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Regioselective Reductive Openings of Acetals; Mechanistic Details and Synthesis of Fluorescently Labeled Compounds

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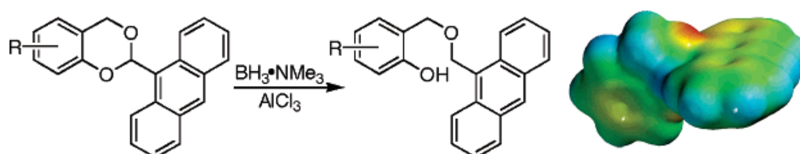

Regioselective reductive openings of mixed phenolic-benzylic acetals, using $BH_3 \cdot NMe_3-AlCl_3$, was investigated, and a mechanism where the outcome is directed by the electrostatic potential of the two oxygen atoms is presented. The regioselective acetal opening was used in the synthesis of a fluorescently labeled analogue to antiproliferative xylosides. The fluorescently labeled xyloside was tested for uptake, antiproliferative activity, and glycosaminoglycan priming in different cell lines. The xyloside was taken up by all cell lines but did not initiate glycosaminoglycan biosynthesis.

Introduction

Proteoglycans, composed of glycosaminoglycan (GAG) chains covalently attached to a core protein, are widely expressed in invertebrate and vertebrate tissues. Many biological functions of proteoglycans are due to interactions between GAG chains and a variety of pathogens and molecules, such as prion protein, viruses, growth factors, cytokines, and factors involved in blood coagulation.[1] The first step in GAG biosynthesis is xylosylation of a serine residue. A specific linker tetrasaccharide, GlcA(β1−3)Gal(β1−3)Gal(β1−4)Xylβ, is then assembled and serves as an acceptor for elongation of GAG chains (Figure 1). Addition of GlcNAc or GalNAc to the nonreducing terminal GlcA residue determines whether heparan sulfate or chondroitin sulfate/dermatan sulfate is initiated. The general structure is then modified through N-deacetylation/N-sulfation, epimerization, and O-sulfation.

Biosynthesis of GAG chains can also take place independently of core protein synthesis by using xylosides as primers. Xylose is an unusual structural component in mammalian cells, i.e., as the linker between the protein and carbohydrate in proteoglycans.

Xylosides with hydrophobic aglycons can penetrate cell membranes and initiate GAG synthesis by serving as acceptors in the first galactosylation step. The composition of the GAG chains assembled on the xylosides 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.

The xyloside-primed GAG chains can be retained inside the cells but are usually mainly secreted into the medium. Xylosides have been used for several purposes, including inhibition of tumor growth[2] and inhibition of scrapie prion protein infectivity.[3] We have previously reported that the GAG-priming 2-(6-hydroxynaphthyl)-β-D-xylopyranoside **1** (Figure 2) selectively inhibits growth of transformed or tumor-derived cells in vitro as well as in vivo. Treatment with this xyloside reduced the average tumor load by 70−97% in a SCID mice model.[4]

To further investigate these effects we decided to synthesize and evaluate the fluorescently labeled analogue **2** (Figure 2).

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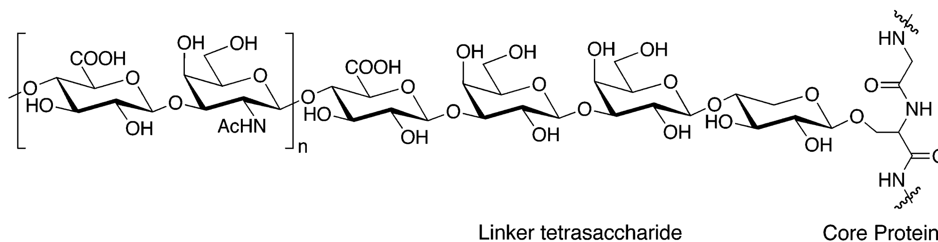


FIGURE 1. Glycosaminoglycan chains consist of a linker tetrasaccharide unit (GlcA(β1−3)Gal(β1−3)Gal(β1−4)Xylβ) coupled to serine residues of the protein chain. The general structure is then modified through N-deacetylation/N-sulfation, epimerization, and O-sulfation.

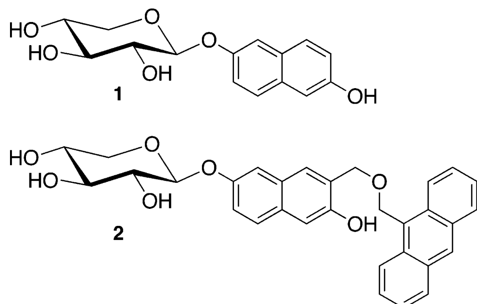
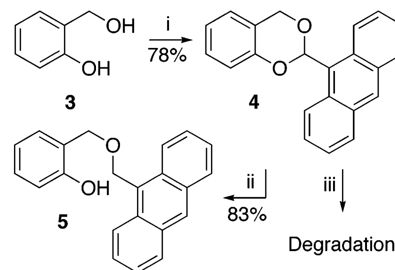


FIGURE 2. Compound **2** was synthesized as a fluorescent analogue of the antiproliferative compound **1**.

SCHEME 1. Synthesis of Compound 5[a]



[a] Reagents and conditions: (i) anthraldehyde dimethyl acetal, *p*TSA, MeCN, 2 h; (ii) $BH_3 \cdot NMe_3$, $AlCl_3$, THF, 0 °C, 3 h; (iii) $BH_3 \cdot NMe_3$, $AlCl_3$, toluene, rt, 5 min.

Regioselective Acetal Openings

We have earlier introduced 9-anthraldehyde acetals as fluorescent protecting groups for carbohydrates.[5] The acetals of both gluco- or galactopyranosides could be regioselectively opened using $NaBH_3CN$/THF/HCl[6] to give 6-*O*-(9-anthracenyl)-methyl ethers. We realized that compound **2** could be synthesized by a reductive regioselective opening of a mixed phenolic-benzylic acetal.

Despite the large number of methods published for reductive regioselective openings of acetals (e.g., $BH_3 \cdot THF-Cu(OTf)_2$,[7] $NaCNBH_3-HCl$,[6,8] $BH_3 \cdot NMe_2H-BF_3 \cdot OEt_2$,[9] $Me_2BBr-BH_3$,[10] $Bu_3SnH-MgBr_2 \cdot OEt_2$,[11] $Me_3SiH-BF_3 \cdot OEt_2$,[12] $Et_3SiH-TFA$,[13] $Et_3SiH-TfOH$,[14] $Et_3SiH-PhBCl_2$,[14] BH_3-Bu_2OTf[15]), there are, to our knowledge, no reductive openings of mixed phenolic-benzylic acetals known, and only one observation of a regioselective opening (i.e., nucleophilic attack).[16] There are also still uncertainties about the mechanism of regioselective openings of normal aliphatic acetals.

