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Synthesis and glycosaminoglycan priming activity of three disaccharides related to the linkage region tetrasaccharide of proteoglycans

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Abstract

To test if disaccharides might serve as primers of oligosaccharide synthesis in animal cells, we synthesized 2-naphthyl *O*-(β-D-galactopyranosyl)-(1 → 4)-β-D-xylopyranoside, 2-naphthyl *O*-(β-D-galactopyranosyl)-(1 → 3)-β-D-galactopyranoside, and 2-naphthyl *O*-(β-D-glucopyranosyluronic acid)-(1 → 3)-β-D-galactopyranoside. These three disaccharides are related to subunits of the linkage tetrasaccharide of heparan sulfate and chondroitin sulfate chains in animal cell proteoglycans. The disaccharides were synthesized with coupling efficiencies of 40–70% using thioglycosides or by activating the monosaccharides with trichloroacetimidate. The structures of these compounds were confirmed by ^{1}H NMR, ^{13}C NMR and elemental analysis. The ability of these disaccharides to prime glycosaminoglycan chains was examined in a Chinese hamster ovary cell mutant, pgsA 745, which lacks xylosyltransferase. The missing enzyme renders the cells dependent on exogenous primers for making glycosaminoglycan chains. 2-Naphthyl *O*-(β-D-galactopyranosyl)-(1 → 3)-β-D-galactopyranoside and 2-naphthyl *O*-(β-D-glucopyranosyluronic acid)-(1 → 3)-β-D-galactopyranoside did not stimulate glycosaminoglycan synthesis, but 2-naphthyl *O*-(β-D-galactopyranosyl)-(1 → 4)-β-D-xylopyranoside at high concentration primed chains. The peracetylated derivative (2-naphthyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3-di-*O*-acetyl-β-D-xylopyranoside) primed chains at lower concentration (100 μM), suggesting that cells took up the compound and removed the acetyl groups apparently in the compartment where glycosaminoglycan synthesis occurs.

Keywords: Proteoglycans; Glycosaminoglycan priming activity

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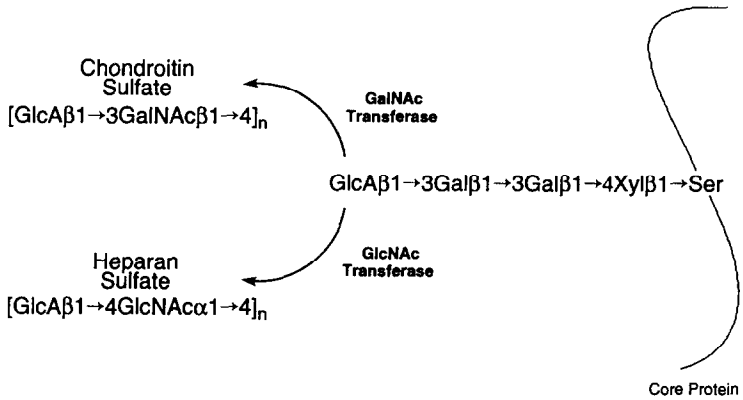

Fig. 1. Biosynthesis of heparan sulfate and chondroitin sulfate proteoglycans on the linkage region tetrasaccharide.

1. Introduction

Heparan sulfate and chondroitin sulfate proteoglycans consist of a core protein and one or more covalently attached glycosaminoglycan (GAG) chains. The assembly of the chains is initiated by the transfer of xylose to specific serine residues within proteoglycan core proteins [1]. Sequential sugar transfer reactions then give rise to the core protein-linkage tetrasaccharide, β-D-GlcA *p*-(1 → 3)-β-D-Gal *p*-(1 → 3)-β-D-Gal *p*-(1 → 4)-β-D-Xyl *p*-(1 → *O*)-serine (Fig. 1). The tetrasaccharide in turn acts as a primer for chain polymerization, which occurs by the alternating transfer of D-GlcNAc *p* or D-GalNAc *p* and D-GlcA *p* residues. The chains then undergo a series of modification reactions that include sulfation and uronic acid epimerization [2].

