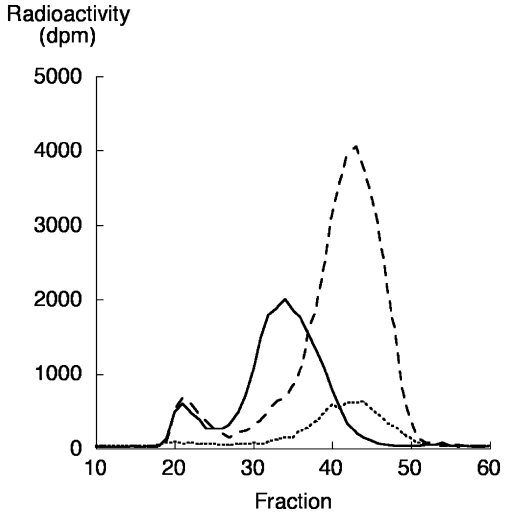

Figure 5. Priming of GAG chains in T24 cells incubated with bis-xyloside **5c** (solid line) compared to the mono-xyloside **5d** (dashed line). The dotted line shows the result from periodate oxidation-alkaline elimination of GAG chains primed by **5c**.

this resulted in shorter chains, similar to those from monodirectional priming (Fig. 5 and Table 3).

Finally we investigated the growth inhibiting properties of a selection of the bis-xylosylated dihydroxynaphthalenes on T24 cells. Despite the fact that several of the mono-xylosylated analogs show antiproliferative activities in the order of 25–100 μM, none of the bis-xylosylated analogs gave any strong growth inhibition (Table 3). The reasons why bis-xylosylated analogs did not exert antiproliferative activity could be several fold. It may be due to the type and the composition of the GAG chains produced by the xylosides. It has earlier been shown that priming of HS is required but insufficient for the antiproliferative activity. It has also been shown that HS chains primed by the antiproliferative xylosides are degraded by nitric oxide at $GlcNH_3^+$. That results in anhydromannose containing degradation products localized to the nuclei in growth inhibited cells. The antiproliferative effect has also been shown to be accompanied by increased apoptosis in tumor derived cells.[7] Furthermore, other studies have shown that CS/DS priming *p*-nitrophenyl β-D-xylopyranoside protects normal fibroblasts against apoptosis through PDGF-BB-induced GAG secretion.[18] The structure of the GAG chains initiated on the antiproliferative xylosides may be different depending on the aglycon structure and this may endow the GAG products with the ability to enter the nucleus and exert an antiproliferative response. Another explanation for the lack of antiproliferative activity of these compounds could be due to either too high polarity or too big size that makes the transport to the nuclei impossible. Polyhydroxylated naphthalenes are well known for their toxic effects, caused by redox cycling between semiquinones and quinones resulting in superoxide radicals and apoptosis.[19–21] Another possibility is thus that the bis-xylosylated analogs, due to the lack of a free hydroxyl group, may not be able to induce toxic effects and therefore are less growth inhibiting.

3. Conclusions

We have synthesized a collection of the ten analogous bis-xylosylated dihydroxynaphthalenes and investigated their chemical and biological properties. The yield of the xylosylation reactions could be correlated to the electrostatic potential for the oxygen atoms of the dihydroxynaphthalenes. All bis-xylosylated compounds were more polar, compared to the mono-xylosylated analogs, and the polarity could be correlated with the distance between the polar carbohydrate residues. The bis-xylosylated compounds were also more stable compared to the mono-xylosylated ones. Our results also indicate that these compounds, except for compounds **2c** and **5c**, initiate priming to a lesser extent than their mono-xylosylated analogs but that the priming proceeds in two directions. None of the tested compounds showed antiproliferative properties.

4. Experimental

4.1. Chemistry

NMR spectra were recorded with a Bruker DRX 400 MHz. ^1H NMR spectra were assigned using

2D-methods (COSY). Chemical shifts are given in ppm downfield from the signal for Me_4Si, with reference to residual $CHCl_3$ or DMSO. Reactions were monitored by TLC using alumina plates coated with silica gel and visualized using either UV light or by charring with *para*-anisaldehyde. Preparative chromatography was performed with silica gel (35–70 μm, 60 Å). CH_2Cl_2 was dried on Al_2O_3. Known and commercially available compounds were in agreement with previously published data (e.g., NMR). Compounds **1**–**10d**, **e** were synthesized as described elsewhere.[22]

4.1.1. 1,4-Dihydroxynaphthalene bis(2,3,4-tri-*O*-acetyl-β-D-xylopyranoside) (1b). 1,4-Dihydroxynaphthalene (50 mg, 0.31 mmol) and 1,2,3,4-tetra-*O*-acetyl-β-D-xylopyranoside (397 mg, 1.25 mmol) were dissolved in CH_2Cl_2 (2.5 mL) and Et_3N (0.03 mL, 0.21 mmol) was added. The mixture was stirred under nitrogen at rt $BF_3{\cdot}OEt_2$ (0.190 mL, 1.50 mmol) dissolved in CH_2Cl_2 (0.625 mL) was added dropwise during 10 min. After 2 h, the reaction mixture was neutralized with Et_3N and concentrated. The residue was chromatographed (SiO_2, heptane/EtOAc 3:4) to give **1b** (128 mg, 64%) as an amorphous brown solid. An analytical sample was recrystallized from ethanol. Mp 113.1–114.4 °C. $[\alpha]_D^{23}$ −97 (*c* 0.35, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 8.09 (dd, 2H, *J* = 6.5, 3.3 Hz, H-5′, H-8′), 7.51 (dd, 2H, *J* = 6.5, 3.3 Hz, H-6′, H-7′), 6.99 (s, 2H, H-2′, H-3′), 5.36 (dd, 2H, *J* = 8.0, 6.1 Hz, H-2), 5.26–5.31 (m, 4H, H-1, H-3), 5.03–5.08 (m, 2H, H-4), 4.26 (dd, 2H, *J* = 12.1, 4.7 Hz, H-5), 3.55 (dd, 2H, *J* = 12.1, 7.8 Hz, H-5), 2.13 (s, 6H, OAc), 2.09 (s, 12H, OAc). ^{13}C NMR ($CDCl_3$): δ 170.1, 170.0, 148.3, 126.8, 126.7, 121.8, 109.1, 99.4, 70.8, 70.3, 68.6, 62.1, 20.9. HRMS calcd for $C_{32}H_{37}O_{16}$ (M+H): 677.2082; found: 677.2081.