To investigate reductive openings of mixed phenolic-benzylic acetals, a model system was designed using 2-anthracen-9-yl-4*H*-benzo[1,3]dioxine (**4**), which was synthesized from salicylic alcohol by treatment with anthraldehyde dimethyl acetal. Compound **4** was subjected to $NaCNBH_3-HCl$ (standard conditions for regioselective opening of benzylidene acetals of carbohydrates), which only resulted in degradation.

The results indicate that these mixed acetals are more sensitive to hydrolysis, and instead we tried $BH_3 \cdot NMe_3$.[17] Borane complexes in combination with Lewis acids (e.g., $AlCl_3$) have been used for reductive openings of acetal-protected carbohydrates and the regioselectivity is mainly directed by the solvent; reaction in THF usually gives the benzylic ether in position 6 of carbohydrates whereas toluene gives 4-benzylic ethers but also degradation.[17] The mechanistic background to this selectivity is unclear.

Treatment of compound **4** with $BH_3 \cdot NMe_3-AlCl_3$ in THF for 3 h at 0 °C gave 2-(anthracen-9-yl-methoxymethyl)-phenol **5** in an excellent 83% yield, whereas reaction in toluene for 5 min resulted in decomposition (Scheme 1). Compound **4** was also treated with $BH_3 \cdot NMe_3$ in THF without $AlCl_3$, which gave no reaction, nor did any reaction occur with $AlCl_3$ in THF. Interestingly, reverse addition order, i.e., first addition of $AlCl_3$ and after 15 min addition of $BH_3 \cdot NMe_3$, gave complete degradation of compound **4**. The more reactive $BH_3 \cdot THF$ complex[18] gave a low yield of 10% as well as degradation of the starting material. Treatment with $AlCl_3$ in toluene resulted in decomposition.

One advantage of studying the regioselective reductive acetal opening using an aromatic system is that the electronic effects of the two oxygen atoms can be altered by substituents of the aromatic moiety.

To provide insight into the mechanism, three more substances were synthesized carrying (i) an electron-donating group (OMe),

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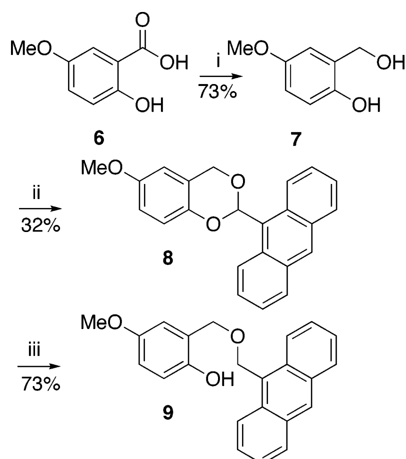
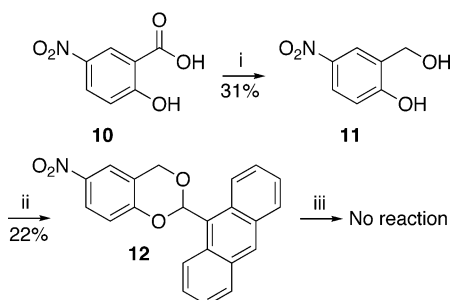
SCHEME 2. Synthesis of Compound 9[a]



[a] Reagents and conditions: (i) BH_3·THF, THF, 0 to 60 °C, 3.5 h; (ii) anthraldehyde dimethyl acetal, *p*TSA, THF, 3.5 h; (iii) BH_3·NMe_3, $AlCl_3$, THF, 0 °C, 3 h.

SCHEME 3. Synthesis of Compound 12[a]



[a] Reagents and conditions: (i) BH_3·THF, THF, 0 to 60 °C, 3.5 h; (ii) anthraldehyde dimethyl acetal, *p*TSA, MeCN, 18 h; (iii) BH_3·NMe_3, $AlCl_3$, THF, 0 °C, 3 h.

(ii) an electron-withdrawing group (NO_2), and (iii) sterically demanding methyl groups in the benzylic position.

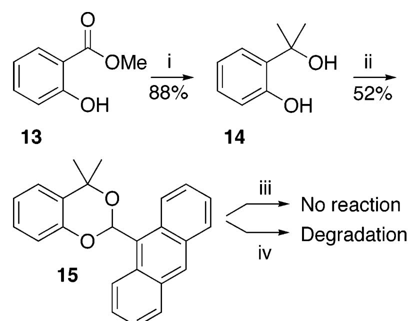
2-Anthracen-9-yl-6-methoxy-4*H*-benzo[1,3]dioxine (**8**) was synthesized from 5-methoxy salicylic acid (**6**), which was first reduced using BH_3·THF to give 2-hydroxymethyl-4-methoxyphenol (**7**) in 73% yield. Compound **7** was then transformed into the anthraldehyde acetal (**8**) in a modest 32% yield and subsequently treated with BH_3·NMe_3−$AlCl_3$ in THF for 3 h at 0 °C to give 2-(anthracen-9-yl-methoxymethyl)-4-methoxyphenol (**9**) in 73% yield (Scheme 2).

2-Anthracen-9-yl-6-nitro-4*H*-benzo[1,3]dioxine (**12**) was synthesized from 5-nitro salicylic acid (**10**) in a similar manner. However, treatment with BH_3·NMe_3−$AlCl_3$ in THF for 3 h at 0 °C resulted in no product and 69% recovery of compound **12** (Scheme 3).

The sterically hindered 2-anthracen-9-yl-4,4-dimethyl-4*H*-benzo[1,3]dioxine (**15**) was synthesized from methyl salicylate (**13**) by treatment with MeMgCl followed by acetal formation. Treatment with BH_3·NMe_3−$AlCl_3$ in THF for 3 h at 0 °C resulted in 99% recovery of **15**, whereas reaction in toluene resulted in decomposition in less than 5 min (Scheme 4).

These results indicate that the regioselectivity of the acetal openings is directed by both electronic effects and steric effects. To further investigate the mechanism, the electrostatic potentials of the four substances were calculated using density functional theory at the B3LYP/6-31G* level and default settings in Spartan '02 for Macintosh.[19] The results show that the benzylic oxygen is the more basic in all four compounds, indicating that this oxygen should react first with a Lewis acid (Table 1 and Figure 3).

SCHEME 4. Synthesis of Compund 15[a]



[a] Reagents and conditions: (i) MeMgCl, Et_2O, 4 h; (ii) anthraldehyde dimethyl acetal, *p*TSA, MeCN, 18 h; (iii) BH_3·NMe_3, $AlCl_3$, THF, 0 °C, 3 h; (iv) BH_3·NMe_3, $AlCl_3$, toluene, rt, 5 min.

TABLE 1. Electrostatic Potential for Acetal Oxygen Atoms (kJ/mol)[a]

compound	phenolic oxygen	benzylic oxygen
4	−141	−150
8	−148	−154
12	−103	−117
15	−146	−151

[a] The electrostatic potentials were calculated using DFT at (B3LYP/6-31G*) level.