Although cells normally assemble GAG chains on xylosylated proteoglycan core proteins, they will also use synthetic β-D-xylopyranosides as primers [3–8]. β-D-Galactopyranosides also will prime GAG chains, but only at much higher concentrations compared to β-D-xylopyranosides [9]. Efficient primers generally contain a hydrophobic aglycon that allows the glycoside to pass across the plasma membrane and into the Golgi compartment where the various glycosyltransferases reside. The priming of oligosaccharides on β-D-xylopyranosides competes with the formation of chains on endogenous proteoglycan core proteins. Thus, β-D-xylopyranosides act as inhibitors of proteoglycan formation. Recent studies indicate that β-D-xylopyranosides can prime other types of glycans in addition to GAG chains [10–16] and thereby inhibit the formation of other glycoconjugates.

In contrast to monosaccharides, the potential of disaccharides as primers or agents to alter proteoglycan biosynthesis in animal cells has not been examined. In this report, we describe the synthesis of three disaccharides derived from the linkage tetrasaccharide, β-D-GlcA *p*-(1 → 3)-β-D-Gal *p*-(1 → *O*)-2-naphthyl, β-D-Gal *p*-(1 → 3)-β-D-Gal *p*-(1 → *O*)-2-naphthyl, and β-D-Gal *p*-(1 → 4)-β-D-Xyl *p*-(1 → *O*)-2-naphthyl. We also investigated the priming activity of these compounds in Chinese hamster ovary cells before and after acetylation.

2. Results and discussion

The synthesis of the three target disaccharides (**4**, **7**, and **11**) has been described previously as reducing sugars or containing a different aglycon [17–23]. In these earlier studies, acetobromo sugars were used as glycosyl donors in coupling reactions, with yields ranging from 20 to 70%. Recent studies have suggested that *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TFMS)-mediated activation of thioglycosides give higher yields in coupling steps [24]. NIS and TFMS activation of thioglycosides was therefore used to synthesize disaccharides **7** and **11**. The trichloroacetimidate [25] method was followed for the synthesis of disaccharide **4**.

Selective chloroacetylation of 2-naphthyl β-D-xylopyranoside [8] (**1**) by the dibutyltin oxide method [26], benzoylation in pyridine, and removal of the chloroacetyl group with thiourea [27] gave 2-naphthyl 2,3-di-*O*-benzoyl-β-D-xylopyranoside (**2**) in 60% overall yield. A similar reaction sequence was used to selectively block the 2-OH and 3-OH of benzyl β-D-xylopyranoside and methyl β-D-xylopyranoside [22,28]. A downfield shift of 2.4 ppm and 2 ppm in the H-2 and H-3 signals, respectively, in the ^{1}H-NMR spectrum of compound **2** compared to 2-naphthyl β-D-xylopyranoside is in accord with selective benzoylation having occurred at the 2-OH and 3-OH positions. One attempt to couple **2** with phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside in the presence of NIS and TFMS met with limited success (5–10% yield), possibly due to the iodination of naphthalene by I^+ generated during the reaction. Condensation of **2** with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate [29] in the presence of 0.5 M trimethylsilyl trifluoromethanesulfonate afforded the disaccharide derivative 2-naphthyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3-di-*O*-benzoyl-β-D-xylopyranoside (**3**) in 64% yield. A similar coupling strategy was followed by Rio et al. [22] for the synthesis of benzyl *O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1 → 4)-2,3-di-*O*-benzoyl-β-D-xylopyranoside (61% yield). The ^{1}H-NMR spectrum of compound **3** showed a peak at δ 4.7 having a coupling constant of $^3J_{H1,H2}$ 7.9 Hz, confirming the β-glycosidic linkage between galactose and xylose. De-*O*-acylation of **3** with base gave the disaccharide, 2-naphthyl *O*-(β-D-galactopyranosyl)-(1 → 4)-β-D-xylopyranoside (**4**), in almost quantitative yield. ^{13}C-NMR signals at δ 102.5 and 100.72 were assigned to C′-1 and C-1. A downfield shift of 6.54 ppm in the resonance of C-4 (δ 76.11) in the ^{13}C-NMR spectra of compound **4** compared to C-4 of 2-naphthyl β-D-xylopyranoside suggested that this site was glycosidically linked.

