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Effects of oxygen–sulfur substitution on glycosaminoglycan-priming naphthoxylosides

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Abstract—Three series of sulfur-containing analogs to the selectively antiproliferative 2-(6-hydroxynaphthyl) β-D-xylopyranoside were synthesized and their biological properties investigated. A short, general route to hydroxynaphthyl disulfides from dihydroxynaphthalenes was developed to utilize the disulfide bond as a sulfur-selective protecting group to enable the orthogonal protection of hydroxyls and thiols. The results indicate that hydrophobic, uncharged oxygen–sulfur substituted naphthoxylosides are taken up by cells and initiate priming of GAG chains to a greater extent compared to the oxygen analogs. No correlation between priming ability and antiproliferative activity was observed.

1. Introduction

Glycosaminoglycan (GAG) chains anchored to core proteins form proteoglycans (PG), a class of extracellular macromolecules having functions ranging from bulk construction material to cell–cell interactions. The biosynthesis of GAG chains starts with the formation of a glycosidic bond between serine residues of the core protein and the unique xylose residue of the resulting GAG chain.[1] A specific linker tetrasaccharide, GlcA(β1–3)Gal(β1–3)Gal(β1–4)Xylβ, is then assembled on which GAG chains are elongated and modified (Fig. 1). The polysaccharide is further modified through N-deacetylation/N-sulfation, O-sulfation, and epimerization.

Xylosides carrying hydrophobic aglycon can enter cells and serve as primers of GAG synthesis.[2] Depending on the structure of the aglycon, different GAG chains are formed. We have previously shown that the GAG-priming 2-(6-hydroxynaphthyl) β-D-xylopyranoside (**1**, Fig. 1) selectively inhibits proliferation of transformed or tumor-derived cells in vitro as well as in vivo.[3] Also, treatment with this xyloside reduced the average tumor load by 70–97% in a SCID mice model.[4] Studies into the toxicity of the 14 isomeric xylosylated dihydroxynaphthalenes revealed differences in antiproliferative activity depending on the aglycon structure.[5,6]

To extend our investigation of the structure-activity relationships (SAR) for naphthoxylosides we are synthesizing sulfur-containing naphthoxylosides. Sulfur is a classic isostere for oxygen and the substitution will expand the range of physical as well as chemical properties for the naphthoxylosides. Both the O-linked naphthoxylosides and the S-linked naphthoxylosides are of interest.

Aryl thiols are more acidic (pK_a 6–8) compared to the corresponding phenols (pK_a 8–11), which could be expected to influence cellular uptake of the xyloside, redox properties, and electron density distribution of the aglycon. Also, the possibility of disulfide bond formation allows the use of aryl thiol *O*-xylosides to create libraries of diverse naphthoxylosides using dynamic combinatorial chemistry (DCC). DCC has recently been introduced as a method to create dynamic libraries, that is, collections of compounds whose constitution is adjusted depending on the conditions.[7] Examples of DCC are the generation and screening of a library of possible concanavalin A vaccines[8] and the discovery of a psammaplin A type antibacterial agent with good activity against

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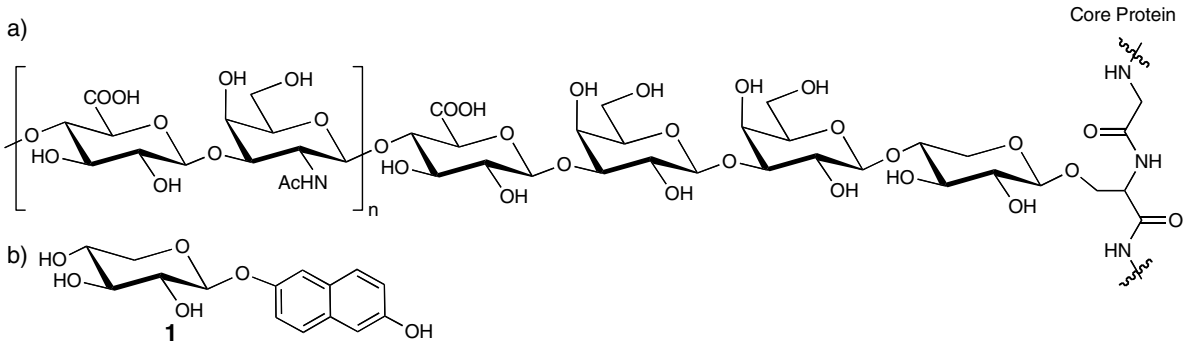


Figure 1. (a) Biosynthesis of glycosaminoglycan (GAG) chains start with xylosylation of a serine residue after which a linker tetrasaccharide (GlcA(β1–3)Gal(β1–3)Gal(β1–4)Xylβ) is assembled. (b) Xylosides carrying hydrophobic aglycon, such as compound **1**, can enter cells and serve as primers of GAG chains.

methicillin-resistant *Staphylococcus aureus*.[9] In both cases the reversibility of the thiol-disulfide linkage was used.

Xylosides with *S*-glycosidic bonds are of great biological interest since the length of the carbon–sulfur bond can result in increased stability toward glycosidases.[10] Also, it has been shown that *S*-xylosides can act as primers of GAG synthesis with comparable or even higher efficiency than the corresponding *O*-xyloside and also show activity in vivo.[11–13] 2-Naphthyl 1-thio-β-D-xylopyranoside shows a higher proportion of HS priming compared to 2-naphthyl β-D-xylopyranoside but both these compounds have poor antiproliferative properties. To infer toxicity on these compounds, a hydroxyl group can be added to the aglycon, thus mimicking the known antiproliferative mono-xylosylated dihydroxynaphthalenes.[3–6]

Synthesis of these sulfur-containing xylosides requires naphthalene moieties carrying both a hydroxyl group and a thiol and the possibility of selective protection of hydroxyl and thiol groups. Here we present a solution phase synthesis of thiol-containing xylosides using a disulfide linker as the thiol-protecting group. The disulfide is a protecting group which is unique for thiols, and it can easily be reduced/deprotected to the thiol by a variety of reagents, both in solution and immobilized on solid-support (e.g., aryl- and alkyl phosphines,[14,15] polystyrene-bound triphenyl phosphine (Ph_3P-PS),[16] nucleophilic hydrides[17,18]) as exemplified in Figure 2.

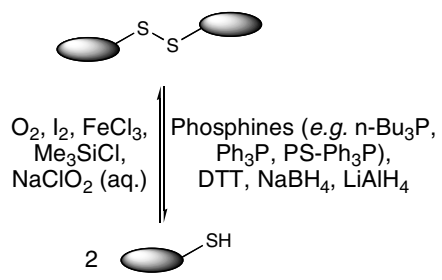
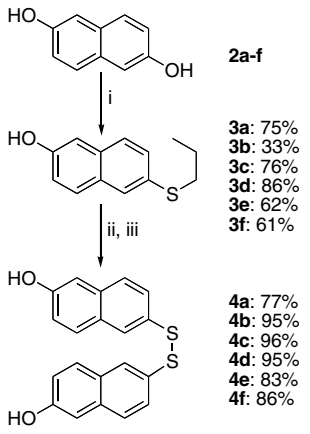


Figure 2. Reversibility of the disulfide bond and a few examples of reagents for the conversions.

2. Synthesis

We envisaged obtaining the disulfide-protected thiols from cleavage of an aryl–alkyl thioether bond by dissolving metal reduction in liquid ammonia.[19–22] Aryl–alkyl thioethers are usually synthesized by coupling of aromatic halides with alkyl thiols,[23] or nucleophilic substitution of alkyl halides with aryl thiols, when the thiol is available.[24] While the yields are normally high, this method requires the availability of a suitable aryl thiol or aryl halide.

Displacement of phenolic hydroxyls under the influence of strong acids is another method that has been shown to give excellent yields for simple compounds.[25,26] However, bis-phenolic compounds were transformed into the analogous bis-thioethers.[27] We therefore first set out to ascertain whether it was possible to obtain mono-hydroxy mono-thioethers using the same method (Scheme 1). The six symmetric naphthalene diols **2a**–**f** were chosen for this study (Fig. 3) with the letters **a**–**f** indicating the substitution pattern of the naphthalene skeleton. After minor optimization, the mono-thiopropyl-naphthols **3a**–**f** (Scheme 1) were obtained in yields from 33% to 86%.



Scheme 1. Reagents and conditions: Synthesis of hydroxynaphthyl disulfides from dihydroxynaphthalenes. (i) *p*-TsOH, *n*-PrSH, toluene, 100 °C; (ii) Na, NH_3, Et_2O; (iii) I_2, MeOH, H_2O. The letters **a**–**f** indicate the substitution pattern of the aromatic residue (cf. Fig. 3).

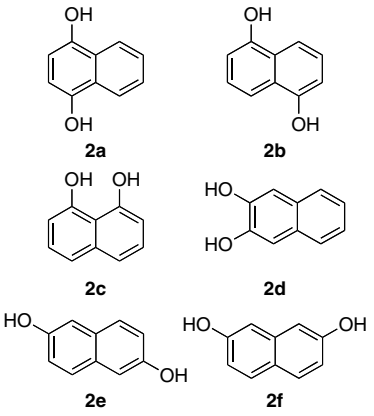


Figure 3. The letters **a**–**f** are used in the following text indicating the substitution pattern on the naphthalene skeleton.

Cleavage of the thioether to give the thiols and, upon oxidation, disulfides **4a**–**f** (Scheme 1), was easily performed by dissolving metal reduction followed by iodine-mediated oxidation. Recrystallization gave the pure products in 40–80% yield without the need for chromatography. However, column chromatography of the residues raised the yields to 77–96%. NMR data for **3a**–**f** and **4a**–**f** are given in Table 1.

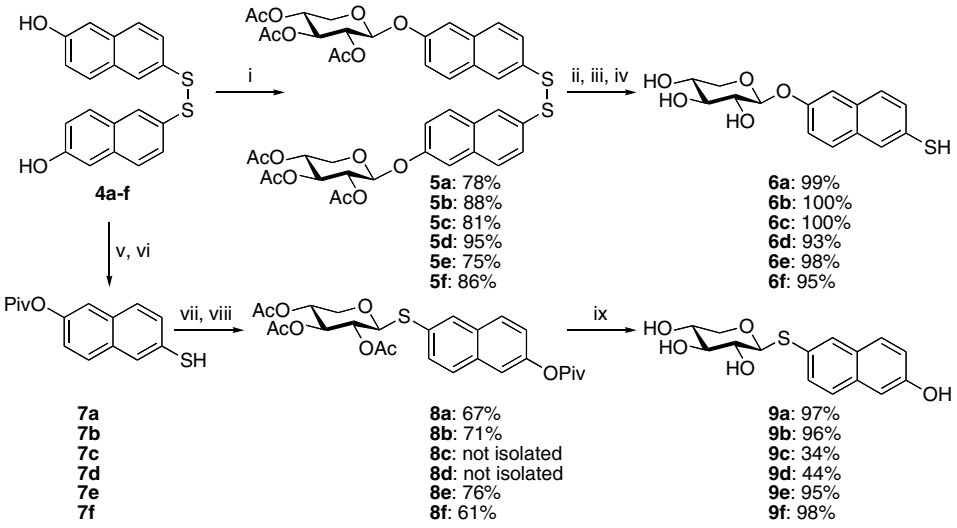
Xylosylation of the hydroxynaphthyl disulfides was first investigated using the peracetylated xylose donor following the method presented by Lee et al.[34] in which anomerization is prevented by the addition of triethylamine. Whilst this method has previously been successful for the xylosylation of several naphthol derivatives,[35] the yields were disappointingly low in this case. As we have noted earlier,[36] trichloroacetimidate donors can give good results for aromatic O-glycosylation reactions. Using a two-fold excess of trichloroacetimidate xylopyranoside donor per phenolic hydroxyl gave excellent results and at 0 °C no Fries-rearrangement was detected (Scheme 2, compounds **5a**–**f**).

To obtain the xylosides with free aryl thiol groups, deacetylation followed by disulfide cleavage was first attempted. Unfortunately, this order of deprotection encountered difficulties due to the very low solubilities of the deprotected bis-xylosylated naphthalene disulfides across a range of solvents. Therefore, an alternative route was developed in which the disulfide was cleaved using Ph_3P-PS in THF with catalytic acid. After stirring at 80 °C overnight, NaOMe–MeOH was added to remove the acetate groups. After 10 min the reaction was neutralized by the addition of acidic ion exchange resin. The solution was filtered and immediately concentrated to give the fully deprotected *O*-xylosides (**6a**–**f**). After filtration only one compound was detected by TLC, except in the case of **6c** for which the cleavage reaction was repeated using fresh Ph_3P-PS and microwave heating to give one product, as judged by TLC. NMR of these products were troublesome due to spontaneous oxidation to the corresponding disulfides in methanolic solution. All attempts to purify the thiols using chromatography or crystallization gave partial

Table 1. Selected ^{1}H NMR data for compounds **3a**–**f** and **4a**–**f**[a,b]

Compound	H-1	H-2	H-3	H-4	H-5	H-6	H-7	H-8
3a[28]	—	6.77, d, 7.9	7.55, d, 7.9	—	8.23, d, 8.5	7.45, m	7.52, dd, 8.4, 1.4	8.43, d, 8.5
4a[29]	—	7.17, d, 7.9	6.60, d, 7.9	—	8.23, dm, 8.3	7.43, ddd, 8.3, 6.8, 1.5	7.37, ddd, 8.3, 6.8, 1.3	8.14, dm, 8.3
3b	—	6.84, dd, 7.5, 0.8	7.35–7.28, m	7.82, d, 8.5	—	7.53, dd, 7.3, 1.0	7.35–7.28, m	8.10, d, 8.5
4b[30]	—	7.54, dd, 7.2, 1.1	7.23, dd, 8.5, 7.2	8.21, d, 8.4	—	6.86, dd, 7.7, 0.7	7.29, dd, 8.5, 7.7	7.75, d, 8.5
3c	—	6.89, dd, 7.2, 1.6	7.28–7.37, m	7.28–7.37, m	7.72, dd, 8.2, 1.1	7.28–7.37, m	7.48, dd, 7.2, 1.2	—
4c[31]	—	7.54, dd, 7.6, 0.9	7.21, t, 7.9	7.67, dd, 8.1, 0.9	7.33, dd, 8.2, 1.3	7.30, t, 8.1	6.86, dd, 7.4, 1.3	—
3d	7.13, s	—	—	7.63, s	7.61, d, 8.1	7.19, dt, 7.0, 1.1	7.27, dt, 6.8, 1.2	7.56, d, 8.2
4d	7.87, s	—	—	7.15, s	7.54, d, 8.3	7.18, dd, 8.2, 1.2	7.30, dd, 8.2, 1.2	7.60, s, 8.3
3e	7.65, s	—	7.04–7.07 m	7.58 d, 7.6	7.07 s	—	7.30 d, 8.6	7.52 d, 8.6
4e[32]	7.08, s	—	7.61, d, 8.1	7.50, dd, 8.7, 1.8	7.81, s	—	7.05, dd, 8.8, 2.3	7.59, d, 9.0
3f	7.01, s	—	7.64, m	7.00, m	7.62, m	7.18, dd, 8.5, 1.9	—	7.52, d, 1.5
4f[33]	7.75, d, 1.8	—	7.69, d, 8.6	7.38, dd, 8.6, 1.9	7.04, dd, 8.8, 2.4	7.67, d, 8.7	—	7.00, d, 2.4

[a] The aromatic protons are reported for each compound.
[b] Given as chemical shift (ppm), multiplicity, and coupling constants (Hz).