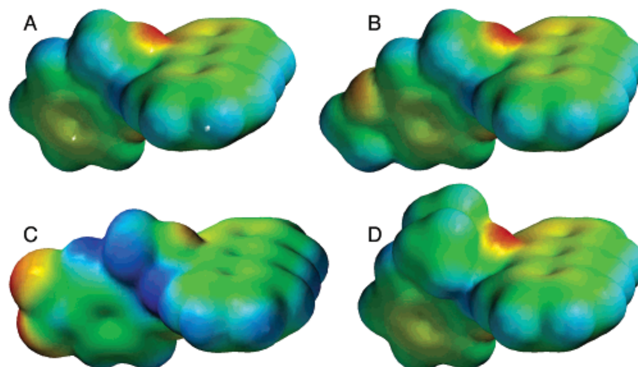


FIGURE 3. Electrostatic potential is displayed as a gradient from −160 kJ/mol in red to 130 kJ/mol in blue: (A) compound **4**, (B) compound **8**, (C) compound **12**, and (D) compound **15**.

Altogether these results indicate that the Lewis acid (i.e., BH_3 or $AlCl_3$ dependent on the addition order) first binds to the more basic oxygen (i.e., the benzylic oxygen). The reductions then proceed, promoted by reaction with $AlCl_3$ (Figure 4).

Probably reduction from the phenolic position (i.e., yielding benzylic alcohols), for example, by reverse order addition, gives decomposition by an unknown mechanism. The preorganization is highly sensitive to the electrostatic potential of the oxygen since the electron-deficient compound **12** does not react under these conditions. The more reactive BH_3·THF complex gives more decomposition, probably as a result of less discrimination between the two oxygen atoms. The lower yield from the

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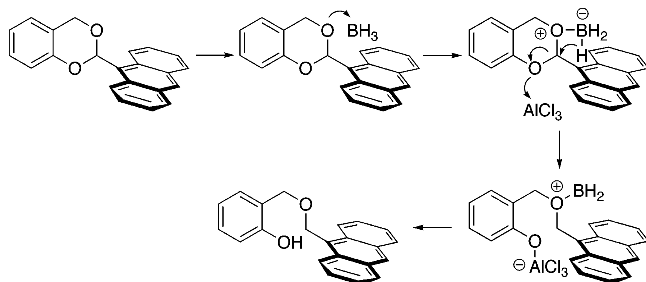
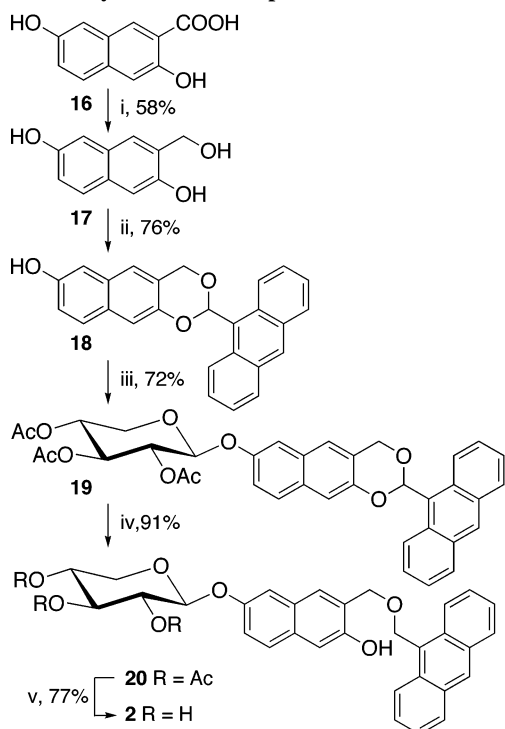


FIGURE 4. Mechanism proposal. The borane first binds to the more basic benzylic oxygen, and the reduction then proceeds, promoted by reaction with $AlCl_3$.

SCHEME 5. Synthesis of Compound 2[a]



[a] Reagents and conditions: (i) BH_3·THF, THF, 0 to 60 °C, 3.5 h; (ii) anthraldehyde dimethyl acetal, *p*TSA, MeCN, 1 h; (iii) 2,3,4-tri-*O*-acetyl-α/*β*-D-xylopyranosyl trichloroacetimidate, TMSOTf, MS 300 AW, CH_2Cl_2, 3 h; (iv) BH_3·NMe_3, $AlCl_3$, THF, 0 °C, 45 min; (v) guanidine-guanidinium nitrate, 30 min.

reduction of the electron-rich compound **8** is probably also a result of less discrimination between the two oxygen atoms. There is also a steric effect as indicated by the unreactive compound **15** (the electrostatic potential of the benzylic oxygen in compound **15** is equal to the highly reactive compound **4**).

No conclusions can be drawn from the reactions in toluene since only degradation was observed.

Synthesis of Fluorescently Labeled Naphthoxylosides

With a well working protocol for regioselective acetal openings we synthesized compound **2** from commercially available 3,7-dihydroxy-2-naphthoic acid (Scheme 5).

3,7-Dihydroxy-2-naphthoic acid (**16**) was reduced by BH_3·THF to give 2,6-dihydroxy-3-(hydroxymethyl)-naphthalene (**17**) in 58% yield.[20] The triol was then treated with anthraldehyde dimethyl acetal in MeCN under acidic conditions (*p*TSA) to give 2-antracen-9-yl-4*H*-naphtho[2,3-*d*][1,3]dioxin-7-ol (**18**) in 76% yield.[5] The acetal was xylosylated in 72% yield using 2,3,4-tri-*O*-acetyl-α/*β*-D-xylopyranosyl trichloroacetimidate to give (2-antracen-9-yl-4*H*-naphtho[2,3-*d*][1,3]dioxin-7-yl) 2,3,4-tri-*O*-acetyl-*β*-D-xylopyranoside (**19**).[21] The xylosylation was carried out in CH_2Cl_2 with a catalytic amount of TMSOTf and molecular sieves in order to minimize degradation of the acid sensitive anthracenylidene acetal.[22] Regioselective opening of the anthraldehyde acetal **19** was then performed using BH_3·NMe_3 and $AlCl_3$ in THF for 45 min to give 6-(2-hydroxy-3-(antracen-9-yl-methoxymethyl))-naphthyl) 2,3,4-tri-*O*-acetyl-*β*-D-xylopyranoside (**20**) in an excellent 91% yield.

Deprotection of the acetyl groups by standard Zemplén conditions (0.05 M NaOMe/MeOH) gave a low yield. Instead guanidine/guanidinium nitrate, a less basic method,[23] was used, giving 6-(2-hydroxy-3-(antracen-9-yl-methoxymethyl))-naphthyl)-*β*-D-xylopyranoside (**2**) in 77% yield.