NIS- and TFMS-activated coupling of phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside [30] with 1-*O*-acetyl 2,4,6-tri-*O*-benzoyl-β-D-galactopyranose [31] afforded disaccharide **5** in 70% yield. A signal at δ 4.5 with a *J* value of 7.8 Hz in the ^{1}H-NMR spectrum confirmed the β-glycosidic linkage between the sugars. The disaccharide derivative, *O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1 → 3)-1-*O*-acetyl-2,4,6-tri-*O*-benzoyl-β-D-galactopyranose was synthesized by Ekborg et al. using 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl bromide as glycosyl donor in the presence of silver trifluoromethanesulfonate [31]. A yield of 53% β-anomer and 28% α-anomer was achieved during the coupling step. Converting **5** to the bromo derivative **5a** was readily achieved with 30% HBr in AcOH. Coupling of **5a** to 2-naphthol in the presence of NaH in acetonitrile gave the disaccharide 2-naphthyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-

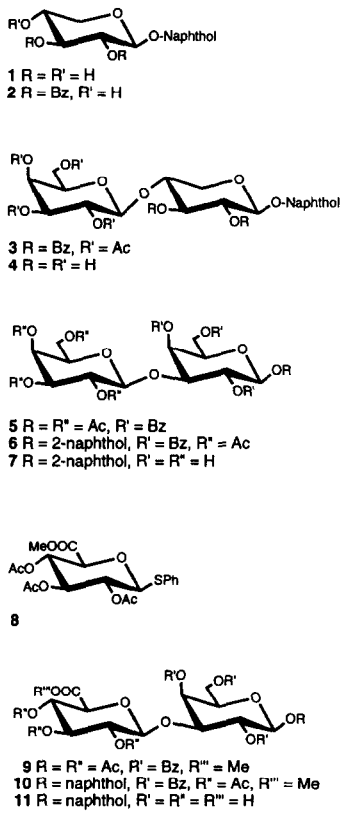

Fig. 2. Structure of monosaccharides and disaccharides.

galactopyranosyl)-(1 → 3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranoside (**6**) in 62% overall yield. De-*O*-acetylation of **6** with 0.5 M NaOMe in MeOH gave the 2-naphthyl *O*-(β-D-galactopyranosyl)-(1 → 3)-β-D-galactopyranoside (**7**) in 90% yield. A downfield shift of 9 ppm (δ 83.12) in the C-3 signal for the reducing-end Gal in the ^{13}C NMR spectrum of **7** compared to C-3 of 2-naphthyl β-D-galactopyranoside confirmed that the galactose residue was linked at this position. The β-anomeric orientations of C-1 and C′-1 were apparent from resonances at δ 100.4 and 105.2.

Thioglycoside **8** [methyl (phenyl 2,3,4-tri-*O*-acetyl-1-thio-β-D-glucopyranosid)-uronate] was prepared by reacting methyl (1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranosyl)-uronate [32] with phenylthiotrimethylsilane in the presence of trimethylsilyl trifluoromethanesulfonate [30]. Very little product formed at room temperature after 24 h, but at 55°C most of the starting material was converted to a product with ^{1}H-NMR resonances at δ 4.74 ppm (*J* 10.0 Hz) for H-1, 7.5–7.3 ppm for aromatic protons, and a peak at 3.75 ppm for the methyl ester, consistent with the structure proposed for compound **8**. However, its specific rotation was different from the previously reported value [33]. An adequate explanation for this difference is not available.

Condensation of **8** with 1-*O*-acetyl-2,4,6-tri-*O*-benzoyl-β-D-galactopyranose [31] in