4.1.2. 1,4-Dihydroxynaphthalene bis(β-D-xylopyranoside) (1c). Compound **1b** (20 mg, 0.031 mmol) was stirred in methanolic NaOMe (0.05 M, 2 mL) for 2 h and then acidified with HOAc and concentrated. The residue was purified using HPLC and then lyophilized to give **1c** (7 mg, 53%). $[\alpha]_D^{22}$ −66 (*c* 0.28, DMSO-d_6). ^{1}H NMR (DMSO-d_6): δ 8.28 (dd, 2H, *J* = 6.5, 3.3 Hz, H-5′, H-8′), 7.52 (dd, 2H, *J* = 6.5, 3.3 Hz, H-6′, H-7′), 7.05 (s, 2H, H-2′, H-3′), 5.50 (d, 2H, *J* = 5.5 Hz, OH), 5.14 (d, 2H, *J* = 4.8 Hz, OH), 5.07 (d, 2H, *J* = 5.0 Hz, OH), 4.87 (d, 2H, *J* = 7.6 Hz, H-1), 3.77 (dd, 2H, *J* = 11.2, 5.2 Hz, H-5), 3.37–3.45 (m, 4H, H-2, H-4), 3.22–3.33 (m, 4H, H-3, H-5). ^{13}C NMR (DMSO-d_6): δ 147.8, 126.3, 125.8, 122.0, 110.0, 102.5, 76.3, 73.3, 69.4, 65.7. HRMS calcd for $C_{20}H_{24}O_{10}Na$ (M+Na): 447.1267; found: 447.1261.

4.1.3. 1,5-Dihydroxynaphthalene bis(2,3,4-tri-*O*-acetyl-β-D-xylopyranoside) (2b). Same method as for **1b**. Chromatographed (SiO_2, heptane/EtOAc 3:4 → 1:3) to give **2b** (112 mg, 55%) as an amorphous white solid. $[\alpha]_D^{23}$ −90 (*c* 0.50, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 7.84 (d, 2H, *J* = 8.6 Hz, H-4′, H-8′), 7.38 (t, 2H, *J* = 8.0 Hz, H-3′, H-7′), 7.10 (d, 2H, *J* = 7.5 Hz, H-2′, H-6′), 5.27–5.38 (m, 6H, H-1, H-2, H-3), 5.03–5.08 (m, 2H, H-4), 4.27 (dd, 2H, *J* = 12.2, 4.6 Hz, H-5), 3.59 (dd, 2H, *J* = 12.1, 7.5 Hz, H-5), 2.14, 2.10, 2.07 (s, 6H each, OAc). ^{13}C NMR ($CDCl_3$): δ 170.03, 169.98, 169.7, 152.4, 127.1, 125.8, 116.6, 109.7, 98.8, 70.6, 70.1, 68.5, 62.0, 21.0, 20.9. HRMS calcd for $C_{32}H_{37}O_{16}$ (M+H): 677.2082; found: 677.2089.

4.1.4. 1,5-Dihydroxynaphthalene bis(β-D-xylopyranoside) (2c). Same method as for **1c**. Purified using HPLC and then lyophilized to give **2c** (13 mg, 49%). $[\alpha]_D^{22}$ −77 (*c* 0.41, DMSO-d_6). ^{1}H NMR (DMSO-d_6): δ 7.94 (d, 2H, *J* = 8.5 Hz, H-4′, H-8′), 7.39 (t, 2H, *J* = 7.8 Hz, H-3′, H-7′), 7.14 (d, 2H, *J* = 7.4 Hz, H-2′, H-6′), 5.49 (d, 2H, *J* = 5.5 Hz, OH), 5.16 (d, 2H, *J* = 4.9 Hz, OH), 5.09 (d, 2H, *J* = 4.9 Hz, OH), 5.01 (d, 2H, *J* = 7.5 Hz, H-1), 3.79 (dd, 2H, *J* = 11.1, 5.1 Hz, H-5), 3.38–3.46 (m, 4H, H-2, H-4), 3.25–3.33 (m, 4H, H-3, H-5). ^{13}C NMR (DMSO-d_6): δ 152.5, 126.5, 125.3, 115.9, 109.7, 101.6, 76.3, 73.2, 69.4, 65.7. HRMS calcd for $C_{20}H_{25}O_{10}$ (M+H): 425.1448; found: 425.1442.