Scheme 2. Reagents and conditions: Synthesis of *O*- and *S*-xylosides from a common hydroxynaphthyl disulfide precursor. (i) 2,3,4-Tri-*O*-acetyl-β-D-xylopyranoside trichloroacetimidate, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 0 °C; (ii) Ph_3P-PS, HCl (aq), THF, 80 °C, sealed tube; (iii) NaOMe–MeOH, MeOH; (iv) Amberlite IR-120H$^+$; (v) Piv_2O, 110 °C; (vi) Ph_3P-PS, HCl (aq), THF, 110 °C, sealed tube, microwave irradiation. Or 80 °C thermal heating; (vii) *t*-BuONa, DMF, 0 °C; (viii) 2,3,4-Tri-*O*-acetyl-α-D-xylopyranosyl bromide, THF. 0 °C to rt; (ix) NaOMe–MeOH, MeOH, CH_2Cl_2. The letters **a**–**f** indicate the substitution pattern of the aromatic residue (cf. Fig. 3).

oxidation to the disulfide and the compounds were therefore not purified further before biological testing. The purity was judged to be at least 90–95% by NMR and TLC analysis.

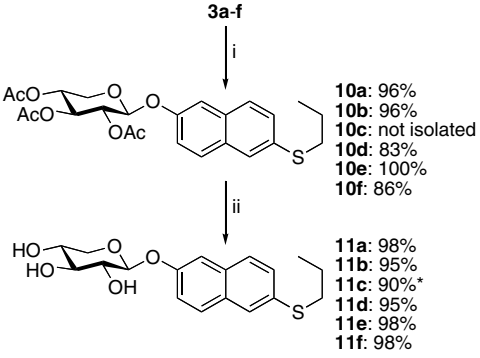
To synthesize the S-xylosides **9a**–**f**, we envisaged a route with protection of the phenol followed by disulfide bond cleavage. Early attempts using acetate as the phenolic protecting group gave rise to problems in the cleavage step. Phenolic acetates are labile under acidic conditions and the use of Ph_3P-PS requires a catalytic amount of acid to lower the reaction times[16] causing deprotection of the phenol. Therefore an alternative protecting group was needed, preferably one which could be removed in the same step as the deprotection of the acetates.

Aryl pivaloates are more stable toward acid compared to the acetates, yet readily cleavable with NaOMe–MeOH. Pivaloylation of disulfide **4e** in hot pivaloyl anhydride over night gave the bis-*O*-pivaloylated derivate as the only product. The pivaloyl groups were stable upon cleavage of the disulfide bond using Ph_3P-PS both at 80 °C in a sealed tube overnight or at 110 °C under microwave irradiation for 15 min (Scheme 2) and this gave full conversion of the pivaloylated hydroxynaphthyl disulfides into single products with slightly lower R_f-values.

Xylosylation of the crude thiols was first attempted using trichloroacetimidate donor but this gave complex mixtures as judged by TLC. Next, peracetylated xylose was tried. Whilst the correct product was obtained, the method gave unreliable and low yields (20–40%), as well as low purity even after multiple chromatographic steps with different mobile phases and recrystallization. The impurities probably arise from Fries-rearrangement and could not be avoided even by using lower amounts of promoter.

Instead, a basic procedure using xylosyl bromide donor was used (Scheme 3). Deprotonation of the thiol using *t*-butoxide at 0 °C followed by addition of the donor gave the thio-xylosides in satisfactory yields.[37] For **8c** and **8d** purification was troublesome and lowered the yields substantially. Therefore these were deprotected prior to full purification and characterization. Deprotection under Zemplén conditions gave the S-xylosides **9a**–**f** (Scheme 2). The difficulties in purification of **8c** and **8d** probably arose from non-quantitative conversion in the pivaloylation step giving traces of hydroxynaphthyl 1-thio-2,3,4-tri-*O*-acetyl-β-D-xylopyranoside, which was converted into final product upon deprotection.

Naphthyl β-D-xylopyranosides carrying aryl *O*-alkyl ethers have previously been reported to prime GAG



Scheme 3. Reagents and conditions: Xylosylation of thiopropyl substituted naphthols. (i) 1,2,3,4-Tetra-*O*-acetyl-β-D-xylopyranoside, $BF_3{\cdot}OEt_2$, Et_3N, CH_2Cl_2, rt; (ii) NaOMe–MeOH, MeOH, CH_2Cl_2. For **10c**: (i) 2,3,4-Tri-*O*-acetyl-β-D-xylopyranoside trichloroacetimidate, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 0 °C; (ii) NaOMe–MeOH, MeOH, CH_2Cl_2. The letters **a**–**f** indicate the substitution pattern of the aromatic residue (cf. Fig. 3). *Yield over two steps.

synthesis. The ethyl ether analog[12] gave a slight increase, while the methyl[4] and butyl derivatives[12] decreased the proportion of HS as compared to priming by 2-naphthyl β-D-xylopyranoside. The synthesis of the hydroxynaphthyl disulfides gave six thiopropyl-substituted naphthols (**3a**–**f**), which in two steps were transformed into naphthoxylosides **11a**–**f** (Scheme 3). Xylosylation of **3a**–**f** using peracetylated xylose in the presence of Et_3N to minimize anomerization was successful and gave high yields and purity. Only for **10c**, the yield was a disappointing 28% but the use of trichloroacetimidate donor and one-pot deacetylation gave the desired xyloside **11c** in 90% over two steps. The remaining five xylosides were easily deacetylated in nearly quantitative yields.

3. Results and discussion

The chemistry and biology of the three different groups of compounds are discussed in separate sections below Table 2.

3.1. Hydroxynaphthyl 1-thio-β-D-xylopyranosides

Gradient HPLC retention times, in contrary to isocratic retention times, can be treated as linear free-energy related parameters,[39], that is, gradient HPLC retention times can be used to substitute log P values in biological evaluations. Correlations between the lipophilicity of naphthoxylosides and their GAG-priming abilities and toxicity have been shown previously.[6,12] The gradient HPLC retention times for the hydroxynaphthyl 1-thio-β-D-xylopyranosides were measured using a C-18 column with a gradient of acetonitrile in water (0.1% trifluoroacetic acid). The retention times were measured for three separate runs per compound and the calculated mean retention times are presented in Table 3.

Compared to the retention times for the analogous mono-xylosylated dihydroxynaphthalenes[6,35] these compounds are generally less polar due to the diminished hydrogen bonding capability of sulfur, but the trend for retention times versus substitution pattern is similar. For example, the compounds with vicinal substituents (i.e., **9c** and **9d**) are generally less polar compared to the other analogs since a larger hydrophobic surface is exposed.

Earlier results have shown that some mono-xylosylated dihydroxynaphthalenes are unstable in the culture media used.[6] For example, 1-(4-hydroxynaphthyl) β-D-xylopyranoside was further hydroxylated to give 4-(1,2-dihydroxynaphthyl) β-D-xylopyranoside in less than 48 h. In contrast, bis-xylosylated analogs were stable in the medium for more than 96 h.[35] To evaluate the stability of the hydroxynaphthyl 1-thio-β-D-xylopyranosides, compounds **9a**–**f** 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, compared to 4,4′-dihydroxybiphenyl used as internal standard, were measured by HPLC. The results are shown in Figure 4.

Table 2. Selected ^{1}H NMR data for xylosides **6a**–**f**, **9a**–**f**, and **11a**–**f**[a,b]

Compound	H-1′	H-1	H-2	H-3	H-4	H-5	H-6	H-7	H-8
6a	5.03, d, 7.5	—	7.05, d, 8.1	7.53, d, 8.5	—	8.15, d, 8.6	7.51, m	7.58, m	8.40, d, 8.4
6b	5.06, d, 7.5	—	7.18, dd, 7.7, 0.8	7.46, dd, 8.3, 7.8	7.77, dt, 8.6, 0.8	—	7.57, dd, 7.2, 1.1	7.31, dd, 8.5, 7.2	8.24, dt, 8.5, 1.0
6c	5.28, d, 7.5	—	7.55, d, 8.3	7.43, t, 8.0	7.25, dd, 7.9, 0.9	7.83, 7.7, 1.0	7.27, t, 8.0	7.61, d, 8.4	—
6d	5.08, d, 7.2	7.47, s	—	—	7.74, s	7.71, m	7.34, m	7.34, m	7.64, m
6e	5.00, d, 7.2	7.70, d, 1.7	—	7.33, dd, 8.5, 1.8	7.65, d, 8.7	7.36, d, 2.3	—	7.25, dd, 9.0, 2.6	7.65, d, 8.7
6f	5.01, d, 7.3	7.27, d, 2.6	—	7.19, dd, 8.9, 2.5	7.72, d, 8.9	7.24, dd, 8.5, 1.9	7.66, d, 7.3	—	7.67, s
9a	4.33, d, 9.2	—	6.78, d, 7.9	7.69, d, 7.9	—	8.23, d, 8.4	7.45, m	7.54, m	8.59, d, 8.6
9b	4.59, d, 9.1[c]	—	7.79, dd, 7.2, 1.2	7.36, m	8.2, d, 8.4	—	6.85, dd, 7.6, 0.9	7.35, m	8.00, d, 8.6
9c	4.59, d, 9.1	—	7.65, dd, 7.3, 1.2	7.34, m	7.77, dd, 8.2, 0.9	7.34, m	7.34, m	6.89, dd, 7.5, 1.4	—
9d	4.61, d, 9.4	8.00, s	—	—	7.20, s	7.71, d, 8.2	7.28, m	7.39, m	7.64, d, 7.8
9e	4.53, d, 9.3	7.09, s	—	7.59, d, 8.6	7.52, dd, 8.6, 1.8	7.93, s	—	7.08, m,	7.67, d, 9.6
9f	7.65, d, 9.2	7.80, d, 1.4	—	7.68, d, 8.5	7.38, dd, 8.5, 1.8	7.06, dd, 8.5, 2.5	7.69, d, 8.5	—	7.05, s
11a	5.08, d, 7.5	—	7.11, d, 8.1	7.61, d, 8.1	—	8.41, m	7.51, m	7.58, m	8.41, m
11b	5.06, d, 7.5	—	7.59, dd, 7.3, 1.1	7.41, dd, 8.5, 7.3	8.29, dt, 8.5, 1.0	—	7.18, dd, 7.8, 0.8	7.45, dd, 8.5, 7.8	8.05, dt, 8.5, 0.8
11c[d]	5.01, d, 7.5	—	7.31, dd, 7.7, 1.1	7.34, m	7.58, dd, 8.0, 1.0	7.50, dd, 8.3, 1.0	7.35, m	7.17, dd, 7.8, 1.1	—
11d	5.14, d, 7.2	7.42, s	—	—	7.69, s	7.74, m	7.36, m	7.36, m	7.74, m
11e	5.01, d, 7.2	7.73, s	—	—	7.40, dd, 8.7, 1.9	7.38, d, 2.4	—	7.71, d, 9.0	7.26, dd, 9.0, 2.4
11f	5.03, d, 7.0	7.33, d, 2.4	—	7.20, dd, 8.9, 2.4	7.73, d, 8.9	7.70, d, 8.7	7.28, dd, 8.7, 1.9	—	7.66, s

[a] The anomeric proton together with the aromatic protons is reported for each compound.
[b] Given as chemical shift (ppm), multiplicity, and coupling constants (Hz).
[c] The anomeric proton for **9b** shows significant higher-order effects consistent with an ABCD-spin system. See example 19, Figure 1 in Dahmén et al.[38]
[d] NMR in CD_3OD–$CDCl_3$ 1:1.