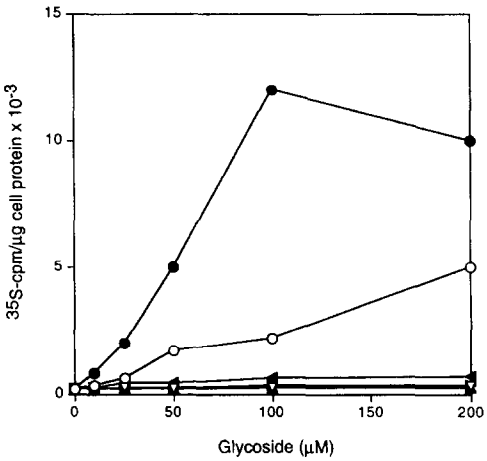

Fig. 3. Priming of glycosaminoglycans by disaccharides in Chinese hamster ovary cells. The indicated compounds were added to semi-confluent monolayers of mutant p*gs*A-745 in F12 growth medium supplemented with $^{35}SO_4$ (Experimental section). After 24 h, [^{35}S]glycosaminoglycans were isolated from the cells and the growth medium. ●, peracetylated β-D-Gal*p*-(1 → 4)-β-D-Xyl*p*-(1 → *O*)-2-naphthyl; ○, non-acetylated β-D-Gal*p*-(1 → 4)-β-D-Xyl*p*-(1 → *O*)-2-naphthyl; ◂, peracetylated β-D-GlcA*p*-(1 → 3)-β-D-Gal*p*-(1 → *O*)-2-naphthyl; ▷, non-acetylated β-D-GlcA*p*-(1 → 3)-β-D-Gal*p*-(1 → *O*)-2-naphthyl; ▲, acetylated β-D-Gal*p*-(1 → 3)-β-D-Gal*p*-(1 → *O*)-2-naphthyl; ▽, non-acetylated β-D-Gal*p*-(1 → 3)-β-D-Gal*p*-(1 → *O*)-2-naphthyl.

presence of NIS and TFMS gave disaccharide **9** in 45% yield. Treatment of **9** with HBr/AcOH afforded the bromosugar, *O*-[methyl (2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)uronate]-(1 → 3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl bromide (**9a**). This was converted directly to the disaccharide derivative, 2-naphthyl *O*-[methyl (2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)uronate]-(1 → 3)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranoside (**10**) by reacting with the sodium salt of 2-naphthol in dry acetonitrile (50% overall yield). A peak at δ 5.52 (*J* 8.1 Hz) for H-1 confirmed the β-configuration and the appearance of peaks in the aromatic region in the ^{1}H-NMR spectrum of **10** confirmed the attachment of the naphthalene ring. Treatment of **10** with 1 M NaOH in tetrahydrofuran–water (1:1, v/v) afforded the disaccharide 2-naphthyl *O*-(β-D-glucopyranosyluronic acid)-(1 → 3)-β-D-galactopyranoside (**11**) in 87% yield. Peaks at δ 5.35 (*J* 8.0 Hz) and 4.46 (*J* 7.1 Hz) in the ^{1}H-NMR spectrum of **11** confirmed the β-glycosidic linkage between the two sugars. Peaks at δ 104.46 (C-1), 102.17 (C′-1) and a peak at δ 83.27 for C-3 in the ^{13}C-NMR spectrum of **11** support the idea that the sugars are linked β-(1 → 3).

Disaccharides were incubated with a Chinese hamster ovary (CHO) cell mutant (*pgs*A745) defective in xylosyltransferase [34]. These cells do not produce GAG chains on core proteins but make chains on exogenous β-D-xylopyranosides [8]. The production of GAGs was measured by determining the incorporation of $^{35}SO_4$ into polysaccharide chains. As shown in Fig. 3, only disaccharide **4** primed GAGs and a relatively high concentration was needed compared to β-D-xylopyranosides. We showed previously that 2-naphthyl β-D-xylopyranoside efficiently primes GAG chains in CHO cells, peaking at

10 μM [8]. Disaccharides **7** and **11** did not prime GAG chains (Fig. 3), even at high concentration (1 mM, data not shown).

The low efficiency of priming by disaccharides **4**, **7** and **11** compared to 2-naphthyl β-D-xylopyranoside might reflect poor uptake of very polar disaccharides [35]. We recently showed that cells will take up acetylated disaccharides efficiently [35]. Intracellular deacetylation generates free glycosides which can act as primers for oligosaccharide biosynthesis. We, therefore, acetylated disaccharides **4**, **7**, and **11** and tested their priming activity in cells. Acetylation of disaccharides **7** and **11** did not improve their priming efficiency. In contrast, acetylation of **4** improved its ability to prime chains compared to the non-acetylated derivative, with peak activity occurring at 100 μM. The acetylated disaccharide did not prime as well as 2-naphthyl β-D-xylopyranoside or its acetylated derivative, possibly due to differences in its uptake or activity as a substrate.