4.1.5. 1,8-Dihydroxynaphthalene bis(2,3,4-tri-*O*-acetyl-β-D-xylopyranoside) (3b). 1,8-Dihydroxynaphthalene (102 mg, 0.64 mmol), 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide (471 mg, 1.39 mmol), and tetrabutylammonium bromide (244 mg, 0.76 mmol) were dissolved in CH_2Cl_2 (14 mL) and NaOH (aq, 1 M, 14 mL) was added. The mixture was stirred vigorously for 2 h and then diluted with EtOAc (70 mL) and the phases were separated. The organic phase was washed with NaOH (1 M) three times, H_2O three times, brine once, dried (Na_2SO_4), and concentrated. The residue was chromatographed (SiO_2, heptane/EtOAc 4:3) to give **3b** (51 mg, 13%) as an amorphous white solid. $[\alpha]_D^{23}$ −133 (*c* 0.50, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 7.49 (dd, 2H, *J* = 8.3, 0.8 Hz, H-4′, H-5′), 7.35 (dd, 2H, *J* = 8.1, 7.8 Hz, H-3′, H-6′), 7.09 (dd, 2H, *J* = 7.7, 0.8 Hz, H-2′, H-7′), 5.62 (d, 1H, *J* = 7.6 Hz, H-2), 5.59 (d, 1H, *J* = 7.6 Hz, H-2), 5.22–5.27 (m, 4H, H-3, H-4), 5.07 (d, 2H, *J* = 7.6 Hz, H-1), 4.32 (dd, 2H, *J* = 12.0, 4.8 Hz, H-5), 3.52 (dd, 2H, *J* = 11.9, 8.3 Hz, H-5), 2.09, 2.07, 1.93 (s, 6H each, OAc). ^{13}C NMR ($CDCl_3$): δ 170.7, 170.1, 169.7, 155.1, 137.0, 126.6, 123.0, 110.9, 102.1, 72.7, 70.7, 69.9, 21.0, 20.95, 20.90. HRMS calcd for $C_{32}H_{37}O_{16}$ (M+H): 677.2082; found: 677.2074.

4.1.6. 1,8-Dihydroxynaphthalene bis(β-D-xylopyranoside) (3c). Same method as for **1c**. Chromatographed (SiO_2, CH_2Cl_2/MeOH 3:1) to give **3c** (23 mg, 82%) as an amorphous white solid. The product was additionally purified using HPLC and then lyophilized before biological tests. $[\alpha]_D^{22}$ −58 (*c* 0.19, DMSO-d_6). ^{1}H NMR (DMSO-d_6): δ 7.55 (d, 2H, *J* = 7.5 Hz, H-4′, H-5′), 7.41 (t, 2H, *J* = 7.9 Hz, H-3′, H-6′), 7.16 (d, 2H, *J* = 7.2 Hz, H-2′, H-7′), 5.01, (d, 2H, *J* = 7.6 Hz, H-1), 4.25–5.25 (br s, 6H, OH), 3.74 (dd, 2H, *J* = 11.2, 5.2 Hz, H-5), 3.24–3.47 (m, 8H, H-2, H-3, H-4, H-5). ^{13}C NMR (DMSO-d_6): δ 154.1, 126.5, 122.3, 111.5, 103.2, 75.9, 73.4, 69.2, 65.9. HRMS calcd for $C_{20}H_{25}O_{10}$ (M+H): 425.1448; found: 425.1446.

4.1.7. 2,3-Dihydroxynaphthalene bis(2,3,4-tri-*O*-acetyl-β-D-xylopyranoside) (4b). 2,3-Dihydroxynaphthalene (31 mg, 0.19 mmol), 2,3,4-tri-*O*-acetyl-α/β-D-xylopyranosyl trichloroacetimidate (262 mg, 0.62 mmol), and

MS 300AW (0.3 g) were dissolved in CH_2Cl_2 (3 mL) and stirred at r.t. under nitrogen for 40 min. TMSOTf (0.05 M in CH_2Cl_2, 0.400 mL) was added dropwise to the mixture which was then stirred for 1 h. Et_3N was added (pH 8) and the mixture was concentrated. The residue was chromatographed (SiO_2, heptane/EtOAc 3:4) to give **4b** (72 mg, 58%) as an amorphous white solid. An analytical sample was recrystallized from heptane/toluene. Mp 99.3–101.1 °C. $[\alpha]_D^{23}$ −47 (*c* 0.49, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 7.69 (dd, 2H, J = 6.1, 3.3 Hz, H-5′, H-8′), 7.44 (s, 2H, H-1′, H-4′), 7.39 (dd, 2H, J = 6.2, 3.3 Hz, H-6′, H-7′), 5.35 (d, 2H, J = 5.6 Hz, H-1), 5.23–5.26 (m, 4H, H-2, H-3), 5.02–5.06 (m, 2H, H-4), 4.30 (dd, 2H, J = 12.2, 4.6 Hz, H-5), 3.59 (dd, 2H, J = 12.2, 7.2 Hz, H-5), 2.10 (s, 18H, OAc). ^{13}C NMR ($CDCl_3$): δ 170.2, 170.0, 169.4, 146.9, 130.4, 127.0, 125.5, 114.5, 99.6, 70.6, 70.3, 68.6, 62.1, 21.0, 20.94, 20.88. HRMS calcd for $C_{32}H_{36}O_{16}Na$ (M+Na): 699.1901; found: 699.1934.