Table 3. Gradient HPLC retention times (min), antiproliferative activity (ED_{50}, μM) of hydroxynaphthyl 1-thio-β-D-xylopyranosides toward HFL-1 cells and T24 cells, and GAG priming capability

Compound	HPLC retention time (min)	ED_{50} HFL-1 (μM)	ED_{50} T24 (μM)	GAG priming	Peak maximum (fraction)
9a	22.25 ± 0.07	130	160	40.7	44
9b	20.44 ± 0.08	135	320	29.8	44
9c	25.82 ± 0.04	120	225	32.6	41
9d	25.52 ± 0.06	100	260	43.5	42
9e	20.42 ± 0.06	145	355	44.3	44
9f	21.81 ± 0.10	125	230	37.4	44

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

It has been shown that the uptake of xylosides in animal cells is fast with a plateau being reached in a matter of minutes.[40] Therefore, decomposition could be of little consequence as long as the decomposition products are not toxic to cells.

For **9a** two more lipophilic UV-active peaks appeared in the HPLC trace as the xyloside decomposed, but since the xyloside was not highly toxic to cells this was not expected to influence the priming studies. For **9c** no major UV-active decomposition products were seen.

For the determination of the antiproliferative activity, normal HFL-1 cells (human fetal lung fibroblasts) and T24 cells (human bladder carcinoma cells) were used. The xylosides were added to the growth medium at various concentrations and cell proliferation was recorded using the crystal violet method.[3] The inhibitory effect of the compounds is expressed as ED_{50} (μM) scored after 96 h of exposure relative to untreated cells (Table 3).

We have earlier shown correlations between the ED_{50} values for HFL-1 cells and HPLC retention times, that is, the toxicity increased with the lipophilicity, indicating effects on cellular uptake. For T24 cells, most compounds followed a similar pattern but some analogs showed a much stronger activity.[6,35] For the hydroxynaphthyl 1-thio-β-D-xylopyranosides we could not find any correlations between HPLC retention times and ED_{50}. However, it is interesting to note that these compounds, contrary to the analogous hydroxynaphthyl β-D-xylopyranosides, are more toxic toward normal HFL-1 cells compared to transformed T24 cells.

To test the ability of the xylosides in priming GAG synthesis, T24 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. 5, Pool I). However, treatment with the xylosides also initiated synthesis of free GAG chains (Fig. 5, Pool II). The proportion of GAG-priming is given as the integrated value of ^{35}S detected per minute for fractions in Pool II (Table 3) divided by the integrated value for fractions of untreated cells.

All the hydroxynaphthyl 1-thio-β-D-xylopyranosides gave very strong priming of GAG chains as indicated in Table 3. The GAG priming capability could not be correlated to the antiproliferative effect. 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. The GAG chains synthesized on the hydroxynaphthyl 1 thio-β-D-xylopyranosides are relatively short.

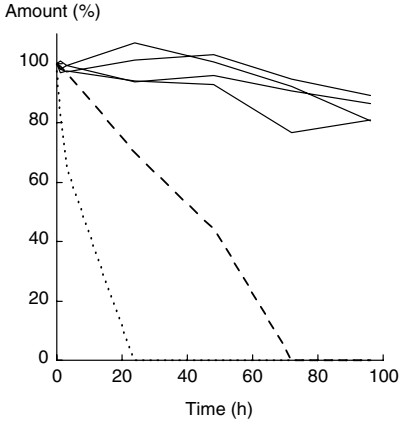


Figure 4. Stability of hydroxynaphthyl 1-thio-β-D-xylopyranosides **6a**–**f** in Ham's F-12 medium at 37 °C. The graph shows that whilst **9b**,**d**–**f** are stable for the first 48 h and then decompose slightly, **9a** (dashed line) and **9c** (dotted line) decompose quickly and totally.

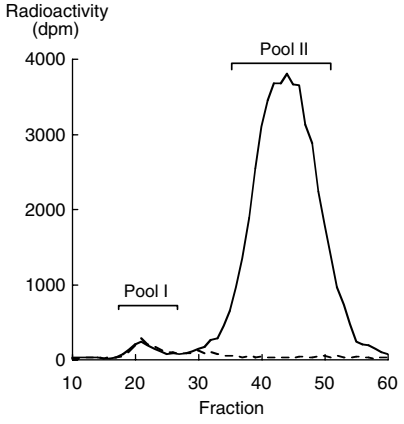


Figure 5. Example of priming of GAG chains in T24 cells incubated with **9e**. Pool II contains xyloside-primed GAG chains. The dashed line shows the results for untreated cells.

3.2. Thionaphthyl β-D-xylopyranosides

The retention times for the thionaphthyl β-D-xylopyranosides were measured as described in Section 3.1 and the calculated mean retention times are presented in Table 4. As expected the retention times for the thionaphthyl β-D-xylopyranosides show that these compounds are less polar compared to the hydroxynaphthyl 1-thio-β-D-xylopyranosides. Aromatic thiols are less hydrophilic and also less prone to be protonated compared to phenols. Also, the difference in lipophilicity between the most and least polar is less than that for the hydroxynaphthyl β-D-xylosides.

These compounds are sensitive toward air oxidation, yielding the analogous disulfides and in all cases complete conversion to the disulfide was seen in less than 48 h with only **6c** and **6d** having half-lives of more than about 1 h in the weakly basic growth medium. The increased stability of these two isomers is most probably due to steric interactions upon disulfide formation. The disulfides had significantly longer retention times as compared to their corresponding thiols, with all disulfides eluting between 36 and 40 min. We could therefore not perform quantitative stability tests for these compounds although no major decomposition was seen over 96 h. Biological evaluation of the pure disulfides was not possible due to their low solubility, hampering both their synthesis and their administration in solution. It is reasonable to suggest that both the free thiol and the disulfide are present in the cells in various proportions depending on the cellular redox conditions. During the proliferation studies no precipitates were seen in the medium, indicating rapid uptake of the thiols into the cells.

The antiproliferative activity toward HFL-1 cells and T24 cells, as well as their GAG priming capability, were evaluated and the data are presented in Table 4. Generally, these compounds show approximately 2–3 times stronger antiproliferative properties compared to the corresponding hydroxynaphthyl 1-thio-β-D-xylopyranoside whilst the GAG priming capability is lowered by 4–6 times. The GAG chains are longer compared to the hydroxynaphthyl 1-thio-β-D-xylopyranosides.

3.3. Propylthio-naphthyl β-D-xylopyranosides

Alkylation of the aromatic thiol gives several effects, such as elimination of the possibility of disulfide formation and introduction of a highly hydrophobic alkylthio group. The retention times for the propylthio naphthyl β-D-xylopyranosides were, as expected, higher than for the other compounds with retention times between 34 and 38 min. The difference in retention times was smaller within the series than for the previously discussed compounds. The antiproliferative activity toward HFL-1 cells and T24 cells, as well as their GAG priming capability, was evaluated and the data are presented in Table 5. All six propylthio-naphthyl β-D-xylopyranosides were stable at 37 °C for 96 h in the growth medium used in the priming and proliferation studies.

The lipophilicities of the propylthio-naphthyl β-D-xylopyranosides are, as expected, higher than for either **6a**–**f** or **9a**–**f**. Also, as seen for **6a**–**f**, substitution of the highly hydrophilic phenolic hydroxyl group for a more lipophilic group decreases the difference in retention time between the isomers. Contrary to the results from both **6a**–**f** or **9a**–**f** and from the previously presented hydroxynaphthyl β-D-xylopyranosides,[6] the isomers in which the substituents on the naphthalene moiety are placed in the 1,8- and 2,3-positions are less lipophilic. This is probably due to "masking" of the highly lipophilic thiopropyl group by the xylose residue.

Table 4. Gradient HPLC retention times (min), antiproliferative activity (ED_{50}, μM) of thionaphthyl β-D-xylopyranosides toward HFL-1 cells and T24 cells, and GAG priming capability

Compound	HPLC retention time (min)	ED_{50} HFL-1 (μM)	ED_{50} T24 (μM)	GAG priming	Peak maximum (fraction)
6a	29.64 ± 0.19	70	55	9.6	38
6b	28.93 ± 0.03	60	110	5.8	33
6c	29.76 ± 0.01	60	45	5.2	37
6d	29.76 ± 0.05	60	145	6.2	38
6e	28.56 ± 0.01	60	135	7.6	37
6f	28.56 ± 0.08	100	360	10.2	38

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

Table 5. Gradient HPLC retention times (min), antiproliferative activity (ED_{50}, μM) of propylthio-naphthyl β-D-xylopyranosides toward HFL-1 cells and T24 cells, and GAG priming capability

Compound	HPLC retention time (min)	ED_{50} HFL-1 (μM)	ED_{50} T24 (μM)	GAG priming	Peak maximum (fraction)
11a	37.90 ± 0.01	30	30	12.4	37
11b	37.92 ± 0.36	20	40	11.1	34
11c	34.77 ± 0.11	100	155	2.5	34
11d	35.33 ± 0.05	30	70	5.5	35
11e	37.13 ± 0.05	30	40	10.5	37
11f	37.00 ± 0.09	25	60	8.1	37

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

Generally, these compounds showed strong antiproliferative properties with only minor discrimination for different cell lines. With a few exceptions the GAG priming capability is higher compared to the thionaphthyl β-D-xylopyranosides. The GAG chains are similar in length to those primed by the thionaphthyl β-D-xylopyranosides.

3.4. Heparan sulfate priming

It has previously been shown that HS priming is an important factor for the selective antiproliferative activity of xylosides.[4,6] HS-deficient CHO cells have been shown to be insensitive to the antiproliferative 2-(6-hydroxynaphthyl) β-D-xylopyranoside (**1**) whilst wild type CHO cells were highly sensitive. The tumor selective antiproliferative **1** initiated priming of HS chains (12% of total GAG chains) in tumor derived T24 cells. The HS chains were subsequently degraded by nitric oxide into HS oligosaccharides and accumulated in the nucleus. This xyloside preferentially inhibited growth of T24 cells at low concentrations with an ED_{50} value of 50 μM. In order to investigate the correlation between aglycon structure, HS priming, and selective antiproliferative activity we investigated the HS priming ability of 2-(6-thionaphthyl) β-D-xylopyranoside (**6e**), where the *O*-glycosidic linkage in **1** has been replaced with an *S*-glycosidic linkage, and 2-(6 hydroxynaphthyl) 1-thio-β-D-xylopyranosides (**9e**) in which the phenolic hydroxyl group has been changed to an aryl thiol group, in tumor derived T24 cells. The amount of HS chains primed in the cells was determined by digestion of GAG pool with HNO_2 at pH 1.5 which cleaves HS chains. Both **6e** and **9e** initiated priming of HS chains to some extent (14% and 8% of total GAGs, respectively). None of these xylosides were selectively antiproliferative toward tumor cells. There was no correlation between HS priming and selective antiproliferative activity. This could however be due to the production of different HS or different HS degradation in these cells.

4. Conclusions

Taken together, the results from this study indicate that hydrophobic, uncharged oxygen–sulfur substituted naphthoxylosides are taken up by cells and initiate priming of GAG chains. No correlation between priming ability and antiproliferative activity was observed. No selective antiproliferative activity in benefit to tumor derived cells was detected. The antiproliferative effect of naphthoxylosides and their priming ability were dependent on the aglycone structure and the structure of glycosidic linkage between the aglycone and the xyloside. Alterations in the substitution pattern of the aglycone affected the priming ability and the antiproliferative activity. No correlation between HS priming and tumor selective antiproliferative activity was observed indicating that HS priming may be necessary for growth inhibition but it is not a sufficient requirement. Previous attempts to determine the mechanism for selective growth inhibition suggest that the priming of HS synthesis, the fine structure of HS chains synthesized by the xyloside, the biotransformation of aglycone such as hydroxylation of the naphthalene ring and the nuclear targeting of xyloside derived products are some of the important factors involved in the antiproliferative activity.

We have also demonstrated a general route for the synthesis of hydroxynaphthyl disulfides via mono-thioalkyl ethers from dihydroxynaphthalenes. This route is of general interest for the synthesis of sulfur substituted aromatic compounds and we are currently investigating the mechanistic aspects of the thioether formation. Also, we have highlighted the strengths and weaknesses of different pyranoside donors,[36] and demonstrated the efficiency of solid support bound reagents, especially for compounds where purification needs to be minimized.

We have also confirmed our previous findings that xylosides are not always stable in the cell growth medium used for studies in vivo. Also, previous findings that xylosides with *S*-glycosidic bonds can be effective primers of GAG synthesis were confirmed.[11,12]

5. Experimental

5.1. Chemistry

CH_2Cl_2, Et_2O, and THF for reactions were dried by passing through a column of Al_2O_3 (neutral, activity grade I). Anhydrous DMF was of commercial grade and used without further purification. NMR-spectra were recorded with a Bruker DRX 400 spectrometer at 400 MHz (^1H) and 100 MHz (^{13}C) and assigned using 2D-methods (COSY, HMQC, HMBC). 2D-experiments were recorded with gradient enhancements using sine shaped gradient pulses. The chemical shifts are reported in ppm with the residual solvent peaks (^1H) and solvent signals (^{13}C) as reference (δH $CDCl_3$ = 7.26 ppm, δC $CHCl_3$ = 77.0, δH CD_3OD = 3.31 ppm, δC CD_3OD = 49.0). High-resolution mass-spectra were recorded on a Micromass Q-Tof (ESI) or JEOL SX-102 (FAB). Reactions using microwave irradiation were performed on a Smith Creator microwave reactor from Personal Chemistry i Uppsala AB. Reactions were monitored by TLC using alumina plates coated with silica gel and visualized using either UV-light or by charring with an ethanolic anisaldehyde solution. Preparative chromatography was performed with silica gel (35–70 μm, 60 Å).