The enhanced level of priming found after acetylation of disaccharide **4** suggests that cells possess the enzymes for removing the blocking groups, thus exposing the sugar in the proper compartment for priming to occur [35]. The failure of acetylated forms of **7** and **11** to prime may reflect high K_m values for GlcA transferase I and α-GlcNAc transferase I, respectively [36]. These enzymes might require more complex structures attached to their reducing termini, such as natural peptide sequences flanking the attachment sites in core proteins [1]. The ability of disaccharide **4** to prime GAG chains renders it a starting point for making a disaccharide-based inhibitor of GAG biosynthesis. The presence of 6 hydroxyl groups in β-D-Gal*p*-(1 → 4)-β-D-Xyl*p*-(1 → *O*)-2-naphthyl provides numerous sites to introduce reactive functional groups or blocking groups that might bind and competitively inhibit glycosyltransferases involved in GAG biosynthesis [37–39].

3. Experimental

General methods.—Melting points were determined with a Fisher–Johns melting point apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer Model 141 polarimeter. ^{1}H-NMR (400 MHz) spectra and ^{13}C-NMR (100.6 MHz) spectra were recorded at 24°C with a Bruker WH-400 spectrometer equipped with an Aspect-3000 computer. Chemical shifts (δ) were measured relative to the signal for Me_4Si. Assignments of NMR data were made by comparing spectra of experimental and parent compounds and by comparing the data with previously published information on related compounds [8,17,22,28,31]. Reaction products were analyzed by TLC on Silica Gel 60-F254 (Merck) and detected with UV light or by charring with 5% (v/v) H_2SO_4 in MeOH. Column chromatography was performed on silica gel 60 Å (Merck, 63–200 μm). Elemental analyses were performed by Robertson Microlit Laboratories, Inc. (Madison, NJ). All solvents were distilled before use and stored over dry molecular sieves. All evaporations were done at 40–50°C under reduced pressure, unless otherwise noted.

2-Naphthyl 2,3-di-O-benzoyl-β-D-xylopyranoside (**2**).—Dibutyltin oxide (0.97 g, 3.9 mmol) was added to a solution of 2-naphthyl β-D-xylopyranoside (**1**, 3.6 mmol) in dry MeOH (40 mL) and the mixture was refluxed for 2.5 h and then concentrated to dryness.

The residue was dissolved in benzene (28 mL) and a solution of chloroacetyl chloride (0.44 mL, 5.5 mmol) in benzene (6 mL) was added dropwise with stirring at room temperature. After 30 min, the mixture was concentrated to dryness and dissolved in pyridine (25 mL). Benzoyl chloride (1.8 mL, 15.5 mmol) in pyridine (5 mL) was added dropwise at 0°C. After stirring for 1 h, excess benzoyl chloride was destroyed by adding MeOH, and the mixture was concentrated to dryness. The residue was dissolved in dichloromethane (60 mL), washed successively with cold aq 10% $KHSO_4$, a cold saturated solution of aq $NaHCO_3$, and cold water. The sample was dried with Na_2SO_4 and concentrated. The residue was dissolved in 1:8 (v/v) pyridine/EtOH (45 mL). Thiourea (2 g, 26 mmol) was added and the mixture was stirred at 80°C overnight. The solvent was removed and the residue was dissolved in dichloromethane (75 mL). The sample was washed with 5% aq NaCl and water (0–5°C), dried with Na_2SO_4 and concentrated. Compound **2** (1.25 g, 72%) was purified by silica gel chromatography using hexanes/ethyl acetate (2:1, v/v); mp 179–181°C (ether); $[\alpha]_D$ +96.2° (*c* 0.9, chloroform). ^{1}H-NMR data ($CDCl_3$): δ 8.1–7.15 (m, 17 H, arom.), 5.66 (dd, 1 H, $J_{1,2}$ 7.00 Hz, $J_{2,3}$ 7.3 Hz, H-2), 5.55 (d, 1 H, $J_{1,2}$ 7.0 Hz, H-1), 5.45 (t, 1 H, $J_{2,3} = J_{3,4} = 7.2$ Hz, H-3), 4.40 (m, 1 H, H-4), 4.10 (dd, 1 H, $J_{4,5e}$ 5.2, $J_{5e,5a}$ 11.3 Hz, H-5e), 3.75 (dd, 1 H, $J_{4,5a}$ 9.2, $J_{5e,5a}$ 11.3 Hz, H-5a), 3.25 (1 H, OH-4). Anal. Calcd for $C_{29}H_{24}O_7$: C, 71.89; H, 4.99. Found: C, 71.75; H, 4.96.