Gradient HPLC retention times can be used to substitute log *P* values in biological evaluations.[24] The gradient HPLC retention times for compounds **1** and **2** 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. The acidity constants (pK_a) of phenolic compounds are usually difficult to predict by computational methods.[25] The pK_a's of compounds **1** and **2** were instead measured using spectroscopic methods at pH 9, 10, and 11. The results are summarized in Table 2. The absorbance spectrum of compound **2** (in MeCN) and fluorescence spectrum (in MeCN, $\lambda_{Ex} = 350$ nm) are presented in Figure 5. The highly unpolar (9-anthracenyl)methyl moiety makes compound **2** highly unpolar as reflected by the retention time. Compound **2** is also much less acidic as a result of the electron-donating properties of the substituent.

TABLE 2. Gradient Retention Times and pK_a Values for Compounds 1 and 2

compound	retention time (min)	pK_a
1	12.49 ± 0.01	9.63 ± 0.02
2	36.86 ± 0.04	10.52 ± 0.09

Biological Results

As compound **2** is highly lipophilic and uncharged, it may penetrate cell membranes, initiate GAG synthesis, and thereby affect various cellular processes. Compound **2** was accordingly tested for uptake, antiproliferative activity, and GAG priming in different cell lines. To investigate the antiproliferative activity of compound **2**, 3T3 A31 cells, 3T3 SV40 cells, and T24 cells were treated with the xyloside for 96 h, and the effect on cell growth was studied using the crystal violet method. Treatment

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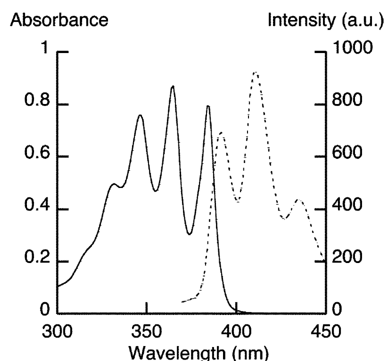

FIGURE 5. Absorbance spectrum of compound **2** (1.15 mM in MeCN, solid line) and fluorescence spectrum of compound **2** (1.15 mM in MeCN, $\lambda_{Ex} = 350$ nm, dotted line).

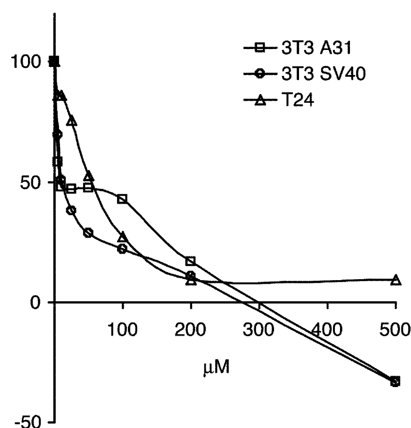

FIGURE 6. Effect of compound **2** on growth of 3T3 A31 (squares), 3T3 SV40 (circles), and T24 (triangles) cells was studied as described in Experimental Section. Cell numbers were determined after 96 h of culturing and expressed as the percentage of growth in the absence of drugs.

with compound **2** inhibited growth of normal 3T3 A31 cells and transformed 3T3 SV40 and T24 cells in a dose-dependent manner (Figure 6).

At a concentration between 100 and 200 μM the transformed T24 and 3T3 SV40 cells were more growth inhibited than normal 3T3 A31 cells. To examine to what extent the growth inhibition was correlated with uptake of the xyloside, the cells were treated with 100 μM xyloside for 16 h and then fixed and studied using fluorescence microscopy. The xyloside was taken up by all used cell lines. In 3T3 A31 cells and 3T3 SV40 cells the xyloside was located largely inside the cytoplasm, whereas in T24 cells the xyloside was accumulated in para- and perinuclear compartments (Figure 7).

To study the GAG priming ability of compound **2**, 3T3 A31 cells were left untreated or were treated with 100 μM xyloside and [^{35}S]sulfate overnight and free GAG chains in the medium and the cell extracts were isolated. 3T3 A31 cells secreted three size-dependent pools (I, II, and III) of radiolabeled material into the culture medium (Figure 8).

Large-sized (I) and intermediate-sized (II) material consisted of alkali-sensitive proteoglycans, whereas the regular-sized (III) chains were free alkali-resistant oligosaccharides. Xyloside treatment suppressed endogenous proteoglycan production to some extent. A very small amount of xyloside-primed GAG chains were recovered from the medium of xyloside-treated cells (pool III). In the cell extracts suppression of endogenous proteoglycan was noted. No cell-associated GAG chains primed by compound **2** were detected, indicating that the xyloside did not initiate GAG priming in these cells.

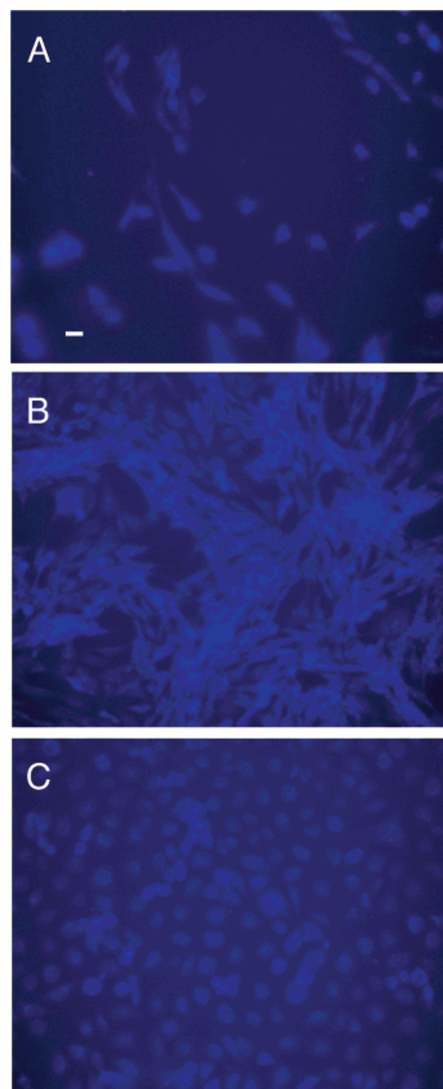

FIGURE 7. Uptake of compound **2** by different cell lines. The panels show fluorescence microscopy of (A) 3T3 A31, (B) 3T3 SV40, and (C) T24 cells treated with 100 μM of compound **2** for 16 h. Bar: 20 μm.

Conclusions

Mixed phenolic-benzylic acetals can be regioselectively opened using $BH_3 \cdot NMe_3-AlCl_3$ in THF at 0 °C to yield a benzylic ether and the free phenol. We are currently investigating the possibilities for inverse openings.

We propose a mechanism where the borane first associates to the more basic oxygen (i.e., the benzylic oxygen). The reductions then proceed, promoted by reaction with $AlCl_3$.

We have used the regioselective acetal opening in the synthesis of a fluorescent analogue to antiproliferative xylosides. The fluorescently labeled compound was taken up by all cell lines but did not initiate glucosaminoglycan biosynthesis.