4.1.8. 2,3-Dihydroxynaphthalene bis(β-D-xylopyranoside) (4c). Same method as for **1c**. Chromatographed (SiO_2, 60:35:5 CH_2Cl_2/MeOH/H_2O) to give **4c** (11 mg, 86%) as an amorphous white solid. The product was additionally purified using HPLC and then lyophilized before biological tests. $[\alpha]_D^{22}$ −16 (*c* 0.18, DMSO-d_6). ^{1}H NMR (DMSO-d_6): δ 7.77 (dd, 2H, J = 6.1, 3.3 Hz, H-5′, H-8′), 7.52 (s, 2H, H-1′, H-4′), 7.36 (dd, 2H, J = 6.2, 3.3 Hz, H-6′, H-7′), 5.22 (d, 2H, J = 4.9 Hz, OH), 5.14 (d, 2H, J = 4.8 Hz, OH), 5.08 (d, 2H, J = 5.0 Hz, OH), 4.99 (d, 2H, J = 7.3 Hz, H-1), 3.79 (dd, 2H, J = 11.2, 5.0 Hz, H-5), 3.23–3.45 (m, 8H, H-2, H-3, H-4, H-5). ^{13}C NMR (DMSO-d_6): δ 147.1, 129.5, 126.6, 124.7, 113.4, 101.9, 76.1, 73.1, 69.3, 65.7. HRMS calcd for $C_{20}H_{24}O_{10}Na$ (M+Na): 447.1267; found: 447.1257.

4.1.9. 2,6-Dihydroxynaphthalene bis(2,3,4-tri-*O*-acetyl-β-D-xylopyranoside) (5b). Same method as for **1b**. Chromatographed (SiO_2, heptane/EtOAc 3:4) to give **5b** (164 mg, 82%) as an amorphous white solid. $[\alpha]_D^{23}$ −35 (*c* 0.50, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 7.67 (d, 2H, J = 8.9 Hz, H-4′, H-8′), 7.33 (d, 2H, J = 2.5 Hz, H-1′, H-5′), 7.17 (dd, 2H, J = 8.8, 2.4 Hz, H-3′, H-7′), 5.20–5.30 (m, 6H, H-1, H-2, H-3), 5.01–5.06 (m, 2H, H-4), 4.27 (dd, 2H, J = 12.1, 4.6 Hz, H-5), 3.58 (dd, 2H, J = 12.1, 7.5 Hz, H-5), 2.10 (s, 12H, OAc), 2.09 (s, 6H, OAc). ^{13}C NMR ($CDCl_3$): δ 170.1, 170.0, 169.6, 153.7, 130.8, 129.0, 119.7, 111.7, 98.8, 70.7, 70.3, 68.6, 62.0, 20.94, 20.92, 20.89. HRMS calcd for $C_{32}H_{37}O_{16}$ (M+H): 677.2082; found: 677.2068.

4.1.10. 2,6-Dihydroxynaphthalene bis(β-D-xylopyranoside) (5c). Same method as for **1c**. Purified using HPLC and then lyophilized to give **5c** (11 mg, 74%). $[\alpha]_D^{22}$ +2 (*c* 0.43, DMSO-d_6). ^{1}H NMR (DMSO-d_6): δ 7.76 (d, 2H, J = 8.9 Hz, H-4′, H-8′), 7.39 (d, 2H, J = 2.5 Hz, H-1′, H-5′), 7.20 (dd, 2H, J = 8.9, 2.4 Hz, H-3′, H-7′), 5.36 (d, 2H, J = 4.8 Hz, OH), 5.12 (d, 2H, J = 4.4 Hz, OH), 5.08 (d, 2H, J = 4.7 Hz, OH), 4.97 (d, 2H, J = 7.2 Hz, H-1), 3.78 (dd, 2H, J = 10.5, 4.5 Hz, H-5), 3.22–3.41 (m, 8H, H-2, H-3, H-4, H-5). ^{13}C NMR (DMSO-d_6): δ 153.7, 129.8, 128.3, 119.3, 110.6, 101.2, 76.5, 73.1, 69.4, 65.8. HRMS calcd for $C_{20}H_{24}O_{10}Na$ (M+Na): 447.1267; found: 447.1267.

4.1.11. 2,7-Dihydroxynaphthalene bis(2,3,4-tri-*O*-acetyl-β-D-xylopyranoside) (6b). Same method as for **1b**. Chromatographed (SiO_2, heptane/EtOAc 3:4) to give **6b** (158 mg, 81%) as an amorphous white solid. $[\alpha]_D^{23}$ −27 (*c* 0.53, $CHCl_3$).^{1}H NMR ($CDCl_3$): δ 7.71 (d, 2H, J = 8.9 Hz, H-4′, H-5′), 7.27 (s, 2H, H-1′, H-8′), 7.08 (dd, 2H, J = 8.9, 2.4 Hz, H-3′, H-6′), 5.31 (d, 2H, J = 5.6 Hz, H-1), 5.21–5.28 (m, 4H, H-2, H-3), 5.01–5.06 (m, 2H, H-4), 4.27 (dd, 2H, J = 12.1, 4.6 Hz, H-5), 3.60 (dd, 2H, J = 12.1, 7.5 Hz, H-5), 2.11 (s, 6H, OAc), 2.10 (s, 12H, OAc). ^{13}C NMR ($CDCl_3$): δ 170.1, 170.0, 169.6, 155.2, 135.3, 129.6, 126.7, 117.6, 110.9, 98.6, 70.7, 70.2, 68.6, 62.0, 21.0, 20.92, 20.89. HRMS calcd for $C_{32}H_{36}O_{16}Na$ (M+Na): 699.1901; found: 699.1931.