2-Naphthyl O-*(2,3,4,6-tetra*-O-*acetyl-β*-D-*galactopyranosyl)-(1 → 4)-2,3-di*-O-*benzoyl-β*-D-*xylopyranoside* (**3**).—A mixture of **2** (0.15 g, 0.3 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate [29] (0.25 g, 0.5 mmol) in toluene–dichloromethane (9:1, 50 mL) was stirred with activated 4 Å molecular sieves (2 g) under argon for 15 min at room temperature. The solution was cooled to −20°C and trimethylsilyl trifluoromethanesulfonate in toluene (0.5 M, 100 μL) was added with stirring at −20°C under an atmosphere of argon. After 40 min, *N,N*-diisopropylethylamine (0.2 mL) was added, the mixture was filtered through Celite, and the filtrate was concentrated to dryness. The residue was purified by silica gel chromatography (hexanes–ethyl acetate, 1:1, v/v) to give **3** (0.16 g, 64%), $[\alpha]_D$ +7° (*c* 0.7, chloroform). ^{1}H NMR data ($CDCl_3$): δ 8.15–7.10 (m, 17 H, aromatic), 5.76 (t, 1 H, $J_{2,3} = J_{3,4} = 7.2$ Hz, H-3), 5.69 (dd, 1 H, $J_{1,2}$ 7.1, $J_{2,3}$ 7.2 Hz, H-2), 5.42 (d, 1 H, $J_{1,2}$ 7.1 Hz, H-1), 5.27 (d, 1 H, $J_{3',4'}$ 3.1 Hz, H′-4), 5.19 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H′-2), 4.99 (dd, 1 H, $J_{2',3'}$ 7.2, $J_{3'4'}$ 3.1 Hz, H′-3), 4.70 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H′-1), 3.64–3.92 (m, 2 H, H-5), 2.15, 2.10, 2.05, 1.98 (4s, 12 H, 4OAc). Anal. Calcd for $C_{43}H_{42}O_{16}$: C, 63.38; H, 5.20. Found: C, 63.52; H, 5.09.

2-Naphthyl O-*(β*-D-*galactopyranosyl)-(1 → 4)-β*-D-*xylopyranoside* (**4**).—A 5 M solution of NaOMe in MeOH (0.5 mL) was added to a solution of **3** (0.11 g, 0.1 mmol) in MeOH (10 mL) and stirred for 48 h at room temperature. The sample was neutralized with IR-120(H^+) resin and concentrated to dryness. The residue was dissolved in water and applied in equal portions to three Sep-Pak C_{18} cartridges (0.7 mL, Water Associates) that had been washed with MeOH and then H_2O. After washing the column with water (7 mL), product was eluted with 5 mL of 40% (v/v) MeOH in H_2O (0.054 g, 92%); $[\alpha]_D$ −15° (*c* 0.7, H_2O–MeOH 1:1). ^{1}H-NMR data (Me_2SO-d_6): δ 7.90–7.20 (m, 7 H, aromatic), 5.08 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.25 (d, 1 H, $J_{1',2'}$ 7.7 Hz, H′-1). ^{13}C-NMR data (Me_2SO-d_6): δ 102.5 (C′-1), 100.72 (C-1), 76.11 (C-4), 75.59 (C′-5),

74.55 (C-2), 73.17 (C-3), 72.97 (C′-3), 69.87 (C′-2), 68.17 (C′-4), 63.59 (C-5), 60.47 (C′-6). Anal. Calcd for $C_{21}H_{26}O_{10}$: C, 57.53; H, 5.98. Found: C, 57.28; H, 6.14.