5.1.1. 4-(Propylthio)naphthalen-1-ol (3a). 1,4-Dihydroxynaphthalene (**2a**) (2.08 g, 13.0 mmol) and *p*-TsOH (1.1 g, 6.3 mmol) were suspended in toluene (76 mL) and propanethiol (1.4 mL, 15.8 mmol) was added. The suspension was stirred at 75 °C for 105 min and then allowed to reach room temperature. The suspension was diluted with Et_2O (40 mL) and washed three times with $NaHCO_3$ (satd aq). The aqueous phases were extracted with Et_2O and the combined organic phases were dried (Na_2SO_4), filtered and concentrated. Column chromatography (SiO_2, toluene–methanol 30:1) gave **3a** as a gray amorphous solid (2.13 g, 75%). Recrystallization

(heptane) gave an analytical sample as white crystals; mp 83 °C (lit. 81–82 °C).[28] ^{13}C NMR (MeOD): δ 155.5, 136.8, 134.8, 127.8, 127.3, 126.8, 125.9, 124.1, 123.1, 108.9, 39.3, 24.0, 13.7. HRMS calcd for $C_{13}H_{15}OS$ (M + 1): 219.0844, found: 219.0864.

5.1.2. 5-(Propylthio)naphthalen-1-ol (3b). 1,5-Dihydroxynaphthalene (**2b**) (1.92 g, 12.0 mmol) and *p*-TsOH (1.0 g, 6.0 mmol) were suspended in toluene (70 mL) and propanethiol (4.3 mL, 47.8 mmol) was added. The suspension was stirred at 100 °C overnight and then worked-up as for **3a**. Column chromatography (SiO_2, toluene–methanol 20:1 → 5:1) gave **3b** as an off-white amorphous solid (872 mg, 33%). An analytical sample was recrystallized (heptane) to give off-white crystals; mp 101–102 °C. ^{13}C NMR (MeOD): δ 155.2, 135.7, 134.7, 129.2, 127.6, 127.0, 125.3, 122.3, 117.1, 109.4, 37.1, 23.7, 13.9. HRMS calcd for $C_{13}H_{15}OS$ (M + 1): 219.0844, found: 219.0863. The bis-substituted compound was also isolated (1.37 g, 41%) and starting material was recovered (414 mg, 22% mmol).

5.1.3. 8-(Propylthio)naphthalen-1-ol (3c). 1,8-Dihydroxynaphthalene (**2c**) (930 mg, 5.8 mmol) and *p*-TsOH (502 mg, 2.9 mmol) were suspended in toluene (33 mL) and propanethiol (2.11 mL, 23.3 mmol) was added. The suspension was stirred at 100 °C overnight and then worked-up as for **3a**. Column chromatography (SiO_2, toluene–methanol 100:1 → 10:1) gave **3c** as an oil (964 mg, 76%). ^{13}C NMR ($CDCl_3$): δ 156.1, 138.2, 133.4, 130.8, 130.2, 128.1, 126.6, 124.4, 121.6, 112.7, 40.5, 23.1, 13.8. HRMS calcd for $C_{13}H_{15}OS$ (M + 1): 219.0844, found: 219.0855. The bis-substituted compound can be obtained using more equivalents of propanethiol. In this experiment the starting material, **2c**, was recovered in 24%.

5.1.4. 3-(Propylthio)naphthalen-2-ol (3d). 2,3-Dihydroxynaphthalene (**2d**) (1.96 g, 12.3 mmol) and *p*-TsOH (1.1 g, 6.3 mmol) were suspended in toluene (70 mL) and propanethiol (4.45 mL, 49.1 mmol) was added. The suspension was stirred at 100 °C overnight and then worked-up as for **3a**. Column chromatography (SiO_2, toluene–methanol 25:1 → 10:1) gave **3d** as a white amorphous solid (2.30 g, 86%). An analytical sample was recrystallized (heptane) to give white crystals; mp 56–57 °C. ^{13}C NMR (MeOD): δ 154.6, 134.8, 130.2, 129.3, 127.9, 127.5, 126.9, 126.8, 124.5, 109.7, 35.3, 23.4, 13.9. HRMS calcd for $C_{13}H_{15}OS$ (M + 1): 219.0844, found: 219.0871. The bis-substituted compound was isolated (187 mg, 5.5%) and some starting material was recovered (158 mg, 8%).

5.1.5. 6-(Propylthio)naphthalen-2-ol (3e). 2,6-Dihydroxynaphthalene (**2e**) (1.92 g, 12.0 mmol) and *p*-TsOH (1.0 g, 6.0 mmol) were suspended in toluene (70 mL) and propanethiol (4.45 mL, 49.1 mmol) was added. The suspension was stirred at 100 °C for 14 h and then worked-up as for **3a**. Column chromatography (SiO_2, toluene–methanol 20:1) gave **3e** as a white amorphous solid (1.63 g, 62%). An analytical sample was recrystallized (heptane) to give white crystals; mp 84–85 °C. ^{13}C NMR (MeOD): δ 156.5, 134.9, 131.7, 130.2, 129.8, 129.6, 129.2, 127.9, 119.9, 110.1, 37.2, 23.7, 13.74. HRMS calcd for $C_{13}H_{15}OS$ (M + 1): 219.0844, found: 219.0859. The bis-substituted compound was also isolated (1.24 g, 38%).

5.1.6. 7-(Propylthio)naphthalen-2-ol (3f). 2,7-Dihydroxynaphthalene (**2f**) (1.49 g, 9.3 mmol) and *p*-TsOH (801.2 mg, 4.6 mmol) were suspended in toluene (53 mL) and propanethiol (1.18 mL, 13.0 mmol) was added. The suspension was stirred at 100 °C overnight and then worked-up as for **3a**. Column chromatography (SiO_2, toluene–acetone 50:1 → 10:1) gave **3f** as a white amorphous solid (1.23 g, 61%). An analytical sample was recrystallized (heptane) to give white crystals; mp 87 °C. ^{13}C NMR (MeOD): δ 157.2, 137.0, 136.3, 130.4, 129.2, 128.2, 125.9, 125.3, 119.0, 109.3, 36.2, 23.7, 13.8. HRMS calcd for $C_{13}H_{15}OS$ (M + 1): 219.0844, found: 219.0863. The bis-substituted compound was isolated in 24% yield (607 mg, 2.2 mmol) and some starting material was isolated (66 mg, 4%).

5.1.7. 4-Hydroxy-1-naphthyl disulfide (4a). Ammonia (35 mL) was condensed into a stirred solution of 4-(propylthio)naphthalen-1-ol (**3a**) (424 mg, 1.94 mmol) in dry Et_2O (11 mL). Small pieces of sodium metal were added, allowing decolorization of the solution between additions, until the blue color persisted for more than 30 min. Excess sodium was destroyed by the careful addition of NH_4Cl. The suspension was diluted with Et_2O (15 mL) and the ammonia was evaporated by placing the reaction flask in a room-temperature water bath. Water (15 mL) was added and the aqueous phase was acidified with HCl (aq concd) and extracted with Et_2O. The combined organic phases were dried ($MgSO_4$), filtered, and concentrated. The crude concentrate was dissolved in MeOH (25 mL) and H_2O (15 mL) was added. Under vigorous stirring, I_2 (10% w/v in MeOH) was added dropwise until the dark color of iodine persisted. Excess iodine was destroyed by the addition of aqueous $NaHSO_3$ (10% w/v). The MeOH was removed under vacuum and the aqueous suspension was extracted three times with Et_2O. The combined organic phases were dried ($MgSO_4$), filtered, and concentrated. Recrystallization (toluene/heptane/MeOH) gave **1c** as off-white crystals (200 mg, 59%); mp 150–152 °C (lit. 152 °C).[29] The mother liquor was chromatographed (SiO_2, toluene–methanol 10:1) giving **4a** (61 mg, 18%) for a total yield of 77%. ^{13}C NMR (MeOD): δ 157.4, 137.2, 136.6, 127.8, 127.3, 127.1, 125.9, 124.1, 123.8, 108.4. HRMS calcd for $C_{20}H_{14}O_2S_2$ (M): 350.0435, found: 350.0438.

5.1.8. 5-Hydroxy-1-naphthyl disulfide (4b). Ammonia (30 mL) was condensed into a stirred solution of 5-(propylthio)naphthalen-1-ol (**3b**) (224 mg, 1.0 mmol) in dry Et_2O (10 mL). The reaction was performed and worked-up as for **4a**. Recrystallization (toluene/heptane/MeOH) gave **4b** as off-white crystals (141 mg, 79%); mp 211–213 °C (lit. 212 °C).[30] The mother liquor was chromatographed (SiO_2, toluene–methanol 5:1) giving **4b** (29 mg, 16%) for a total yield of 95%. ^{13}C NMR (MeOD): δ 155.3, 135.6, 134.1, 132.1, 128.2, 127.2, 125.1, 125.0, 117.2, 109.6. HRMS calcd for $C_{20}H_{14}O_2S_2$ (M): 350.0435, found: 350.0431.

5.1.9. 8-Hydroxy-1-naphthyl disulfide (4c). Ammonia (25 mL) was condensed into a stirred solution of 8-(propylthio)naphthalen-1-ol (**3c**) (231 mg, 1.1 mmol) in dry Et_2O (8 mL). The reaction was performed and worked-up as for **4a**. Recrystallization (toluene/heptane) gave **4c** as yellow crystals (127 mg, 69%); mp 170 °C (lit. 168–169 °C).[31] The mother liquor was chromatographed (SiO_2, toluene–methanol 10:1) giving **4c** (50 mg, 27%) for a total yield of 96%. ^{13}C NMR (MeOD): δ 155.8, 138.3, 134.0, 127.5, 126.9, 126.7, 124.3, 122.5, 120.9, 110.6. HRMS calcd for $C_{20}H_{14}O_2S_2$ (M): 350.0435, found: 350.0427.

5.1.10. 3-Hydroxy-2-naphthyl disulfide (4d). Ammonia (30 mL) was condensed into a stirred solution of 3-(propylthio)naphthalen-2-ol (**3d**) (620 mg, 2.84 mmol) in dry Et_2O (15 mL). The reaction was performed and worked-up as for **4a**. Recrystallization (toluene/heptane/MeOH) gave **4d** as yellow crystals (198 mg, 40%); mp 160–163 °C. The mother liquor was chromatographed (SiO_2, toluene–methanol 5:1) to give **4d** (274 mg, 55%) for a total yield of 95%. ^{13}C NMR (MeOD): δ 154.1, 135.5, 130.2, 128.2, 127.5, 127.2, 127.0, 126.6, 124.7, 110.1. HRMS calcd for $C_{20}H_{14}O_2S_2$ (M): 350.0435, found: 350.0427.

5.1.11. 6-Hydroxy-2-naphthyl disulfide (4e). Ammonia (30 mL) was condensed into a stirred solution of 6-(propylthio)naphthalen-2-ol (**3e**) (327 mg, 1.50 mmol) in dry Et_2O (10 mL). The reaction was performed and worked-up as for **4a**. Recrystallization (toluene/heptane/MeOH) gave **4e** as off-white crystals (156 mg, 56%); mp 222 °C (lit. 221–222 °C).[32] The mother liquor was chromatographed (SiO_2, toluene–methanol 10:1) to give **4e** (63 mg, 24%) for a total yield of 83%. ^{13}C NMR (MeOD): δ 157.5, 136.1, 132.2, 130.4, 129.89, 129.87, 128.7, 128.6, 120.3, 110.1. HRMS calcd for $C_{20}H_{14}O_2S_2$ (M): 350.0435, found: 350.0439.

5.1.12. 7-Hydroxy-2-naphthyl disulfide (4f). Ammonia (30 mL) was condensed into a stirred solution of 7-(propylthio)naphthalen-2-ol (**3f**) (411 mg, 1.88 mmol) in dry Et_2O (10 mL). The reaction was performed and worked-up as for **4a**. Recrystallization (toluene/heptane/MeOH) gave **4f** as off-white crystals (266 mg, 80%); mp 208 °C (lit. 205 °C).[33] The mother liquor was chromatographed (SiO_2, toluene–methanol 5:1) to give **4f** (17 mg, 5%) for a total yield of 86%. ^{13}C NMR (MeOD): δ 157.6, 136.7, 136.0, 130.6, 130.0, 129.1, 126.1, 123.7, 119.9, 109.7. HRMS calcd for $C_{20}H_{14}O_2S_2$ (M): 350.0435, found: 350.0432.

5.1.13. 4-(2,3,4-Tetra-*O*-acetyl-β-D-xylopyranoside)-1-naphthyl disulfide (5a). 4-Hydroxy-1-naphthyl disulfide (**4a**) (20.3 mg, 0.06 mmol) and 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside trichloroacetimidate (96.1 mg, 0.23 mmol) were suspended in CH_2Cl_2 (1.5 mL) and cooled to 0 °C. $BF_3{\cdot}OEt_2$ (3 μL, 0.02 mmol) was added and the mixture was stirred for 20 min at 0 °C. Et_3N was added and the solution was concentrated and filtered through a short column of SiO_2 (CH_2Cl_2–acetone 20:1). Column chromatography (SiO_2, heptane–EtOAc 1:1) gave **5a** as an off-white amorphous solid (39.3 mg, 78%). $[\alpha]_D^{20}$ − 50.4° (*c* 0.53, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 8.28 (m, 2H, H-8), 8.18 (m, 2H, H-5), 7.51 (m, 4H, H-6, H-7), 7.40 (d, 2H, *J* 8.1 Hz, H-3), 6.87 (d, 2H, *J* 8.1 Hz, H-2), 5.38 (m, 4H, H-1′, H-2′), 5.31 (dt, 2H, *J* 7.2, 0.9 Hz, H-3′), 5.06 (dt, 2H, *J* 7.4, 4.6 Hz, H-4′), 4.27 (dd, 2H, *J* 12.2, 4.5 Hz, H-5′), 3.62 (dd, 2H, *J* 12.2, 7.4 Hz, H-5′), 2.16, 2.12, 2.10 (s, 6H each, –OAc). ^{13}C NMR ($CDCl_3$): δ 169.98, 169.96, 169.7, 154.0, 134.6, 133.8, 127.6, 126.6, 126.4, 126.1, 122.3, 108.1, 98.3, 70.3, 69.9, 68.4, 62.0, 21.0, 20.9, 20.9. HRMS calcd for $C_{42}H_{42}O_{16}S_2Na$ (M+Na): 889.1812, found: 889.1805.