4.1.12. 2,7-Dihydroxynaphthalene bis(β-D-xylopyranoside) (6c). Same method as for **1c**. Chromatographed (SiO_2, 60:35:5 CH_2Cl_2/MeOH/H_2O) to give **6c**(13 mg, 99%) as an amorphous white solid. The product was additionally purified using HPLC and then lyophilized before biological tests. $[\alpha]_D^{22}$ +12 (*c* 0.38, DMSO-d_6). ^{1}H NMR (DMSO-d_6): δ 7.77 (d, 2H, J = 8.9 Hz, H-4′, H-5′), 7.35 (d, 2H, J = 2.4 Hz, H-1′, H.8′), 7.08 (dd, 2H, J = 8.9, 2.4 Hz, H-3′, H-6′), 5.01 (d, 2H, J = 7.3 Hz, H-1), 5.00–5.50 (br s, 6H, OH), 3.79 (dd, 2H, J = 9.5, 3.5 Hz, H-5), 3.23–3.43 (m, 8H, H-2, H-3, H-4, H-5). ^{13}C NMR (DMSO-d_6): δ 155.5, 129.0, 116.9, 109.6, 100.9, 76.5, 73.1, 69.4, 65.8. HRMS calcd for $C_{20}H_{24}O_{10}Na$ (M+Na): 447.1267; found: 447.1278.

4.1.13. 1,2-Dihydroxynaphthalene bis(2,3,4-tri-*O*-acetyl-β-D-xylopyranoside) (7b). 1,2-Naphthoquinone (28 mg, 0.18 mmol) was dissolved in ethanol (3 mL). $NaBH_4$ (73 mg, 1.93 mmol) suspended in ethanol (3 mL) was added dropwise. The mixture was stirred at r.t. under nitrogen. After 1.5 h, the mixture was poured into ice-water (50 mL), acidified with HCl (0.5 M), extracted with CH_2Cl_2, dried ($MgSO_4$), and concentrated to give crude 1,2-dihydroxynaphthalene. The 1,2-dihydroxynaphthalene, 2,3,4-tri-*O*-acetyl-α/β-D-xylopyranosyl trichloroacetimidate (268 mg, 0.64 mmol), and MS 300AW (0.3 g) were dissolved in CH_2Cl_2 (3 mL) and stirred at rt under nitrogen for 80 min. TMSOTf (0.05 M in CH_2Cl_2, 0.400 mL) was added dropwise to the mixture. After 1 h, TMSOTf (0.05 M in CH_2Cl_2, 0.080 mL) was added and the mixture was stirred for another 80 min. Et_3N was added (pH 8) and the mixture was concentrated. The residue was chromatographed (SiO_2, heptane/EtOAc 3:4) to give **7b** (38 mg, 33%) as an amorphous white solid. An analytical sample was purified on Sephadex LH-20 (1:1 CH_2Cl_2/MeOH). $[\alpha]_D^{23}$ −25 (*c* 0.61, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 8.18 (d, 1H, J = 8.5 Hz, H-8′), 7.77 (d, 1H, J = 8.1 Hz, H-5′), 7.61 (d, 1H, J = 9.0 Hz, H-4′), 7.49 (ddd, 1H, J = 8.4, 6.9, 1.2 Hz, H-7′), 7.41 (ddd, 1H, J = 8.1, 6.9, 1.3 Hz, H-6′), 7.33 (d, 1H, J = 9.0 Hz, H-3′), 5.51 (d, 1H, J = 6.2 Hz, H-1), 5.36 (dd, 1H, J = 8.1, 6.2 Hz, H-2), 5.22–5.31 (m, 4H, H-1, H-2, H-3), 5.03–5.09 (m, 2H, H-4), 4.30 (dd, 1H, J = 12.1, 4.7 Hz, H-5), 4.16 (dd, 1H, J = 12.1, 4.8 Hz, H-5), 3.53 (dd, 1H, J = 12.1, 7.6 Hz, H-5), 3.36 (dd, 1H, J = 12.1, 8.0 Hz, H-5), 2.13, 2.11,

2.10, 2.09, 2.08, 2.05 (s, 3H each, OAc). ^{13}C NMR ($CDCl_3$): δ 170.1, 169.9, 144.8, 127.8, 126.8, 125.6, 125.5, 122.3, 118.5, 100.7, 100.1, 71.4, 71.3, 71.0, 70.5, 68.8, 62.5, 62.4, 31.2, 21.0. HRMS calcd for $C_{32}H_{36}O_{16}Na$ (M+Na): 699.1901; found: 699.1870.

4.1.14. 1,2-Dihydroxynaphthalene bis(β-D-xylopyranoside) (7c). Same method as for **1c**. Chromatographed (SiO_2, 5:1 CH_2Cl_2/MeOH) to give **7c** (8 mg, 70%) as an amorphous white solid. The product was additionally purified using HPLC and then lyophilized before biological tests. $[\alpha]_D^{22}$ −47 (*c* 0.23, DMSO-d_6). ^{1}H NMR (DMSO-d_6): δ 8.19 (d, 1H, *J* = 8.5 Hz, H-8′), 7.84 (d, 1H, *J* = 8.1 Hz, H-5′), 7.70 (d, 1H, *J* = 8.9 Hz, H-4′), 7.39–7.51 (m, 3H, H-3′, H-6′, H-7′), 5.07 (d, 1H, *J* = 7.6 Hz, H-1), 4.92 (d, 1H, *J* = 7.5 Hz, H-1), 4.90–5.90 (br s, 6H, OH), 3.80 (dd, 1H, *J* = 11.2, 5.2 Hz, H-5), 3.62 (dd, 1H, *J* = 11.3, 5.3 Hz, H-5), 3.21–3.46 (m, 7H, H-2, H-3, H-4, H-5), 2.95 (t, 1H, *J* = 11.3 Hz, H-3). ^{13}C NMR ($CDCl_3$): δ 145.5, 139.9, 130.0, 129.4, 127.3, 126.1, 124.8, 122.5, 118.9, 104.4, 102.9, 76.3, 75.7, 74.0, 73.5, 69.5, 69.4, 65.9. HRMS calcd for $C_{20}H_{25}O_{10}$ (M+H): 425.1448; found: 425.1442.