Experimental Section

6-(2-Hydroxy-3-(anthracen-9-yl-methoxymethyl)-naphthyl)-β-D-xylopyranoside (2). Guanidinium nitrate (127 mg, 1.04 mmol) was dissolved in MeOH/CH_2Cl_2 (10 mL, 9:1) and methanolic

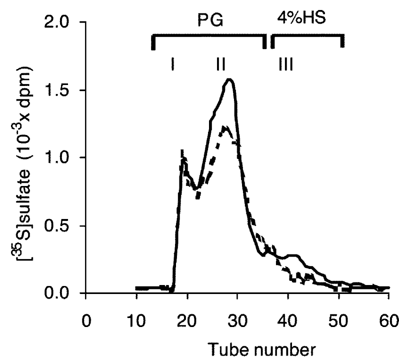

FIGURE 8. Secreted proteoglycan and GAG in 3T3 A31 cells treated with compound **2**. 3T3 A31 cells grown to confluence were labeled with [^{35}S]sulfate for 24 h in the absence (solid line) or presence (dashed line) of 100 μM of compound **2**. Polyanionic macromolecules from the culture medium were isolated by recovery on DEAE-cellulose followed by hydrophobic interaction chromatography on octyl-Sepharose and gel permeation chromatography on Superose 6. Proteoglycans and heparan sulfate were determined as described in Experimental Section.

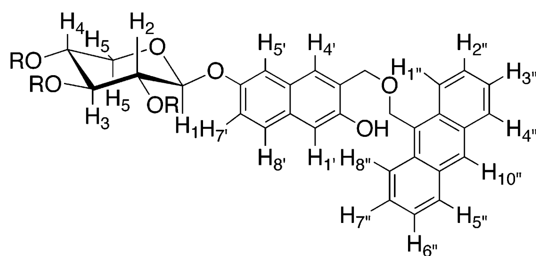

FIGURE 9. Numbering of compounds **2** and **20**.

MeONa (0.20 mL, 1 M) was added to give a clear stock solution. Compound **20** (23 mg, 0.037 mmol) was dissolved in the stock solution (2 mL). The mixture was stirred at room temperature for 30 min, acidified with HOAc (0.05 mL), diluted with CH_2Cl_2 (17 mL), filtered on SiO_2 and concentrated. The residue was chromatographed (SiO_2, CH_2Cl_2/MeOH 10:1) to give **2** (14.4 mg, 77%) as a light yellow solid. $[\alpha]^{21}_D$ +7 (*c* 0.01, DMSO-d_6). ^{1}H NMR (DMSO-d_6): δ 9.75 (s, 1 H, OH), 8.66 (s, 1 H, H-10″), 8.46 (d, 2 H, *J* 8.7 Hz, H-1″, H-8″), 8.12 (dd, 2 H, *J* 7.7, 1.4 Hz, H-4″, H-5″), 7.69 (s, 1 H, H-1′), 7.60 (d, 1 H, *J* 9.0 Hz, H-8′), 7.52−7.59 (m, 4 H, H-2″, H-3″, H-6″, H-7″), 7.26 (d, 1 H, *J* 2.3 Hz, H-5′), 7.11 (s, 1 H, H-4′), 7.09 (dd, 1 H, *J* 9.0, 2.4 Hz, H-7′), 5.61 (s, 2 H, CH_2-ant), 5.30 (d, 1 H, *J* 4.8 Hz, OH), 5.08 (d, 1 H, *J* 4.3 Hz, OH), 5.03 (d, 1 H, *J* 4.8 Hz, OH), 4.89 (d, 1 H, *J* 7.4 Hz, H-1), 4.87 (s, 2 H, CH_2-nap), 3.73 (dd, 1 H, *J* 11.0, 5.0 Hz, H-5), 3.20−3.30 (m, 4 H, H-2, H-3, H-4, H-5). ^{13}C NMR (DMSO-d_6): δ 152.9, 152.0, 131.0, 130.5, 129.7, 129.2, 128.8, 128.3, 128.1, 128.0, 126.9, 126.3, 126.2, 125.2, 124.6, 118.8, 110.6, 108.4, 101.2, 76.5, 73.1, 69.4, 67.4, 65.7, 64.2. HRMS calcd for $C_{31}H_{28}O_7Na$ (M + Na): 535.1733, found 535.1721.

2-Anthracen-9-yl-4*H*-benzo[1,3]dioxine (4). To a solution of **3** (110 mg, 0.89 mmol) and anthraldehyde dimethyl acetal (247 mg, 0.98 mmol) in MeCN (10 mL) was added *p*TSA (5 mg). The mixture was stirred at room temperature for 2 h, then diluted with CH_2Cl_2, washed with saturated aqueous $NaHCO_3$ twice, and then dried (Na_2SO_4). The residue was chromatographed (SiO_2, toluene) to give **4** (217 mg, 78%) as a yellow solid. ^{1}H NMR ($CDCl_3$): δ 8.65 (d, 2 H, *J* 8.9 Hz, H-1′, H-8′), 8.56 (2, 1 H, H-10′), 8.04 (dd, 2 H, *J* 8.1, 1.7 Hz, H-4′, H-5′), 7.46−7.53 (m, 4 H, H-2′, H-3′, H-6′, H-7′), 7.41 (s, 1 H, H-2), 7.27−7.25 (m, 1 H, H-6), 7.18 (d, 1 H, *J* 6.9 Hz, H-5), 7.09 (dt, 1 H, *J* 7.5, 1.1 Hz, H-7), 7.00 (dd, 1 H, *J* 8.2, 0.7 Hz, H-8), 5.45, 5.23 (ABq, 1 H each, *J* 14.8 Hz, H-4). ^{13}C NMR ($CDCl_3$): δ 154.0, 131.8, 130.5, 130.2, 129.3, 128.4, 126.6, 125.4, 125.2, 124.9, 121.8, 121.4, 117.7, 98.0, 68.2. HRMS calcd for $C_{22}H_{17}O_2$ (M + H): 313.1229, found 313.1221.

2-(Anthracen-9-yl-methoxymethyl)-phenol (5). To a solution of **4** (20 mg, 0.07 mmol) and BH_3·NMe_3 (34 mg, 0.46 mmol) in THF (2 mL) was added $AlCl_3$ (69 mg, 0.51 mmol in 1 mL THF) during 5 min at 0 °C. The mixture was stirred at 0 °C for 3 h, then diluted with CH_2Cl_2, and washed with saturated aqueous $NaHCO_3$. The organic phase was dried (Na_2SO_4) and concentrated. The residue was chromatographed (SiO_2, heptane/EtOAc 10:1) to give **5** (17 mg, 83%) as a white solid. ^{1}H NMR ($CDCl_3$): δ 8.51 (s, 1 H, H-10′), 8.29 (dd, 2 H, *J* 8.8, 0.7 Hz, H-1′, H-8′), 8.04 (ddd, 2 H, *J* 8.4, 0.7, 0.6 Hz, H-4′, H-5′), 7.48−7.58 (m, 4 H, H-2′, H-3′, H-6′, H-7′), 7.40 (s, 1 H, OH), 7.27−7.22 (m, 1 H, H-4), 7.05 (dd, 1 H, *J* 7.5, 1.4 Hz, H-3), 6.85−6.92 (m, 2 H, H-6, H-5), 5.60 (s, 2 H, CH_2-ant), 4.86 (s, 2 H, CH_2-ben). ^{13}C NMR ($CDCl_3$): δ 156.6, ?31.6, 131.3, 129.9, 129.4, 129.3, 128.8, 126.9, 125.3, 124.0, 122.5, 120.2, 116.9, 71.8, 64.5. HRMS calcd for $C_{22}H_{18}O_2Na$ (M + Na): 337.1204, found 337.1193.