5.1.14. 5-(2,3,4-Tetra-*O*-acetyl-β-D-xylopyranoside)-1-naphthyl disulfide (5b). 5-Hydroxy-1-naphthyl disulfide (4b) (20.4 mg, 0.06 mmol) and 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside trichloroacetimidate (97.4 mg, 0.23 mmol) were suspended in CH_2Cl_2 (1.5 mL) and cooled to 0 °C. $BF_3{\cdot}OEt_2$ (3 μL, 0.02 mmol) was added and the reaction was performed and worked-up as for **5a** to give **5b** as an off-white amorphous solid (44.2 mg, 88%). $[\alpha]_D^{20}$ − 74.2° (*c* 0.88, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 8.13 (dt, 2H, *J* 7.5, 0.9 Hz, H-8), 8.05 (d, 2H, *J* 8.6 Hz, H-4), 7.65 (dd, 2H, *J* 7.2, 1.2 Hz, H-6), 7.43 (t, 2H, *J* 8.2 Hz, H-3), 7.32 (dd, 2H, *J* 7.3, 1.2 Hz, H-7), 7.12 (dd, 2H, *J* 7.7, 0.7 Hz, H-2), 5.37 (m, 4H, H-1′, H-2′), 5.31 (m, 2H, H-3′), 5.07 (dt, 2H, *J* 7.4, 4.6 Hz, H-4′), 4.28 (dd, 2H, *J* 12.2, 4.6 Hz, H-5′), 3.62 (dd, 2H, *J* 12.1, 7.5 Hz, H-5′), 2.15, 2.12, 2.09 (s, 6H each, –OAc). ^{13}C NMR ($CDCl_3$): δ 170.0, 169.7, 152.8, 133.9, 133.4, 130.9, 126.6, 126.6, 125.5, 123.0, 120.0, 109.4, 98.8, 70.6, 70.1, 68.5, 62.0, 20.95, 20.94, 20.92. HRMS calcd for $C_{42}H_{42}O_{16}S_2Na$ (M+Na): 889.1812, found: 889.1812.

5.1.15. 8-(2,3,4-Tetra-*O*-acetyl-β-D-xylopyranoside)-1-naphthyl disulfide (5c). 8-Hydroxy-1-naphthyl disulfide (**4c**) (17.9 mg, 0.05 mmol) and 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside trichloroacetimidate (85.2 mg, 0.20 mmol) were suspended in CH_2Cl_2 (1.5 mL) and cooled to 0 °C. $BF_3{\cdot}OEt_2$ (3 μL, 0.02 mmol) was added and the reaction was performed and worked-up as for **5a** to give **5c** as an off-white amorphous solid (35.9 mg, 81%). $[\alpha]_D^{20}$ − 109.5° (*c* 0.21, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.78 (dd, 2H, *J* 7.7, 1.0 Hz, H-2), 7.59 (dd, 2H, *J* 8.2, 0.8 Hz, H-4), 7.56 (dd, 2H, *J* 8.3, 0.8 Hz, H-5), 7.41 (t, 2H, *J* 8.0 Hz, H-6), 7.27 (t, 2H, *J* 7.9 Hz, H-3), 7.22 (dd, 2H, *J* 7.9, 1.0 Hz, H-7), 5.55 (m, 4H, H-1′, H-2′), 5.30 (t, 2H, *J* 7.1 Hz, H-3′), 5.09 (dt, 2H, *J* 7.0, 4.5 Hz, H-4′), 4.42 (dd, 2H, *J* 12.2, 4.5 Hz, H-5′), 3.71 (dd, 2H, *J* 12.2, 6.8 Hz, H-5′), 2.12, 2.06, 2.05 (s, 6H each, –OAc). ^{13}C NMR ($CDCl_3$): δ 170.3, 170.0, 169.4, 153.6, 136.6, 132.1, 126.4, 126.3, 126.1, 123.83, 123.81, 123.0, 109.9, 99.2, 70.9, 70.2, 68.7, 62.1, 21.0. HRMS calcd for $C_{42}H_{42}O_{16}S_2Na$ (M+Na): 889.1812, found: 889.1809.

5.1.16. 3-(2,3,4-Tetra-*O*-acetyl-β-D-xylopyranoside)-2-naphthyl disulfide (5d). 3-Hydroxy-2-naphthyl disulfide (**4d**) (19.8 mg, 0.06 mmol) and 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside trichloroacetimidate (96.4 mg, 0.23 mmol) were suspended in CH_2Cl_2 (1.5 mL) and cooled to 0 °C. $BF_3{\cdot}OEt_2$ (3 μL, 0.02 mmol) was added and the

reaction was performed and worked-up as for **5a** to give **5d** as an off-white amorphous solid (46.6 mg, 95%). $[\alpha]_D^{20}$ +29.5° (*c* 0.98, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.88 (s, 2H, H-4), 7.72 (d, 2H, *J* 8.2 Hz, H-5), 7.63 (d, 2H, *J* 8.1 Hz, H-8), 7.48 (s, 2H, H-1), 7.40 (dt, 2H, *J* 8.2, 1.2 Hz, H-7), 7.33 (dt, 2H, *J* 8.1, 1.2 Hz, H-6), 5.50 (d, 2H, *J* 4.9 Hz, H-1′), 5.31 (m, 4H, H-2′, H-3′), 5.04 (dt, 2H, *J* 6.2, 4.1 Hz, H-4′), 4.40 (dd, 2H, *J* 12.4, 4.0 Hz, H-5′), 3.73 (dd, 2H, *J* 12.4, 6.4 Hz, H-5′), 2.17, 2.17, 2.15 (s, 6H each, –OAc). ^{13}C NMR ($CDCl_3$): δ 170.0, 169.99, 169.5, 151.6, 133.1, 130.3, 127.3, 127.0, 126.6, 126.3, 125.6, 125.4, 111.2, 98.7, 69.6, 69.2, 68.1, 61.5, 21.1, 21.0. HRMS calcd for $C_{42}H_{42}O_{16}S_2Na$ (M+Na): 889.1812, found: 889.1805.

5.1.17. 6-(2,3,4-Tetra-*O*-acetyl-β-D-xylopyranoside)-2-naphthyl disulfide (5e). 6-Hydroxy-2-naphthyl disulfide (**4e**) (20.2 mg, 0.06 mmol) and 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside trichloroacetimidate (92.2 mg, 0.22 mmol) were suspended in CH_2Cl_2 (1.5 mL) and cooled to 0 °C. $BF_3{\cdot}OEt_2$ (3 μL, 0.02 mmol) was added and the reaction was performed and worked-up as for **5a** to give **5e** as an off-white amorphous solid (37.4 mg, 75%). $[\alpha]_D^{20}$ −22.7° (*c* 0.72, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.91 (d, 2H, *J* 1.8 Hz, H-1), 7.69 (d, 2H, *J* 8.7 Hz, H-3), 7.67 (d, 2H, *J* 8.9 Hz, H-7), 7.60 (dd, 2H, *J* 8.7, 1.9 Hz, H-4), 7.32 (d, 2H, *J* 2.5 Hz, H-5), 7.17 (dd, 2H, *J* 8.9, 2.4 Hz, H-8), 5.33 (d, 2H, *J* 5.6 Hz, H-1′), 5.24 (m, 4H, H-2′, H-3′), 5.03 (dt, 2H, *J* 7.3, 4.8 Hz, H-4′), 4.27 (dd, 2H, *J* 12.2, 4.6 Hz, H-5′), 3.60 (dd, 2H, *J* 12.2, 7.3 Hz, H-5′), 2.11, 2.10, 2.10 (s, 6H each, –OAc). ^{13}C NMR ($CDCl_3$): δ 170.0, 169.99, 169.5, 154.8, 133.6, 133.0, 130.2, 129.5, 128.4, 127.4, 127.2, 119.7, 111.4, 98.5, 70.6, 70.1, 68.6, 62.0, 20.94, 20.92, 20.88. HRMS calcd for $C_{42}H_{42}O_{16}S_2Na$ (M+Na): 889.1812, found: 889.1817.

5.1.18. 7-(2,3,4-Tetra-*O*-acetyl-β-D-xylopyranoside)-2-naphthyl disulfide (5f). 7-Hydroxy-2-naphthyl disulfide (**4f**) (19.8 mg, 0.06 mmol) and 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside trichloroacetimidate (91.0 mg, 0.22 mmol) were suspended in CH_2Cl_2 and cooled to 0 °C. $BF_3{\cdot}OEt_2$ (3 μL, 0.02 mmol) was added and the reaction was performed and worked-up as for **5a** to give **5f** as an off-white amorphous solid (42.0 mg, 86%). $[\alpha]_D^{20}$ −23.0° (*c* 0.93, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.87 (d, 2H, *J* 1.8 Hz, H-1), 7.74 (d, 4H, *J* 8.8 Hz, H-3, H-5), 7.52 (dd, 2H, *J* 8.8, 1.8 Hz, H-4), 7.26 (d, 2H, *J* 2.4 Hz, H-8), 7.15 (dd, 2H, *J* 9.0, 2.4 Hz, H-6), 5.31 (d, 2H, *J* 5.6 Hz, H-1′), 5.24 (m, 4H, H-2′, H-3′), 5.03 (dt, 2H, *J* 7.3, 4.8 Hz, H-4′), 4.25 (dd, 2H, *J* 12.1, 4.6 Hz, H-5′), 3.59 (dd, 2H, 12.1, 7.3 Hz, H-5′), 2.11, 2.10, 2.09 (s, 6H each, –OAc). ^{13}C NMR ($CDCl_3$): δ 170.0, 169.99, 169.5, 155.1, 135.3, 134.5, 129.7, 129.1, 128.8, 125.2, 124.1, 119.2, 110.9, 98.4, 70.6, 70.1, 68.5, 62.0, 20.94, 20.91, 20.87. HRMS calcd for $C_{42}H_{42}O_{16}S_2Na$ (M+Na): 889.1812, found: 889.1816.

5.1.19. 1-(4-Thionaphthyl) β-D-xylopyranoside (6a). 4-(2,3,4-Tetra-*O*-acetyl-β-D-xylopyranoside)-1-naphthyl disulfide (**5a**) (14.0 mg, 0.02 mmol) and polystyryldiphenylphosphine (Ph_3P-PS) (24 mg, 3 mmol/g) were suspended in THF (1.4 mL) and HCl (0.12 mL, 0.1 M, aq) was added. The mixture was heated at 80 °C in a sealed tube under careful stirring overnight. The mixture was removed from heat and MeOH (1 mL) was added followed by NaOMe–MeOH (0.5 mL, 1 M) and the reaction mixture was stirred for 10 min, neutralized with Amberlite IR-120H$^+$ then carefully filtered and immediately concentrated to give **6a** as an off-white amorphous solid (9.9 mg, 99%). No further purification was performed. $[\alpha]_D^{20}$ −37.2° (*c* 0.18, MeOD). ^{13}C NMR (CD_3OD): δ 152.4, 133.3, 128.8, 126.6, 126.4, 125.3, 124.8, 122.5, 120.6, 109.3, 101.9, 76.4, 73.4, 69.6, 65.6. HRMS calcd for $C_{15}H_{16}O_5SNa$ (M+Na): 331.0616, found: 331.0649.

5.1.20. 1-(5-Thionaphthyl) β-D-xylopyranoside (6b). 5-(2,3,4-Tetra-*O*-acetyl-β-D-xylopyranoside)-1-naphthyl disulfide (**5b**) (14.4 mg, 0.02 mmol) and Ph_3P-PS (25 mg, 3 mmol/g) were suspended in THF (1.4 mL) and HCl (0.12 mL, 0.1 M, aq) was added and the reaction was performed as for **6a** to give **6b** as an off-white amorphous solid (10.2 mg, quant.). $[\alpha]_D^{20}$ −50.2° (*c* 0.28, MeOD). ^{13}C NMR (CD_3OD): δ 153.4, 132.7, 128.6, 127.9, 126.5, 125.8, 124.7, 120.2, 118.7, 109.4, 101.8, 76.4, 73.4, 69.6, 65.6. HRMS calcd for $C_{15}H_{16}O_5SNa$ (M+Na): 331.0616, found: 331.0654.

5.1.21. 1-(8-Thionaphthyl) β-D-xylopyranoside (6c). 8-(2,3,4-Tetra-*O*-acetyl-β-D-xylopyranoside)-1-naphthyl disulfide (**5c**) (8.2 mg, 0.01 mmol) and Ph_3P-PS (14 mg, 3 mmol/g) were suspended in THF (1.4 mL) and HCl (0.12 mL, 0.1 M, aq) was added. Disulfide cleavage, deprotection, and work-up was performed as for **6a** after which TLC-analysis showed a substantial amount of disulfide. Cleavage was performed again using new Ph_3P-PS under microwave irradiation (110 °C for 15 min). Gave **6c** as an off-white amorphous solid (5.8 mg, quant.). $[\alpha]_D^{20}$ −44.2° (*c* 0.20, MeOD). ^{13}C NMR (CD_3OD): δ 153.6, 136.6, 131.7, 125.9, 125.6, 124.0, 122.8, 122.5, 109.6, 102.3, 76.7, 73.4, 69.9, 65.8. HRMS calcd for $C_{15}H_{16}O_5SNa$ (M+Na): 331.0616, found: 331.0654.