4.1.15. 1,3-Dihydroxynaphthalene bis(2,3,4-tri-*O*-acetyl-β-D-xylopyranoside) (8b). Same method as for **1b**. Chromatographed (SiO_2, heptane/EtOAc 3:4) to give **8b** (45 mg, 22%) as an amorphous white solid. $[\alpha]_D^{23}$ −45 (*c* 0.50, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 8.04 (d, 1H, *J* = 8.4 Hz, H-8′), 7.69 (d, 1H, *J* = 8.2 Hz, H-5′), 7.47 (ddd, 1H, *J* = 8.1, 6.9, 1.2 Hz, H-6′), 7.37 (ddd, 1H, *J* = 8.2, 6.9, 1.1 Hz, H-7′), 7.06 (d, 1H, *J* = 1.9 Hz, H-4′), 6.80 (d, 1H, *J* = 2.2 Hz, H-2′), 5.20–5.36 (m, 6H, H-1, H-2, H-3), 5.02–5.05 (m, 2H, H-4), 4.27 (ddd, 2H, *J* = 12.1, 4.6, 1.4 Hz, H-5), 3.58–3.65 (m, 2H, H-5), 2.15 (s, 3H, OAc), 2.11 (s, 9H, OAc), 2.10, 2.08 (s, 3H each, OAc). ^{13}C NMR ($CDCl_3$): δ 170.1, 170.0, 169.9, 169.63, 169.56, 154.4, 153.5, 134.8, 127.6, 127.1, 124.6, 122.7, 121.8, 105.7, 102.6, 98.5, 98.4, 70.7, 70.2, 69.8, 68.6, 68.4, 62.1, 61.8, 20.93, 20.90. HRMS calcd for $C_{32}H_{36}O_{16}Na$ (M+Na): 699.1901; found: 699.1874.

4.1.16. 1,3-Dihydroxynaphthalene bis(β-D-xylopyranoside) (8c). Same method as for **1c**. Chromatographed (SiO_2, 60:35:5 CH_2Cl_2/MeOH/H_2O) to give **8c** (24 mg, 88%) as an amorphous white solid. The product was additionally purified using HPLC and then lyophilized before biological tests. $[\alpha]_D^{22}$ −35 (*c* 0.28, DMSO-d_6). ^{1}H NMR (DMSO-d_6): δ 8.18 (d, 1H, *J* = 8.4 Hz, H-8′), 7.77 (d, 1H, *J* = 8.2 Hz, H-5′), 7.47 (ddd, 1H, *J* = 8.1, 6.8, 1.1 Hz, H-6′), 7.35 (ddd, 1H, *J* = 8.2, 6.9, 1.1 Hz, H-7′), 7.11 (d, 1H, *J* = 1.9 Hz, H-4′), 6.83 (d, 1H, *J* = 2.1 Hz, H-2′), 5.01 (d, 1H, *J* = 7.5 Hz, H-1), 4.99 (d, 1H, *J* = 7.6 Hz, H-1), 4.50–5.50 (br s, 6H, OH), 3.77–3.84 (m, 2H, H-5), 3.24–3.46 (m, 8H, H-2, H-3, H-4, H-5). ^{13}C NMR (DMSO-d_6): δ 155.1, 153.7, 134.4, 127.0, 126.7, 121.7, 104.0, 102.8, 101.7, 101.0, 76.5, 76.2, 73.14, 73.08, 69.4, 69.3, 65.8. HRMS calcd for $C_{20}H_{24}O_{10}Na$ (M+Na): 447.1267; found: 447.1269.

4.1.17. 1,6-Dihydroxynaphthalene bis(2,3,4-tri-*O*-acetyl-β-D-xylopyranoside) (9b). Same method as for **1b**. Chromatographed (SiO_2, heptane/EtOAc 3:4) to give **9b** (187 mg, 95%) as an amorphous white solid. $[\alpha]_D^{23}$ −73 (*c* 0.50, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 8.07 (d, 1H, *J* = 9.2 Hz, H-8′), 7.43 (d, 1H, *J* = 8.3 Hz, H-4′), 7.36 (m, 1H, H-3′), 7.33 (d, 1H, *J* = 2.4 Hz, H-5′), 7.17 (dd, 1H, *J* = 9.2, 2.5 Hz, H-7′), 6.97 (dd, 1H, *J* = 7.6, 0.7 Hz, H-2′), 5.21–5.39 (m, 6H, H-1, H-2, H-3), 5.01–5.08 (m, 2H, H-4), 4.26 (ddd, 2H, *J* = 12.1, 4.6, 1.7 Hz, H-5), 3.59 (ddd, 2H, *J* = 12.1, 7.4, 4.4 Hz, H-5), 2.13, 2.11 (s, 3H each, OAc), 2.10 (s, 9H, OAc), 2.07 (s, 3H, OAc). ^{13}C NMR ($CDCl_3$): δ 170.1, 170.01, 169.98, 169.97, 169.7, 169.5, 155.1, 152.6, 135.7, 126.8, 123.9, 122.3, 122.1, 118.6, 111.2, 107.7, 98.8, 98.5, 70.7, 70.6, 70.20, 70.16, 68.6, 62.1, 62.0, 20.93, 20.91, 20.87. HRMS calcd for $C_{32}H_{37}O_{16}$ (M+H): 677.2082; found: 677.2086.