2-Hydroxymethyl-4-methoxy-phenol (7). Compound **6** (296 mg, 1.76 mmol) was dissolved in THF (1.3 mL) and cooled to 0 °C under argon. BH_3·THF (1 M, 4.4 mL) was added dropwise during 30 min. The mixture was stirred at 0 °C for 45 min and then left to attain room temperature. After 1 h BH_3·THF (1 M, 0.9 mL) was added and the mixture was stirred at room temperature for 1 h and then at 60 °C for 1.5 h. The mixture was then added to a mixture of ice and HCl (0.5 M, 100 mL) and extracted with Et_2O. The organic phase was washed with saturated aqueous $NaHCO_3$ twice, then dried (Na_2SO_4), and concentrated to give **7** (197 mg, 73%) as a light yellow solid. ^{1}H NMR data was in agreement with published data.[26]

2-Anthracen-9-yl-6-methoxy-4*H*-benzo[1,3]dioxine (8). To a solution of **7** (98 mg, 0.64 mmol) and anthraldehyde dimethyl acetal (180 mg, 0.71 mmol) in THF (7 mL) was added *p*TSA (5 mg). The mixture was stirred at room temperature for 3.5 h, then diluted with CH_2Cl_2, washed with saturated aqueous $NaHCO_3$ twice, and then dried (Na_2SO_4). The residue was chromatographed (SiO_2, heptane/EtOAc 10:1 → 5:1) to give **8** (70 mg, 32%) as a light yellow solid. ^{1}H NMR ($CDCl_3$): δ 8.64 (d, 2 H, *J* 8.9 Hz, H-1′, H-8′), 8.55 (s, 1 H, H-10′), 8.03 (dd, 2 H, *J* 8.1, 1.7 Hz, H-4′, H-5′), 7.45−7.53 (m, 4 H, H-2′, H-3′, H-6′, H-7′), 7.34 (s, 1 H, H-2), 6.94 (d, 1 H, *J* 8.9 Hz, H-8), 6.84 (dd, 1 H, *J* 8.9, 3.0 Hz, H-7), 6.71 (d, 1 H, *J* 2.9 Hz, H-5), 5.41, 5.19 (ABq, 1 H each, *J* 14.9 Hz, H-4), 3.84 (s, 3 H, CH_3). ^{13}C NMR ($CDCl_3$): δ 154.6, 148.0, 131.8, 130.4, 130.2, 129.4, 129.3, 126.6, 125.2, 125.0, 121.9, 118.4, 114.5, 109.9, 98.0, 68.1, 56.1. HRMS calcd for $C_{23}H_{19}O_3$ (M + H): 343.1334, found 343.1333.

2-(Anthracen-9-yl-methoxymethyl)-4-methoxy-phenol (9). According to the same procedure as for **5**: **8** (22 mg, 0.07 mmol), BH_3·NMe_3 (30 mg, 0.41 mmol), THF (2 mL), $AlCl_3$ (62 mg, 0.47 mmol in 1 mL THF). Chromatographed (SiO_2, heptane/EtOAc 10:1 → 5:1) to give **9** (16 mg, 73%) as a white solid. ^{1}H NMR ($CDCl_3$): δ 8.51 (s, 1 H, H-10′), 8.29 (d, 2 H, *J* 8.3 Hz, H-1′, H-8′), 8.04 (d, 2 H, *J* 8.4 Hz, H-4′, H-5′), 7.54−7.58 (m, 2 H, H-2′, H-7′), 7.47−7.51 (m, 2 H, H-3′, H-6′), 6.95 (s, 1 H, OH), 6.84 (d, 1 H, *J* 8.8 Hz, H-6), 6.79 (dd, 1 H, *J* 8.8, 2.8 Hz, H-5), 6.60 (d, 1 H, *J* 2.8 Hz, H-3), 5.60 (s, 2 H, CH_2-ant), 4.80 (s, 2 H, CH_2-ben), 3.74 (s, 3 H, CH_3). ^{13}C NMR ($CDCl_3$): δ 153.2, 150.4, 131.6, 131.3, 129.4, 129.3, 127.4, 126.9, 125.4, 124.0, 123.2, 117.5, 115.1, 114.2, 71.6, 64.4, 56.0. HRMS calcd for $C_{23}H_{20}O_3Na$ (M + Na): 367.1310, found 367.1311.

2-Hydroxymethyl-4-nitro-phenol (11). According to the same procedure as for **7**: **10** (299 mg, 1.63 mmol), THF (1.3 mL), BH_3·THF (1 M, 4.4 mL), BH_3·THF (1 M, 0.85 mL), ice and HCl (0.5 M, 85 mL) to give **11** (87 mg, 31%) as a yellow solid. ^{1}H NMR data was in agreement with published data.[27]

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2-Anthracen-9-yl-6-nitro-4*H*-benzo[1,3]dioxine (12). To a solution of **11** (51 mg, 0.30 mmol) and anthraldehyde dimethyl acetal (82 mg, 0.33 mmol) in MeCN (4 mL) was added *p*TSA (5 mg). The mixture was stirred at room temperature for 18 h, then diluted with CH_2Cl_2, washed with saturated aqueous $NaHCO_3$ twice, and then dried (Na_2SO_4). The residue was chromatographed (SiO_2, heptane/EtOAc 10:1 → 5:1) to give **12** (23 mg, 22%) as a yellow solid. ^{1}H NMR ($CDCl_3$): δ 8.60 (s, 1 H, H-10′), 8.52 (d, 2 H, *J* 8.9 Hz, H-1′, H-8′), 8.15−8.19 (m, 2 H, H-7, H-5), 8.06 (dd, 2 H, *J* 8.0, 1.7 Hz, H-4′, H-5′), 7.48−7.56 (m, 4 H, H-2′, H-3′, H-6′, H-7′), 7.46 (s, 1 H, H-2), 7.05 (d, 1 H, *J* 8.7 Hz, H-8), 5.48, 5.29 (ABq, 1 H each, *J* 15.1 Hz, H-4). ^{13}C NMR ($CDCl_3$): δ 159.2, 131.7, 131.1, 130.2, 129.5, 128.2, 128.0, 127.0, 125.3, 125.0, 124.7, 124.4, 122.0, 121.5, 118.3, 99.0, 67.9. HRMS calcd for $C_{22}H_{15}NO_4Na$ (M + Na): 380.0899, found 380.0900.