5.1.22. 2-(3-Thionaphthyl) β-D-xylopyranoside (6d). 3-(2,3,4-Tetra-*O*-acetyl-β-D-xylopyranoside)-2-naphthyl disulfide (**5d**) (14.5 mg, 0.02 mmol) and Ph_3P-PS (25 mg, 3 mmol/g) were suspended in THF (1.4 mL) and HCl (0.12 mL, 0.1 M, aq) was added and the reaction was performed as for **6a** to give **6d** as an off-white amorphous solid (9.6 mg, 93%). $[\alpha]_D^{20}$ −31.9° (*c* 0.24, MeOD). ^{13}C NMR (CD_3OD): δ 161.0, 132.3, 130.0, 126.6, 126.5, 125.8, 125.1, 124.6, 124.0, 110.8, 101.9, 76.0, 73.1, 69.5, 65.5. HRMS calcd for $C_{15}H_{16}O_5SNa$ (M+Na): 331.0616, found: 331.0590.

5.1.23. 2-(6-Thionaphthyl) β-D-xylopyranoside (6e). 6-(2,3,4-Tetra-*O*-acetyl-β-D-xylopyranoside)-2-naphthyl disulfide (**5e**) (13.3 mg, 0.02 mmol) and Ph_3P-PS (23 mg, 3 mmol/g) were suspended in THF (1.4 mL) and HCl (0.12 mL, 0.1 M, aq) was added and the reaction was performed as for **6a** to give **6e** as an off-white amorphous solid (9.3 mg, 98%). $[\alpha]_D^{20}$ −14.2° (*c* 0.30, MeOD). ^{13}C NMR (CD_3OD): δ 155.0, 132.2, 130.3, 127.9, 127.8, 127.4, 126.9, 126.2, 119.3, 110.6, 101.5,

76.3, 73.4, 69.6, 65.6. HRMS calcd for $C_{15}H_{16}O_5SNa$ (M+Na): 331.0616, found: 331.0636.

5.1.24. 2-(7-Thionaphthyl) β-D-xylopyranoside (6f). 7-(2,3,4-Tetra-*O*-acetyl-β-D-xylopyranoside)-2-naphthyl disulfide (**5f**) (15.1 mg, 0.02 mmol) and Ph_3P-PS (26 mg, 3 mmol/g) were suspended in THF (1.4 mL) and HCl (0.12 mL, 0.1 M, aq) was added and the reaction was performed as for **6a** to give **6f** as an off-white amorphous solid (10.2 mg, 95%). $[\alpha]_D^{20}$ −16.2° (*c* 0.25, MeOD). ^{13}C NMR (CD_3OD): δ 155.9, 134.9, 130.0, 128.8, 127.9, 127.5, 125.4, 125.0, 117.9, 109.3, 101.3, 76.3, 73.4, 69.6, 65.6. HRMS calcd for $C_{15}H_{16}O_5SNa$ (M+Na): 331.0616, found: 331.0633.

5.1.25. 1-(4-*O*-Pivaloyl-naphthyl) 1-thio-2,3,4-tri-*O*-acetyl-β-D-xylopyranoside (8a). 4-Hydroxy-1-naphthyl disulfide (**4a**) (19.3 mg, 0.06 mmol) was suspended in pivalic anhydride (Piv_2O) (1.0 mL) and heated to 110 °C overnight. The solution was concentrated under high vacuum, the residue was dissolved in THF (0.7 mL), and Ph_3P-PS (82.3 mg, 3 mmol/g) was added followed by HCl (0.06 mL, 0.1 M, aq). The mixture was heated to 110 °C for 15 min under microwave irradiation, then carefully filtered and immediately concentrated. The residue was dissolved in DMF (0.4 mL) and the solution was cooled to 0 °C. *t*-BuONa (9.0 mg, 0.08 mmol) was added and the solution was stirred at 0 °C for 5 min. 2,3,4-Tri-*O*-acetyl-α-D-xylopyranosyl bromide (26.0 mg, 0.08 mmol) in THF (0.2 mL) was added and the solution was stirred at 0 °C for 30 min after which it was filtered through a short column of SiO_2 (Heptane–EtOAc 1:1) and concentrated. The residue was chromatographed (SiO_2, Heptane–EtOAc 2:1) to give **8a** as an off-white amorphous solid (26.6 mg, 67%). $[\alpha]_D^{20}$ −59.7° (*c* 0.49, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 8.49 (d, 1H, *J* 8.0 Hz, H-8), 7.91 (d, 1H, *J* 8.3 Hz, H-5), 7.83 (d, 1H, *J* 7.9 Hz, H-3), 7.61 (m, 1H, H-7), 7.58 (m, 1H, H-6), 7.21 (d, 1H, *J* 7.9 Hz, H-2), 5.17 (t, 1H, *J* 8.2 Hz, H-3′), 5.07 (t, 1H, 8.2 Hz, H-2′), 4.97 (dt, 1H, *J* 8.4, 5.0 Hz, H-4′), 4.77 (d, 1H, *J* 8.5, H-1′), 4.24 (dd, 1H, *J* 11.7, 4.9 Hz, H-5′), 3.31 (dd, *J* 11.7, 8.9 Hz, H-5′), 2.14, 2.07, 2.04 (s, 3H each, –OAc), 1.50 (s, 9H, –OPiv). ^{13}C NMR ($CDCl_3$): δ 176.7, 170.0, 169.8, 169.4, 148.2, 135.3, 133.5, 127.7, 127.3, 127.0, 126.8, 126.2, 121.7, 117.9, 87.2, 72.1, 70.3, 68.4, 65.3, 39.6, 27.3, 20.8, 20.73, 20.72. HRMS calcd for $C_{26}H_{30}O_9SNa$ (M+Na): 541.1508, found: 541.1528.

5.1.26. 1-(5-*O*-Pivaloyl-naphthyl) 1-thio-2,3,4-tri-*O*-acetyl-β-D-xylopyranoside (8b). 5-Hydroxy-1-naphthyl disulfide (**4b**) (47.8 mg, 0.14 mmol) was pivaloylated as for **8a**. The residue was dissolved in THF (1.4 mL) and Ph_3P-PS (204.4 mg, 3 mmol/g) was added followed by HCl (0.12 mL, 0.1 M, aq). The mixture was heated to 110 °C for 15 min under microwave irradiation then carefully filtered and immediately concentrated. The residue was dissolved in DMF (0.4 mL) and the solution was cooled to 0 °C. *t*-BuONa (30.0 mg, 0.27 mmol) was added and the solution was stirred at 0 °C for 10 min. 2,3,4-Tri-*O*-acetyl-α-D-xylopyranosyl bromide (83.6 mg, 0.25 mmol) in THF (0.2 mL) was added and the solution was allowed to regain rt. After 2 h the solution was diluted with EtOAc and washed with $NaHCO_3$ (satd aq), brine, and H_2O. The solution was dried ($MgSO_4$), filtered, and concentrated. The residue was chromatographed (SiO_2, Heptane–EtOAc 1:1) to give **8b** as an off-white amorphous solid (90.8 mg, 71%). $[\alpha]_D^{20}$ −80.4° (*c* 0.45, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 8.36 (dt, 1H, *J* 8.7, 0.9 Hz, H-4), 7.90 (dt, 1H, *J* 8.5, 0.9 Hz, H-8), 7.84 (dd, 1H, *J* 7.3, 1.1 Hz, H-6), 7.57 (dd, 1H, *J* 8.7, 7.5 Hz, H-3), 7.47 (dd, 1H, *J* 8.5, 7.3 Hz, H-7), 7.26 (dd, 1H, *J* 7.5, 0.9 Hz, H-2), 5.18 (t, 1H, *J* 8.1 Hz, H-3′), 5.08 (t, 1H, *J* 8.1 Hz, H-2′), 4.97 (dt, 1H, *J* 8.3, 4.9 Hz, H-4′), 4.83 (d, 1H, *J* 8.3 Hz, H-1′), 4.26 (dd, 1H, *J* 11.7, 4.9, Hz, H-5′), 3.34 (dd, 1H, *J* 11.7, 8.9 Hz, H-5′), 2.13, 2.08, 2.04 (s, 3H each, –OAc), 1.50 (s, 9H, –OPiv). ^{13}C NMR ($CDCl_3$): δ 177.0, 169.9, 169.8, 169.4, 147.2, 135.3, 133.6, 130.3, 127.8, 126.3, 126.1, 123.5, 122.6, 118.5, 87.0, 72.0, 70.2, 68.4, 65.2, 39.5, 27.3, 20.8, 20.7. HRMS calcd for $C_{26}H_{30}O_9SNa$ (M+Na): 541.1508, found: 541.1497.

5.1.27. 2-(6-*O*-Pivaloyl-naphthyl) 1-thio-2,3,4-tri-*O*-acetyl-β-D-xylopyranoside (8e). 6-Hydroxy-2-naphthyl disulfide (**4e**) (51.4 mg, 0.15 mmol) was pivaloylated as for **8a**. The residue was dissolved in THF (1.4 mL) and Ph_3P-PS (222.1 mg, 3 mmol/g) was added followed by HCl (0.12 mL, 0.1 M, aq). The mixture was heated to 110 °C for 15 min under microwave irradiation then carefully filtered and immediately concentrated. The residue was dissolved in DMF (0.4 mL) and the solution was cooled to 0 °C. *t*-BuONa (6.3 mg, 0.06 mmol) was added and the solution was stirred at 0 °C for 7 min. 2,3,4-Tri-*O*-acetyl-α-D-xylopyranosyl bromide (89.5 mg, 0.26 mmol) in THF (0.2 mL) was added and the solution was allowed to regain rt. After 3 h the solution was worked-up as for **8b**. The residue was chromatographed (SiO_2, Heptane–EtOAc 1:1) to give **8e** as an off-white amorphous solid (104.2 mg, 76%). $[\alpha]_D^{20}$ −59.9° (*c* 0.34, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.99 (s, 1H, H-1), 7.81 (d, 1H, *J* 8.8 Hz, H-8), 7.75 (d, I H, *J* 8.6 Hz, H-4), 7.56 (dd, 1H, *J* 8.6, 1.6 Hz, H-3), 7.52 (d, 1H, *J* 2.1 Hz, H-5), 7.23 (dd, 1H, *J* 8.8, 2.1 Hz, H-7), 5.21 (t, 1H, *J* 7.9, H-3′), 5.00 (t, 1H, *J* 7.9 Hz, H-2′), 4.94 (dt, 1H, *J* 8.1, 4.9 Hz, H-4′), 4.92 (d, H 1, *J* 8.1 Hz, H-1′), 4.32 (dd, 1H, *J* 11.9, 4.8 Hz, H-5′), 3.46 (dd, 1H, *J* 11.9, 8.6 Hz, H-5′), 2.13, 2.07, 2.06 (s, 3H each, –OAc), 1.41 (s, 3H, –OPiv). ^{13}C NMR ($CDCl_3$): δ 177.1, 169.9, 169.8, 169.4, 149.4, 133.2, 132.0, 131.4, 130.5, 129.3, 129.1, 128.3, 122.0, 118.4, 86.3, 71.8, 69.9, 68.4, 65.1, 39.2, 27.2, 20.8, 20.7. HRMS calcd for $C_{26}H_{30}O_9SNa$ (M+Na): 541.1508, found: 541.1492.

5.1.28. 2-(7-*O*-Pivaloyl-naphthyl) 1-thio-2,3,4-tri-*O*-acetyl-β-D-xylopyranoside (8f). 7-Hydroxy-2-naphthyl disulfide (**4f**) (46.7 mg, 0.13 mmol) was pivaloylated as for **8a**. The residue was dissolved in THF (1.4 mL) and Ph_3P-PS (197.0 mg, 3 mmol/g) was added followed by HCl (0.12 mL, 0.1 M, aq). The mixture was heated to 110 °C for 15 min under microwave irradiation then carefully filtered and immediately concentrated. The residue was dissolved in DMF (0.4 mL) and the solution was cooled to 0 °C. *t*-BuONa (29.3 mg, 0.26 mmol) was added and the solution was stirred at 0 °C for

15 min. 2,3,4-Tri-*O*-acetyl-α-D-xylopyranosyl bromide (82.3 mg, 0.24 mmol) in THF (0.2 mL) was added and the solution was allowed to regain rt. After 2 h the solution was worked-up as for **8b**. The residue was chromatographed (SiO_2, Heptane–EtOAc 1:1) to give **8f** (76.8 mg, 61%). $[\alpha]_D^{20}$ −38.7° (*c* 0.45, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.93 (d, 1H, *J* 1.3 Hz, H-1), 7.83 (d, 1H, *J* 8.9 Hz, H-5), 7.79 (d, 1H, *J* 8.6 Hz, H-4), 7.52 (dd, 1H, *J* 8.5, 1.8 Hz, H-3), 7.49 (d, 1H, *J* 2.2 Hz, H-8), 7.22 (dd, 1H, *J* 8.9, 2.3, H-6), 5.21 (t, 1H, *J* 7.9, H-3′), 5.00 (t, 1H, *J* 7.9 Hz, H-2′), 4.94 (dt, 1H, *J* 8.4, 4.9 Hz, H-4′), 4.92 (d, H 1, *J* 8.2 Hz, H-1′), 4.32 (dd, 1H, *J* 11.8, 4.8 Hz, H-5′), 3.46 (dd, 1H, *J* 11.8, 8.6 Hz, H-5′), 2.13, 2.07, 2.06 (s, 3H each, –OAc), 1.41 (s, 3 H, –OPiv). ^{13}C NMR ($CDCl_3$): δ 177.2, 169.9, 169.8, 169.4, 149.4, 133.9, 131.3, 130.6, 130.6, 129.4, 129.1, 128.4, 122.0, 118.2, 86.2, 71.8, 69.9, 68.4, 65.2, 39.2, 27.2, 20.8, 20.73, 20.72. HRMS calcd for $C_{26}H_{30}O_9SNa$ (M+Na): 541.1508, found: 541.1525.