4.1.18. 1,6-Dihydroxynaphthalene bis(β-D-xylopyranoside) (9c). Same method as for **1c**. Chromatographed (SiO_2, 60:35:5 CH_2Cl_2/MeOH/H_2O) to give **9c** (9 mg, 85%) as an amorphous white solid. The product was additionally purified using HPLC and then lyophilized before biological tests. $[\alpha]_D^{22}$ −45 (*c* 0.31, DMSO-d_6). ^{1}H NMR (DMSO-d_6): δ 8.22 (d, 1H, *J* = 9.2 Hz, H-8′), 7.45 (d, 1H, *J* = 8.3 Hz, H-4′), 7.39 (d, 1H, *J* = 2.5 Hz, H-5′), 7.37 (t, 1H, *J* = 8.1 Hz, H-3′), 7.19 (dd, 1H, *J* = 9.2, 2.4 Hz, H-7′), 6.98 (d, 1H, *J* = 7.3 Hz, H-2′), 5.03 (d, 1H, *J* = 7.2 Hz, H-1), 4.99 (d, 1H, *J* = 7.6 Hz, H-1), 4.80–5.50 (br s, 6H, OH), 3.76–3.80 (m, 2H, H-5), 3.24–3.45 (m, 8H, H-2, H-3, H-4, H-5). ^{13}C NMR (DMSO-d_6): δ 155.4, 152.9, 135.3, 126.7, 123.9, 121.3, 120.7, 118.0, 109.9, 107.4, 101.6, 100.8, 76.5, 76.3, 73.2, 73.1, 69.4, 65.8, 65.7. HRMS calcd for $C_{20}H_{25}O_{10}$ (M+H): 425.1448; found: 425.1450.

4.1.19. 1,7-Dihydroxynaphthalene bis(2,3,4-tri-*O*-acetyl-β-D-xylopyranoside) (10b). Same method as for **1b**. Chromatographed (SiO_2, heptane/EtOAc 3:4) to give **10b** (194 mg, 96%) as an amorphous white solid. $[\alpha]_D^{23}$ −71 (*c* 0.50, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 7.74 (d, 1H, *J* = 9.0 Hz, H-5′), 7.61 (d, 1H, *J* = 2.5 Hz, H-8′), 7.49 (d, 1H, *J* = 8.3 Hz, H-2′), 7.26–7.30 (m, 1H, H-3′), 7.18 (dd, 1H, *J* = 8.9, 2.5 Hz, H-6′), 7.03 (d, 1H, *J* = 7.4 Hz, H-4′), 5.42–5.46 (m, 2H, $J_{H\text{-}1}$ = 6.7 Hz, H-1, H-2), 5.31–5.37 (m, 2H, H-3), 5.22–5.26 (m, 2H, $J_{H\text{-}1}$ = 6.7 Hz, H-1, H-2), 5.05–5.14 (m, 2H, H-4), 4.22–4.31 (m, 2H, H-5), 3.81 (dd, 1H, *J* = 11.9, 9.0 Hz, H-5), 3.61 (dd, 1H, *J* = 11.9, 8.6 Hz, H-5), 2.13 (s, 3H, OAc), 2.09 (s, 9H, OAc), 2.07, 2.06 (s, 3H each, OAc). ^{13}C NMR ($CDCl_3$): δ 170.3, 170.2, 170.03, 170.00, 169.8, 169.6, 154.6, 152.2, 131.0, 129.6, 126.5, 124.3, 122.5, 119.7, 109.1, 104.4, 99.8, 97.8, 71.7, 71.2, 70.9, 70.7, 69.0, 68.8, 62.6, 62.3, 21.1, 21.0, 20.9, 20.8. HRMS calcd for $C_{32}H_{37}O_{16}$ (M+H): 677.2082; found: 677.2049.

4.1.20. 1,7-Dihydroxynaphthalene bis(β-D-xylopyranoside) (10c). Same method as for **1c**. Chromatographed (SiO_2, 60:35:5 CH_2Cl_2/MeOH/H_2O) to give **10c** (15 mg, 98%) as an amorphous white solid. The product was additionally purified using HPLC and then lyophilized before biological tests. $[\alpha]_D^{22}$ −62 (*c* 0.28, DMSO-d_6). ^{1}H NMR (DMSO-d_6): δ 7.82 (d, 1H, *J* = 9.0 Hz,

H-5′), 7.75 (d, 1H, J = 2.5 Hz, H-7′), 7.50 (d, 1H, J = 8.3 Hz, H-4′), 7.28 (t, 1H, J = 7.9 Hz, H-3′), 7.24 (dd, 1H, J = 8.9, 2.6 Hz, H-6′), 7.08 (d, 1H, J = 7.3 Hz, H-2′), 5.57 (d, 1H, J = 5.5 Hz, OH), 5.41 (d, 1H, J = 4.9 Hz, OH), 5.17 (d, 1H, J = 4.9 Hz, OH), 5.14 (d, 1H, J = 4.6 Hz, OH), 5.11 (d, 1H, J = 4.4 Hz, OH), 5.09 (d, 1H, J = 7.1 Hz, H-1), 5.08 (d, 1H, J = 5.0 Hz, OH), 4.96 (d, 1H, J = 7.5 Hz, H-1), 3.79 (dd, 2H, J = 11.0, 5.1 Hz, H-5), 3.37–3.47 (m, 4H, H-2, H-4), 3.26–3.35 (m, 4H, H-3, H-5). ^{13}C NMR (DMSO-d_6): δ 154.6, 151.8, 130.1, 129.0, 126.3, 123.9, 121.4, 118.9, 110.1, 104.8, 101.6, 100.5, 76.6, 76.3, 73.2, 73.1, 69.4, 65.7, 65.6. HRMS calcd for $C_{20}H_{24}O_{10}Na$ (M+Na): 447.1267; found: 447.1261.