2-(1-Hydroxy-1-methyl-ethyl)-phenol (14). To methylmagnesium chloride (12 mL, 3 M in THF) stirred at 0 °C under argon was added **13** (0.50 mL, 3.9 mmol) dissolved in Et_2O (3 mL) during 10 min. The mixture was stirred at 0 °C for 1.5 h and then left to attain room temperature. After 2.5 h ice (14 g) was added and the mixture was acidified with 10% aqueous HCl. The water phase was extracted with Et_2O and the combined organic phases were washed with saturated aqueous $NaHCO_3$, dried (Na_2SO_4), and concentrated. The residue was chromatographed (SiO_2, heptane/EtOAc 2:1) to give **14** (515 mg, 88%) as a clear oil. ^{1}H NMR was in agreement with published data.[28]

2-Anthracen-9-yl-4,4-dimethyl-4*H*-benzo[1,3]dioxine (15). According to the same procedure as for **12**: **14** (100 mg, 0.66 mmol), anthraldehyde dimethyl acetal (182 mg, 0.72 mmol), MeCN (7 mL), *p*TSA (5 mg). Chromatographed (SiO_2, toluene) to give **15** (116 mg, 52%) as a light yellow solid. ^{1}H NMR (DMSO-d_6): δ 8.73−8.75 (m, 3 H, H-10′, H-1′, H-8′), 8.12−8.16 (m, 2 H, H-4′, H-5′), 7.52−7.55 (m, 5 H, H-2′, H-3′, H-6′, H-7′, H-2), 7.45 (dd, 1 H, *J* 7.8, 1.5 Hz, H-5), 7.23 (ddd, 1 H, *J* 8.1, 7.4, 1.6 Hz, H-7), 7.09 (dt, 1 H, *J* 7.6, 1.2 Hz, H-6), 6.90 (dd, 1 H, *J* 8.1, 1.1 Hz, H-8), 1.86, 1.66 (s, 3 H each, CH_3). ^{13}C NMR (DMSO-d_6): δ 152.2, 131.0, 130.2, 129.7, 129.4, 128.9, 127.8, 126.8, 126.4, 126.3, 125.1, 124.9, 121.5, 116.9, 92.0, 76.6, 30.6, 28.7. HRMS calcd for $C_{24}H_{20}O_2Na$ (M + Na): 363.1361, found 363.1350.

2,6-Dihydroxy-3-(hydroxymethyl)-naphthalene (17). Compound **16** (395 mg, 1.93 mmol) was dissolved in THF (1.5 mL) and cooled to 0 °C under argon. BH_3·THF (1 M, 5 mL) was added dropwise during 10 min. The mixture was stirred at 0 °C for 35 min and then left to attain room temperature. After 1 h BH_3·THF (1 M, 1 mL) was added and the mixture was stirred at room temperature for 1 h and then at 60 °C for 1.5 h. The mixture was then added to a mixture of ice and HCl (0.5 M, 100 mL) and extracted with Et_2O. The organic phase was washed with saturated aqueous $NaHCO_3$ twice, then dried (Na_2SO_4), and concentrated to give **17** (213 mg, 58%) as a light yellow solid. ^{1}H NMR (CD_3OD): δ 7.65 (s, 1 H, H-4), 7.46 (d, 1 H, *J* 8.8 Hz, H-8), 7.02 (d, 1 H, *J* 2.1 Hz, H-5), 7.00 (s, 1 H, H-1), 6.95, (dd, 1 H, *J* 8.8, 2.5 Hz, H-7), 4.73 (s, 2 H, CH_2). ^{13}C NMR (CD_3OD): δ 156.6, 155.0, 134.3, 133.5, 132.8, 130.7, 128.5, 121.6, 112.6, 112.3, 64.0. HRMS calcd for $C_{11}H_{10}O_3Na$ (M + Na): 213.0528, found 213.0537.

2-Antracen-9-yl-4*H*-naphtho[2,3-*d*][1,3]dioxin-7-ol (18). To a solution of **17** (466 mg, 2.45 mmol) and anthraldehyde dimethyl acetal (740 mg, 2.95 mmol) in MeCN (20 mL) was added *p*TSA (5 mg). The mixture was stirred at room temperature for 1 h, then neutralized by addition of Et_3N, and concentrated with toluene 3 times. The residue was chromatographed (SiO_2, heptane/EtOAc 2:1) to give **18** (704 mg, 76%) as a light yellow solid. ^{1}H NMR (DMSO-d_6): δ 9.55 (s, 1 H, OH), 8.70−8.80 (m, 3 H, H-1′, H-8′, H-10′), 8.10−8.15 (m, 2 H, H-4′, H-5′), 7.66 (s, 1 H, H-2), 7.62 (d, 1 H, *J* 8.5 Hz, H-9), 7.58 (s, 1 H, H-5), 7.50−7.55 (m, 4 H, H-2′, H-3′, H6′, H-7′), 7.24 (s, 1 H, H-10), 7.11 (d, 1 H, *J* 2.2 Hz, H-6), 7.04 (dd, 1 H, *J* 8.8, 2.3 Hz, H-8), 5.67, 5.34 (ABq, 1 H each, *J* 15.3 Hz, H-4). ^{13}C NMR (DMSO-d_6): δ 153.9, 149.3, 131.0, 130.0, 129.8, 129.4, 128.9, 128.0, 127.6, 127.0, 126.3, 125.1, 125.0, 123.0, 122.3, 119.1, 111.3, 108.3, 97.3, 67.6. HRMS calcd for $C_{26}H_{19}O_3$ (M + H): 379.1334, found 379.1320.