5.1.29. 1-(4-Hydroxynaphthyl) 1-thio-β-D-xylopyranoside (9a). 1-(4-*O*-Pivaloyl-naphthyl) 1-thio-2,3,4-tri-*O*-acetyl-β-D-xylopyranoside (**8a**) (12.7 mg, 0.02 mmol) was dissolved in CH_2Cl_2 (1 mL). MeOH (1 mL) and NaOMe–MeOH (0.5 mL, 1 M) were added, the mixture was stirred for 3 h, neutralized with Amberlite IR-120H$^+$, filtered, and concentrated. The residue was filtered (SiO_2, CH_2Cl_2–MeOH 5:1) to give **9a** as an off-white amorphous solid (7.3 mg, 97%). $[\alpha]_D^{20}$ −73.7° (*c* 0.41, MeOH). ^{13}C NMR (CD_3OD): δ 156.3, 137.5, 137.2, 127.8, 127.4, 127.1, 125.8, 123.7, 119.7, 108.7, 91.4, 79.3, 74.2, 71.0, 70.5. HRMS calcd for $C_{15}H_{16}O_5SNa$ (M+Na): 331.0616, found: 331.0623.

5.1.30. 1-(5-Hydroxynaphthyl) 1-thio-β-D-xylopyranoside (9b). 1-(5-*O*-Pivaloyl-naphthyl) 1-thio-2,3,4-tri-*O*-acetyl-β-D-xylopyranoside (**8a**) (21.8 mg, 0.04 mmol) was deprotected and purified as for **9a**. Gave **9b** as an off-white amorphous solid (7.3 mg, 97%). $[\alpha]_D^{20}$ −100.9° (*c* 0.66, MeOH). ^{13}C NMR (CD_3OD): δ 155.0, 136.5, 133.2, 131.5, 127.8, 126.9, 125.1, 124.0, 117.8, 109.3, 90.5, 79.2, 74.3, 70.9, 70.3. HRMS calcd for $C_{15}H_{16}O_5SNa$ (M+Na): 331.0616, found: 331.0586.

5.1.31. 1-(8-Hydroxynaphthyl) 1-thio-β-D-xylopyranoside (9c). 8-Hydroxy-1-naphthyl disulfide (**4c**) (26.9 mg, 0.08 mmol) was pivaloylated as for **8a**. The residue was dissolved in THF (0.7 mL) and Ph_3P-PS (120.0 mg, 3 mmol/g) was added followed by HCl (0.06 mL, 0.1 M, aq). The mixture was heated to 110 °C for 15 min under microwave irradiation then carefully filtered and immediately concentrated. The residue was dissolved in DMF (0.4 mL) and the solution was cooled to 0 °C. *t*-BuONa (15.6 mg, 0.14 mmol) was added and the solution was stirred at 0 °C for 5 min. 2,3,4-Tri-*O*-acetyl-α-D-xylopyranosyl bromide (42.0 mg, 0.12 mmol) in THF (0.2 mL) was added and the solution was stirred at 0 °C for 30 min after which it was filtered through a pad of SiO_2 (Heptane–EtOAc 1:1) and concentrated. The residue was dissolved in CH_2Cl_2 (2 mL) and MeOH (2 mL), and NaOMe–MeOH (1 mL, 1 M) was added. The solution was stirred for 2 h, neutralized with Amberlite IR-120H$^+$, filtered, and concentrated. Chromatography (SiO_2, CH_2Cl_2–MeOH 5:1) gave **9c** as an off-white amorphous solid (13.1 mg, 34%). $[\alpha]_D^{20}$ −89.2° (*c* 0.24, MeOH). ^{13}C NMR (CD_3OD): δ 155.7, 137.9, 133.9, 130.4, 128.5, 127.8, 126.4, 124.8, 121.5, 112.8, 90.7, 79.3, 73.9, 70.8, 70.5. HRMS calcd for $C_{15}H_{16}O_5SNa$ (M+Na): 331.0616, found: 331.0627.

5.1.32. 2-(3-Hydroxynaphthyl) 1-thio-β-D-xylopyranoside (9d). 3-Hydroxy-2-naphthyl disulfide (**4d**) (28.5 mg, 0.08 mmol) was pivaloylated as for **8a**. The residue was dissolved in THF (0.7 mL) and Ph_3P-PS (121.2 mg, 3 mmol/g) was added followed by HCl (0.06 mL, 0.1 M, aq). The mixture was heated to 110 °C for 15 min under microwave irradiation then carefully filtered and immediately concentrated. The residue was dissolved in DMF (0.4 mL) and the solution was cooled to 0 °C. *t*-BuONa (15.5 mg, 0.14 mmol) was added and the solution was stirred at 0 °C for 5 min. 2,3,4-Tri-*O*-acetyl-α-D-xylopyranosyl bromide (43.3 mg, 0.13 mmol) in THF (0.2 mL) was added and the solution was stirred at 0 °C for 25 min after which it was worked-up, deprotected, and purified as for **9a**. Gave **9d** as an off-white amorphous solid (17.3 mg, 44%). $[\alpha]_D^{20}$ −33.9° (*c* 0.32, MeOH). ^{13}C NMR (CD_3OD): δ 154.1, 134.2, 128.6, 127.0, 126.4, 125.6, 125.5, 123.2, 120.7, 109.1, 87.6, 77.7, 72.4, 69.4, 69.3. HRMS calcd for $C_{15}H_{16}O_5SNa$ (M+Na): 331.0616, found: 331.0629.

5.1.33. 2-(6-Hydroxynaphthyl) 1-thio-β-D-xylopyranoside (9e). 2-(6-*O*-Pivaloyl-naphthyl) 1-thio-2,3,4-tri-*O*-acetyl-β-D-xylopyranoside (**8e**) (18.5 mg, 0.04 mmol) was deprotected and purified as for **9a**. Gave **9e** as an off-white amorphous solid (10.5 mg, 95%). $[\alpha]_D^{20}$ −35.4° (*c* 0.58, MeOH). ^{13}C NMR (CD_3OD): δ 155.8, 134.4, 132.0, 130.5, 128.8, 128.5, 126.3, 126.2, 118.5, 108.4, 89.0, 77.9, 72.3, 69.5, 69.1. HRMS calcd for $C_{15}H_{16}O_5SNa$ (M+Na): 331.0616, found: 331.0580.

5.1.34. 2-(7-Hydroxynaphthyl) 1-thio-β-D-xylopyranoside (9f). 2-(7-*O*-Pivaloyl-naphthyl) 1-thio-2,3,4-tri-*O*-acetyl-β-D-xylopyranoside (**8f**) (22.2 mg, 0.04 mmol) was deprotected and purified as for **9a**. Gave **9f** as an off-white amorphous solid (13.0 mg, 98%). $[\alpha]_D^{20}$ −31.9° (*c* 0.71, MeOH). ^{13}C NMR (CD_3OD): δ 155.7, 135.2, 131.1, 128.9, 128.8, 127.7, 127.4, 126.1, 118.3, 108.1, 88.7, 77.8, 72.4, 69.5, 69.0. HRMS calcd for $C_{15}H_{16}O_5SNa$ (M+Na): 331.0616, found: 331.0630.

5.1.35. 1-(4-(Propylthio)naphth-1-yl)-2,3,4-tri-*O*-acetyl-β-D-xylopyranoside (10a). 4-(Propylthio)naphthalen-1-ol (**3a**) (545 mg, 2.5 mmol) and 1,2,3,4-tetra-*O*-acetyl-β-D-xylopyranoside (1.59 g, 5.0 mmol) were dissolved in CH_2Cl_2 (37 mL). Et_3N (350 μL, 2.5 mmol) was added followed by $BF_3{\cdot}OEt_2$ (1.56 mL, 12.4 mmol) and the solution was stirred for 2 h. Excess Et_3N was added and the solution was concentrated. Column chromatography (SiO_2, heptane–EtOAc 3:1) gave **10a** as an off-white amorphous solid (1.14 g, 96%). $[\alpha]_D^{20}$ −62.1° (*c* 0.58, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 8.46 (d, 1H, *J* 8.3 Hz, H-8), 8.17 (d, 1H, *J* 8.4 Hz, H-5), 7.60 (m, 1H, H-7), 7.59 (d, 1H, *J* 8.1 Hz, H-3), 7.53 (m, 1H, H-6),

7.02 (d, 1H, *J* 8.1 Hz, H-2), 5.38 (m, 1H, H-1′, H-2′), 5.31 (m, 1H, H-3′), 5.07 (dt, 1H, *J* 7.4, 4.6 Hz, H-4′), 4.28 (dd, 1H, *J* 12.2, 4.6 Hz, H-5′), 3.62 (dd, 1H, *J* 12.2, 7.4 Hz, H-5′), 2.85 (t, 2H, *J* 7.3 Hz, –SCH_2–), 2.16 (s, 3H, –OAc), 2.12 (s, 3H, –OAc), 2.09 (s, 3H, –OAc), 1.61 (tq, 2H, *J* 7.4, 7.3 Hz, –CH_2Me), 1.00 (t, 3H, *J* 7.4 Hz, –CH_3). ^{13}C NMR ($CDCl_3$): *δ* 170.0, 169.7, 152.3, 134.7, 130.8, 127.3, 127.28, 126.26, 126.2, 125.8, 122.3, 108.7, 98.5, 70.4, 70.0, 68.5, 61.9, 37.7, 22.9, 20.94, 20.93, 20.91, 13.5. HRMS calcd for $C_{24}H_{28}O_8SNa$ (M+Na): 499.1403, found: 499.1420.

5.1.36. 1-(5-(Propylthio)naphth-1-yl) 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside (10b). Synthesized as for **10a**. 5-(Propylthio)naphthalen-1-ol (**3b**) (515 mg, 2.4 mmol) gave **10b** as off-white amorphous solid (1.08 g, 96%). $[\alpha]_D^{20}$ −74.0° (*c* 0.52, $CHCl_3$). ^{1}H NMR ($CDCl_3$): *δ* 8.12 (d, 1H, *J* 8.6 Hz, H-8), 8.05 (d, 1H, *J* 8.6 Hz, H-4), 7.57 (dd, I H, *J* 7.3, 1.1, H-2), 7.45 (dd, 1H, *J* 8.6, 7.8 Hz, H-7), 7.41 (dd, 1H, *J* 8.6, 7.3 Hz, H-3), 7.11 (d, 1 H, *J* 7.8 Hz, H-6), 5.38 (m, 2H, H-1′, H-2′), 5.31 (t, 1H, *J* 7.2 Hz, H-3′), 5.07 (dt, 1H, *J* 7.3, 4.6 Hz, H-4′), 4.28 (dd, 1H, *J* 12.2, 4.6 Hz, H-5′), 3.61 (dd, 1H, *J* 12.2, 7.3 Hz, H-5′), 2.96 (t, 2H, *J* 7.2 Hz, –SCH_2–), 2.15 (s, 3H, –OAc), 2.11 (s, 3H, –OAc), 2.09 (s, 3H, –OAc), 1.69 (tq, 2H, *J* 7.4, 7.2 Hz, –CH_2Me), 1.05 (t, 3H, *J* 7.4 Hz, –CH_3). ^{13}C NMR ($CDCl_3$): *δ* 169.9, 169.8, 169.5, 152.6, 134.0, 134.0, 128.2, 126.2, 125.9, 125.4, 120.3, 119.8, 109.1, 98.6, 70.4, 70.0, 68.4, 61.8, 36.2, 22.5, 20.78, 20.76, 13.5. HRMS calcd for $C_{24}H_{28}O_8SNa$ (M+Na): 499.1403, found: 499.1394.

5.1.37. 1-(8-(Propylthio)naphth-1-yl) 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside (10c). Synthesis of **10c** using the method described above for **10a** gave a poor yield (28%) and therefore synthesis of the desired product **11c** (see below) was performed using a trichloroacetimidate donor and full isolation of the acetylated product was not performed prior to deacetylation. Data for **10c** are nonetheless reported here. $[\alpha]_D^{20}$ −105.8° (*c* 0.51, $CHCl_3$). ^{1}H NMR ($CDCl_3$): *δ* 7.51 (d, 1H, *J* 8.2 Hz, H-5), 7.49 (d, 1H, *J* 8.3 Hz, H-4), 7.35 (t, 1H, *J* 7.8 Hz, H-3), 7.34 (t, 1H, *J* 7.6 Hz, H-6), 7.19 (d, 1H, *J* 7.6 Hz, H-7), 7.10 (dd, 1H, *J* 7.8, 0.9 Hz, H-2), 5.57 (dd, 1H, *J* 8.2, 6.3 Hz, H-2′), 5.39 (d, 1H, *J* 6.3 Hz, H-1′), 5.29 (t, 1H, *J* 8.2 Hz, H-3′), 5.14 (dt, 1H, *J* 7.9, 4.9 Hz, H-4′), 4.36 (dd, 1H, *J* 12.2, 4.9 Hz, H-5′), 3.63 (dd, 1H, *J* 12.2, 7.9 Hz, H-5′), 2.89 (t, 2H, *J* 7.7 Hz, –SCH_2–), 2.10, 2.10, 2.05 (s, 3H each, –OAc), 1.79 (tq, 2H, *J* 7.7, 7.7 Hz, –CH_2Me), 1.12 (t, 3H, *J* 7.4 Hz, –CH_3). ^{13}C NMR ($CDCl_3$): *δ* 170.3, 169.9, 169.5, 154.1, 136.3, 136.2, 125.9, 125.8, 124.2, 123.6, 123.55, 121.6, 109.6, 99.2, 71.7, 70.3, 68.9, 62.3, 34.8, 21.2, 21.0, 20.9, 20.8, 14.1. HRMS calcd for $C_{24}H_{28}O_8SNa$ (M+Na): 499.1403, found: 499.1427.