4.1.21. HPLC of naphthoxylosides. High-performance liquid chromatography was run on a Hewlett–Packard Series II 1090 Liquid Chromatograph and a YMC Hydrosphere C18 column (15 cm × 4.6 mm, 5 μm). The system was controlled by the Hewlett–Packard ChemStation for LC software suite. The mobile phase consisted of H_2O + 0.1% trifluoroacetic acid (TFA) with a gradient of acetonitrile from 1 min increasing by 1.2% per minute until 30 min. The mean retention times were calculated from three separate runs per compound.

4.1.22. Stability of naphthoxylosides. A solution of xyloside (20 mM in DMSO/water 1:1) was diluted in Ham's F-12 medium to about 0.20 mM. 4,4′-Dihydroxybiphenyl (0.1 mM) was used as an internal standard. The samples were heated to 37 °C, and analytical samples were taken at t = 0, 1, 3, 24, 48, 72, and 96 h and analyzed by HPLC.

4.2. Biology

The human bladder carcinoma cell-line T24 was obtained from ATCC, Rockville, MD. Regular cell culture media, L-glutamine, penicillin–streptomycin, trypsin, and donor calf serum were obtained from Life Technologies. Dulbecco's modified Eagle's medium (DMEM) and Ham's F-12 medium were purchased from Sigma. $Na_2{}^{35}SO_4$ (1310 Ci/mmol) was obtained from Amersham International, UK. Epidermal growth factor was purchased from Genzyme, Cambridge, MA and crystal violet from Merck, Germany. The prepacked Superose 6 HR 10/30 and Dextran T-500 were from Pharmacia-LKB, Sweden, and DE-53 DEAE-cellulose was from Whatman. Water for HPLC-analysis was from a Millipore Milli-Q system.

4.2.1. Cell culture and radiolabeling. Cells were cultured as monolayers in modified Eagle's medium (MEM) supplemented with 10% (v/v) fetal bovine serum, 2 mM L-glutamine, 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 preincubated for 1 h in low-sulfate, $MgCl_2$-labeling medium supplemented with 2 mM glutamine. The pre-incubation medium was replaced by fresh medium containing 50 mCi/mL of [^{35}S]sulfate and 0.1 mM xyloside. 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).

4.2.2. 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 7 M urea, 10 mM Tris, pH 7.5, 0.1% Triton X-100, 10 mM NEM, and passed over a 1 mL-column of DEAE equilibrated with 6 M urea, 0.5 M NaOAc, pH 5.8, 5 μg/mL ovalbumin, 0.1% Triton X-100. After sample application, the columns were washed successively with 10 mL-portions of (a) equilibration buffer (see above), (b) 6 M urea, 10 mM Tris, pH 8.0, 5 μg/mL ovalbumin, 0.1% Triton X-100, and (c) 50 mM Tris, pH 7.5. Bound material was eluted with 5× 1 mL of 4 M guanidine–HCl, 50 mM NaOAc, pH 5.8, 5 μg/mL ovalbumin, 0.2% Triton X-100. Radioactive fractions were pooled, precipitated with 5 vol of 95% ethanol 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, material was dissolved in 4 M guanidine–HCl, 50 mM NaOAc, 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.

4.2.3. Degradative method. Xyloside-primed GAG chains separated by Superose 6 were pooled and precipitated with 5 vol of 95% ethanol 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, material was subjected to periodate oxidation using 0.02 M $NaIO_4$, 0.1 M sodium formate, pH 3.0, at 4 °C for 24 h.[23] The reaction was stopped by addition of mannitol. The samples were then desalted using PD10 columns, lyophilized, and subjected to alkali treatment at pH 12 for 30 min.[23]

4.2.4. In vitro growth assay using crystal violet method. The procedure has been described elsewhere.[4] Cells were seeded into 96-well microculture plates at 3000 cells/well in DMEM supplemented with insulin (10 ng/mL), transferrin (25 ng/mL), and 10% fetal calf serum. After 4 h of plating the cells were placed in serum-free Ham's F-12 medium supplemented with insulin (10 ng/mL) and transferrin (25 ng/mL) for an additional 24 h. Cells were then allowed to proliferate supported by 10 ng/mL of epidermal growth factor in the presence of 0.025, 0.05, 0.1, 0.2 and 0.4 mM of xylosides. Controls without xylosides were included. The total exposure-time was 96 h. Cells were then fixed in 1% glutaraldehyde dissolved in Hanks' balanced salt solution (NaCl 80 g/L, KCl 4 g/L, glucose 10 g/L, KH_2PO_4 600 mg/L, $NaHPO_4$ 475 mg/L) for 15 min, then cell nuclei were stained with 0.1% crystal violet. After washing and cell lysis for 24 h in Triton X-100, the amount of bound dye was measured at A_{600} in a microplate photometer (Titertek multiscan). The inhibitory

effect of the compounds is expressed as the percentage of growth in the absence of drugs.

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Supplementary data

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