(2-Antracen-9-yl-4*H*-naphtho[2,3-*d*][1,3]dioxin-7-yl) 2,3,4-tri-*O*-acetyl-*β*-D-xylopyranoside (19). A solution of **18** (99 mg, 0.263 mmol), 2,3,4-tri-*O*-acetyl-α/*β*-D-xylopyranosyl trichloroacetimidate (244 mg, 0.581 mmol) and ground MS 300 AW (0.3 g) in CH_2Cl_2 (7 mL) was stirred at room temperature under argon for 1 h. TMSOTf (0.05 M in CH_2Cl_2, 0.250 mL) was added dropwise to the mixture. After 1 h TMSOTf (0.05 M in CH_2Cl_2, 0.050 mL) was added and stirred for 1 h. Et_3N (3 mL) was added (pH ∼8) and the mixture was concentrated. The residue was chromatographed (SiO_2, heptane/EtOAc 2:1) to give **19** (120 mg, 72%) as a light yellow solid. $[\alpha]^{21}_D$ −16 (*c* 0.89, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 8.65 (δ, 2 H, *J* 8.6 Hz, H-1″, H-8″), 8.57 (s, 1 H, H-10″), 8.05 (dd, 2 H, *J* 7.5, 1.9 Hz, H-4″, H-5″), 7.66 (d, 1 H, *J* 9.0 Hz, H-9′), 7.56 (s, 1 H, H-5′), 7.45−7.55 (m, 5 H, H-2′, H-2″, H-3″, H-6″, H-7″), 7.35 (d, 1 H, *J* 2.2 Hz, H-6′), 7.33 (s, 1 H, H-10′), 7.16 (dd, 1 H, *J* 8.9, 2.4 Hz, H-8′), 5.61, 5.42 (ABq, 1 H each, *J* 10.0 Hz, H-4′), 5.20−5.30 (m, 3 H, H-1, H-2, H-3, J_{H1-H2} 5.6 Hz), 5.06 (dt, 1 H, *J* 7.2, 4.6 Hz, H-4), 4.30 (dd, 1 H, *J* 12.1, 4.6 Hz, H-5), 3.62 (dd, 1 H, *J* 12.1, 7.5, H-5), 2.13, 2.12, 2.11 (s, 3 H each, OAc). ^{13}C NMR ($CDCl_3$): δ 170.2, 170.1, 169.7, 153.5, 151.3, 131.8, 130.6, 130.4, 130.2, 129.7, 129.3, 128.8, 126.7, 126.4, 125.2, 124.8, 123.6, 123.2, 119.7, 112.7, 111.41, 111.37, 99.0, 98.9, 98.5, 70.8, 70.4, 68.70, 68.67, 62.1, 21.03, 21.00. HRMS calcd for $C_{37}H_{32}O_{10}Na$ (M + Na): 659.1893, found 659.1900.

6-(2-Hydroxy-3-(anthracen-9-yl-methoxymethyl)-naphthyl) 2,3,4-tri-*O*-acetyl-*β*-D-xylopyranoside (20). To a solution of **19** (70 mg, 0.1 mmol) and BH_3·NMe_3 (50 mg, 0.69 mmol) in THF (3 mL) was added $AlCl_3$ (110 mg, 0.83 mmol in 2 mL THF) during 5 min at 0 °C. The mixture was stirred at 0 °C for 45 min, then diluted with CH_2Cl_2, and washed with saturated aqueous $NaHCO_3$. The organic phase was dried (Na_2SO_4) and concentrated. The residue was chromatographed (SiO_2, heptane/EtOAc 2:1 → 1:1) to give **20** (64 mg, 91%) as a light yellow solid. $[\alpha]^{21}_D$ −16 (*c* 0.79, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ (s, 1 H, H-10″), 8.29 (d 2 H, *J* 8.8 Hz, H-1″, H-8″), 8.05 (d, 2 H, *J* 8.1 Hz, H-4″, H-5″), 7.66 (d, 1 H, *J* 8.1 Hz, H-8′), 7.45−7.60 (m, 5 H, H-4′, H-2″, H-3″, H-6″, H-7″), 7.36 (s, 1 H, H-1′), 7.27 (d, 1 H, *J* 2.4 Hz, H-5′), 7.23 (s, 1 H, OH), 7.14 (dd, 1 H, *J* 8.9, 2.4 Hz, H-7′), 5.63 (s, 2 H, CH_2-ant), 5.20−5.30 (m, 3 H, H-1, H-2, H-3), 5.04 (dt, 1 H, *J* 7.5, 4.7 Hz, H-4), 4.98 (s, 2 H, CH_2-nap), 4.26 (dd, 1 H, *J* 12.1, 4.7 Hz, H-5), 3.57 (dd, 1 H, *J* 12.1, 7.6, H-5), 2.12, 2.11, 2.09 (s, 3 H each, OAc). ^{13}C NMR ($CDCl_3$): δ 170.2, 170.1, 169.6, 153.5, 153.1, 131.6, 131.3, 129.44, 129.36, 128.9, 128.3, 127.6, 127.2, 126.9, 125.9, 125.4, 123.9, 119.7, 111.9, 111.5, 99.1, 71.7, 70.9, 70.4, 68.8, 64.6, 62.1, 21.02, 21.00, 20.98. HRMS calcd for $C_{37}H_{34}O_{10}Na$ (M + Na): 661.2050, found 661.2022.

High-pressure liquid chromatography was run on a Supelco LC-18-DB column (15 cm × 4.6 mm, 5 μm). The mobile phase consisted of H_2O + 0.1% TFA with a gradient of MeCN from 1 min increasing by 1.2% per minute. The retention times are the mean values for three separate runs for each compound.

Acidity constants (pK_a) were determined spectrophotometrically. Compound **2** (1.4 mg) was dissolved in MeOH (1.5 mL); 0.040 mL of this solution was added to 1 mL each of HCl (aq, 0.1 M), NaOH (aq, 0.1 M), and pH 9, 10, and 11 buffers; and the absorption was measured at 365 nm. The pK_a was calculated from at least three measurements at each pH.

Absorbance and Fluorescence. Compound **2** (1.18 mg) was dissolved on DMSO (0.100 mL), and 0.010 mL of this solution was added to 2.00 mL MeCN.[29]

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Cell Culture, Radiolabeling and Extraction Procedures. Cells were cultured as monolayers in Dulbeccos Modified Earles Medium (DMEM) 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 preincubation medium was replaced by fresh medium containing 50 mCi/mL of [^{35}S]sulfate and the xyloside. Dilutions were made from 20 mM stock solutions in DMSO/water (1:1, v/v). 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).

Isolation of Xyloside-Primed Radiolabeled GAG. The procedures have been described in detail previously.[30] Free xyloside-primed GAG chains were separated from proteoglycans by hydrophobic interaction chromatography on octyl-Sepharose followed by gel permeation FPLC on Superose 6. Radioactivity was determined in a beta counter.

Degradation Procedures. GAG chains were released from proteoglycans by treatment with 0.5 M NaOH, 0.1 M $NaBH_4$ at room temperature overnight. Samples were neutralized with HOAc. Heparan sulfate chains were degraded by deaminative cleavage by using the pH 1.5-HNO_2 method.[31] The samples were lyphilized and redissolved for analysis by gel-permeation chromatography on Superose 6 to determine the proportions of total heparan sulfate.

In Vitro Growth Assay using Crystal Violet Method. The procedure has been described elsewhere.[30] The inhibitory effect of the compounds is expressed as the percentage of growth in the absence of drugs.

Fluorescence Microscopy. Cells were seeded at a concentration of 10,000 cells/well in chamber slides with covers, allowed to adhere overnight, and then treated with 100 μM of compound **2** for 16 h. After the treatment cells were washed with phosphate-buffered saline three times and fixed with acetone for 2−4 min. The fixed cells were then visualized in a microscope. Excitation was obtained at 250 nm, and the emitted light was filtered at 461 nm. Images were digitized for annotation, and printing.

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Supporting Information Available: Additional experimental details; NMR spectra of compounds **2**, **4**, **5**, **8**, **9**, **12**, **15**, and **17**−**20**; and computational data. This material is available free of charge via the Internet at http://pubs.acs.org.

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