5.1.38. 1-(3-(Propylthio)naphth-2-yl) 2,3,4-tri-*O*-acetyl β-D-xylopyranoside (10d). Synthesized as for **10a**. 3-(Propylthio)naphthalen-2-ol (**3d**) (520 mg, 2.4 mmol) gave **10d** as off-white amorphous solid (941 mg, 83%). $[\alpha]_D^{20}$ −33.3° (*c* 0.69, $CHCl_3$). ^{1}H NMR ($CDCl_3$): *δ* 7.70 (m, 2H, H-6, H-7), 7.57 (s, 1H, H-1), 7.42 (s, 1H, H-4), 7.39 (m, 2H, H-5, H-8), 5.38 (d, 1H, *J* 5.0 Hz, H-1′), 5.28 (m, 1H, H-2′), 5.24 (m, 1H, H-3′), 5.02 (dt, 1H, *J* 6.3, 4.1 Hz, H-4′), 4.35 (dd, 1H, *J* 12.3, 4.1 Hz, H-5′), 3.67 (dd, 1H, *J* 12.3, 6.4 Hz, H-5′), 2.96 (dt, 2H, *J* 7.6, 2.9 Hz, –SCH_2–), 2.17, 2.15, 2.13 (s, 3H each, –OAc), 1.76 (tq, 2H, *J* 7.6, 7.3 Hz, –CH_2Me), 1.10 (t, 3H, *J* 7.3 Hz, –CH_3). ^{13}C NMR ($CDCl_3$): *δ* 169.9, 169.85, 169.4, 152.1, 131.9, 130.2, 129.0, 126.9, 126.5, 125.6, 125.4, 125.0, 110.8, 98.5, 69.5, 69.1, 68.1, 61.3, 33.5, 21.9, 20.9, 20.86, 20.8, 13.7. HRMS calcd for $C_{24}H_{28}O_8SNa$ (M+Na): 499.1403, found: 499.1422.

5.1.39. 1-(6-(Propylthio)naphth-2-yl) 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside (10e). Synthesized as for **10a**. 6-(Propylthio)naphthalen-2-ol (**3e**) (509 mg, 2.3 mmol) gave **10e** as an off-white amorphous solid (1.11 g, quantitative). $[\alpha]_D^{20}$ −21.8° (*c* 0.50, $CHCl_3$). ^{1}H NMR ($CDCl_3$): *δ* 7.71 (d, 1H, *J* 1.8 Hz, H-1), 7.68 (d, 1H, *J* 9.0 Hz, H-8), 7.65 (d, 1H, *J* 8.6 Hz, H-4), 7.43 (d, 1H, *J* 8.6, 1.8 Hz, H-3), 7.32 (d, 1H, *J* 2.4 Hz, H-5), 7.17 (dd, 1H, *J* 9.0, 2.4 Hz, H-7) 5.32 (d, 1H, *J* 5.6 Hz, H-1′), 5.27 (m, 1 H, H-3′), 5.25 (m, 1H, H-2′), 5.05 (dt, 1H, *J* 7.1, 4.7 Hz, H-4′), 4.28 (dd, 1H, *J* 12.2, 4.7, H-5′), 3.60 (dd, 1H, *J* 12.2, 7.1, H-5′), 2.98 (t, 2H, *J* 7.4 Hz, –SCH_2–), 2.12, 2.11, 2.11 (s, 3H each, –OAc), 1.71 (tq, 2H, *J* 7.4, 7.3 Hz, –CH_2Me), 1.05 (t, 3 H, *J* 7.3 Hz, –CH_3). ^{13}C NMR ($CDCl_3$): *δ* 169.9, 169.8, 169.4, 154.1, 132.9, 132.4, 130.4, 128.8, 128.4, 127.6, 127.0, 119.3, 111.3, 98.5, 70.5, 70.1, 68.4, 61.8, 35.9, 22.5, 20.79, 20.77, 20.7, 13.4. HRMS calcd for $C_{24}H_{28}O_8SNa$ (M+Na): 499.1403, found: 499.1393.

5.1.40. 1-(7-(Propylthio)naphth-2-yl) 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside (10f). Synthesized as for **10a**. 7-(Propylthio)naphthalen-2-ol (**3f**) (523 mg, 2.4 mmol) gave **10f** as an off-white amorphous solid (976 mg, 86%). $[\alpha]_D^{20}$ −11.9° (*c* 0.39, $CHCl_3$). ^{1}H NMR ($CDCl_3$): *δ* 7.71 (d, 1H, *J* 8.9 Hz, H-4), 7.68 (d, 1H, *J* 8.5 Hz, H-5), 7.61 (d, 1H, *J* 1.7 Hz, H-8), 7.32 (dd, 1H, *J* 8.5, 1.7, H-6), 7.26 (d, 1H, *J* 2.5, H-1) 7.12 (dd, 1H, *J* 8.9, 2.5 Hz, H-3), 5.33 (d, 1H, *J* 5.6, H-1′), 5.28 (m, 1 H, H-3′), 5.25 (m, 1H, H-2′), 5.05 (dt, 1H, *J* 7.3, 4.6 Hz, H-4′), 4.28 (dd, 1H, *J* 12.2, 4.6 Hz, H-5′), 3.61 (dd, 1H, *J* 12.2, 7.3 Hz, H-5′), 3.00 (t, 2H, *J* 7.2 Hz, –SCH_2–), 2.12, 2.11, 2.11 (s, 3H each, –OAc), 1.73 (tq, 2H, *J* 7.4, 7.2 Hz, –CH_2Me), 1.07 (t, 3 H, *J* 7.4 Hz, –CH_3). ^{13}C NMR ($CDCl_3$): *δ* 169.9, 169.9, 169.4, 154.8, 135.7, 134.6, 129.5, 128.1, 128.0, 125.8, 125.2, 118.2, 110.4, 98.4, 70.5, 70.0, 68.4, 61.8, 35.2, 22.4, 20.79, 20.76, 20.7, 13.5. HRMS calcd for $C_{24}H_{28}O_8SNa$ (M+Na): 499.1403, found: 499.1403.

5.1.41. 1-(4-(Propylthio)naphth-1-yl) β-D-xylopyranoside (11a). 1-(4-(Propylthio)naphth-1-yl) 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside (**10a**) (828 mg, 1.74 mmol) was suspended in MeOH (19 mL) and NaOMe/MeOH (1 mL, 1 M) was added. The mixture was stirred for 90 min after which it was neutralized with Amberlite IR-120 H^+. The mixture was filtered, concentrated, and filtered through SiO_2 (CH_2Cl_2–MeOH 5:1) to give **11a** as a clear oil (597 mg, 98%). $[\alpha]_D^{20}$ −71.1° (*c* 0.26, MeOH). ^{13}C NMR (CD_3OD): *δ* 153.0, 134.5, 130.9, 126.6, 126.0, 125.2, 124.9, 122.6, 108.8, 101.7, 76.3, 73.4, 69.6, 65.6,

37.0, 22.3, 12.2. HRMS calcd for $C_{18}H_{22}O_5SNa$ (M+Na): 373.1086, found: 373.1080.

5.1.42. 1-(5-(Propylthio)naphth-1-yl) β-D-xylopyranoside (11b). 1-(5-(Propylthio)naphth-1-yl) 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside (**10b**) (819 mg, 1.72 mmol) was deprotected and purified as for **11a**. Gave **11b** as a white amorphous solid (572 mg, 95%). $[\alpha]_D^{20}$ −69.9° (*c* 0.29, MeOH). ^{13}C NMR (CD_3OD): δ 154.8, 135.2, 134.8, 129.2, 128.0, 127.1, 126.1, 122.2, 120.1, 110.9, 103.3, 77.8, 74.8, 71.0, 67.0, 37.0, 23.5, 13.7. HRMS calcd for $C_{18}H_{22}O_5SNa$ (M+Na): 373.1086, found: 373.1080.

5.1.43. 1-(8-(Propylthio)naphth-1-yl) β-D-xylopyranoside (11c). 8-(Propylthio)naphthalen-1-ol (**3c**) (32.4 mg, 0.15 mmol) and 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside trichloroacetimidate (120.4 mg, 0.29 mmol) were suspended in CH_2Cl_2 (4.5 mL) and cooled to 0 °C. $BF_3{\cdot}OEt_2$ (4 μL, 0.03 mmol) was added and the mixture was stirred for 18 min at 0 °C. Excess Et_3N was added and the solution was concentrated and filtered through SiO_2 (Toluene–EtOAc 4:3). The crude product was deprotected and purified as for **11a**. Gave **11c** as a white amorphous solid (46.6 mg, 90%). $[\alpha]_D^{20}$ −124.1° (*c* 0.23, MeOH). ^{13}C NMR (CD_3OD–$CDCl_3$ 1:1): δ 155.9, 137.5, 134.9, 127.0, 126.7, 126.0, 124.7, 124.2, 123.7, 111.0, 104.6, 76.8, 74.5, 70.7, 66.9, 35.6, 22.2, 14.2. HRMS calcd for $C_{18}H_{22}O_5SNa$ (M+Na): 373.1086, found: 373.1078.

5.1.44. 1-(3-(Propylthio)naphth-2-yl) β-D-xylopyranoside (11d). 1-(3-(Propylthio)naphth-2-yl) 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside (**10d**) (823 mg, 1.73 mmol) was deprotected and purified as for **11a**. Gave **11d** as a white amorphous solid (573 mg, 95%). $[\alpha]_D^{20}$ −66.9° (*c* 0.21, MeOH). ^{13}C NMR (CD_3OD): δ 154.2, 133.9, 131.4, 129.5, 127.9, 127.7, 127.6, 126.8, 125.8, 111.1, 102.8, 77.6, 74.6, 71.0, 66.9, 34.7, 23.2, 13.9. HRMS calcd for $C_{18}H_{22}O_5SNa$ (M+Na): 373.1086, found: 373.1088.

5.1.45. 1-(6-(Propylthio)naphth-2-yl) β-D-xylopyranoside (11e). 1-(6-(Propylthio)naphth-2-yl) 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside (**10e**) (819 mg, 1.72 mmol) was deprotected and purified as for **11a**. Gave **11e** as a white amorphous solid (590 mg, 98%). $[\alpha]_D^{20}$ −34.4° (*c* 0.26, MeOH). ^{13}C NMR (CD_3OD): δ 156.6, 134.1, 133.7, 131.6, 129.6, 129.3, 128.7, 128.1, 120.6, 111.9, 102.9, 77.7, 74.8, 71.1, 67.0, 36.6, 23.6, 13.6. HRMS calcd for $C_{18}H_{22}O_5SNa$ (M+Na): 373.1086, found: 373.1096.

5.1.46. 1-(7-(Propylthio)naphth-2-yl) β-D-xylopyranoside (11f). 1-(7-(Propylthio)naphth-2-yl) 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside (**10f**) (820 mg, 1.72 mmol) was deprotected and purified as for **11a**. Gave **11f** as a white amorphous solid (592 mg, 98%). $[\alpha]_D^{20}$ −29.5° (*c* 0.33, MeOH). ^{13}C NMR (CD_3OD): δ 157.2, 136.7, 136.3, 130.2, 129.3, 129.0, 126.4, 126.2, 119.5, 111.0, 102.8, 77.7, 74.8, 71.1, 67.0, 35.9, 23.5, 13.7. HRMS calcd for $C_{18}H_{22}O_5SNa$ (M+Na): 373.1086, found: 373.1076.

5.1.47. 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 all UV-absorbing (254 nm) peaks had eluted. The mean retention times and standard deviations were calculated from three separate runs per compound.

5.1.48. Stability of naphthoxylosides. A solution of xyloside (20 mM in H_2O–DMSO 1:1) was diluted in fresh Ham's F-12 cell growth medium to about 0.2 mM. 4,4′-Dihydroxybiphenyl (0.1 mM) was used as an internal standard. The samples were incubated at 37 °C and samples were taken at t = 0, 1, 3, 24, 48, 72, and 96 h, and analyzed by HPLC.

5.2. Biology

The human bladder carcinoma cell-line T24 and human lung fibroblasts, HFL-1, were obtained from ATCC. Regular cell culture media, L-glutamine, penicillin–streptomycin, trypsin, and donor calf serum were obtained from Life Technologies. Dulbecco's modified Eagles 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. Epidermal growth factor was purchased from Genzyme and crystal violet from Merck. The prepacked Superose 6 HR 10/30 and Dextran T-500 were from Pharmacia-LKB, and DE-53 DEAE–cellulose was from Whatman. Water for HPLC-analysis was from a Millipore Milli-Q system.

5.2.1. Cell culture and radiolabeling. Cells were cultured as monolayers in Modified Earles 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).

5.2.2. Isolation of xyloside-primed radiolabeled GAG. The procedures have been described in detail previously.[3] [^{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, and 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, and 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 4 M guanidine–HCl, 50 mM NaOAc, pH 5.8, and 5 μg/mL ovalbumin. 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.

5.2.3. 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 deaminative cleavage using nitrous acid at pH 1.5 to degrade the HS chains.[41] The samples were then subjected to gel permeation chromatography on Superose 6 and the radioactivity was determined in a β-counter.

5.2.4. In vitro growth assay using crystal violet method. The procedure has been described elsewhere.[3] Cells were seeded into 96-well microculture plates at 3000 cells/well for T24 cells and 5000 cells/well for HFL-1 cells 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 25, 50, 100, 200, and 500 μM 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, and $NaHPO_4$ 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 ED_{50} (μM) scored after 96 h of exposure.

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Supplementary data

Priming and cell proliferation data and ^1H NMR spectra of compounds **6a–f**, **9a–f**, and **11a–f**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.05.008.

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