O-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-(1 → 3)-1-O-acetyl-2,4,6-tri-O-benzoyl-β-D-galactopyranose (**5**).—A mixture of 1-*O*-acetyl-2,4,6-tri-*O*-benzoyl-β-D-galactopyranose [31] (1 g, 1.9 mmol), phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside [30] (1.2 g, 2.7 mmol), and activated 4 Å molecular sieves (2 g) in dry dichloromethane (50 mL) was stirred for 1 h at room temperature under argon. The mixture was cooled to 0–5°C (ice-bath) and NIS (1.12 g, 5 mmol) was added while stirring the sample in the cold. A solution of 0.15 M TMSF in dichloromethane (10 mL) was added dropwise over 30 min with stirring. After 1 h the reaction mixture was filtered through Celite and washed successively in the cold with a 5% aq solution of $Na_2S_2O_3$, saturated aq $NaHCO_3$, and water. The sample was dried with Na_2SO_4 and concentrated. Silica gel chromatography of the residue using hexanes–ethyl acetate (3:1, 2:1, and 1.5:1 stepwise, v/v) gave **5** (1.12 g, 70%) as an amorphous powder, $[\alpha]_D$ +42° (*c* 0.7, chloroform). ^{1}H-NMR data ($CDCl_3$): δ 8.18–7.40 (m, 15 H, aromatic), 6.06 (d, 1 H, $J_{3,4}$ 3.5 Hz, H-4), 5.98 (d, 1 H, $J_{1,2}$ 8.6 Hz, H-1), 5.87 (dd, 1 H, $J_{1,2}$ 8.6, $J_{2,3}$ 9.9 Hz, H-2), 5.36 (dd, $J_{3',4'}$ 3.2, $J_{4',5'}$ 1 Hz, H′-4), 5.10 (dd, 1 H, $J_{1',2'}$ 7.8, $J_{2',3'}$ 9.2 Hz, H′-2), 4.95 (dd, 1 H, $J_{2',3'}$ 9.3, $J_{3',4'}$ 3.2 Hz, H′-3), 4.50 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H′-1), 4.02 (m, 2 H, H-3 and H-5), 2.12, 2.09, 2.04, 2.00 (4s, 15 H, 5OAc). Anal. Calcd for $C_{43}H_{44}O_{19}$: C, 59.72; H, 5.13, Found: C, 59.42; H, 5.02.

2-Naphthyl O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1 → 3)-2,4,6-tri-O-benzoyl-β-D-galactopyranoside (**6**).—HBr (30%) in AcOH (1 mL) was added to a solution of **5** (0.25 g, 0.3 mmol) in dichloromethane (1 mL) and the reaction was stirred for 30 min at room temperature. After evaporating the solution, the residue was dissolved in dichloromethane and washed successively with a cold saturated solution of aq $NaHCO_3$ and water. The sample was dried with Na_2SO_4 and concentrated to give *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl bromide (**5a**). This was used without further purification.

2-Naphthol (70 mg, 0.5 mmol) and NaH (11.2 mg, 0.5 mmol) in acetonitrile (3 mL) were stirred at room temperature for 5 min. A solution of **5a** (250 mg, 0.3 mmol) was added to the mixture at 0–5°C and stirred for 1 h at room temperature. The sample was concentrated and the residue was purified by column chromatography on silica (hexanes–ethyl acetate, 1.5:1, v/v) to give **6** (0.17 g, 62%), $[\alpha]_D$ +35° (*c* 0.5, $CHCl_3$). ^{1}H-NMR data ($CDCl_3$): δ 8.20–7.16 (m, 22 H, aromatic), 6.01 (d, 1 H, $J_{3',4'}$ 3.6 Hz, H′-4), 5.89 (dd, 1 H, $J_{1,2}$ 7.9 Hz, $J_{2,3}$ 9.2 Hz, H-2), 5.40 (dd, $J_{3',4'}$ 3.4 Hz, $J_{4',5'}$ 1 Hz, H′-4), 5.34 (d, 1 H, $J_{1,2}$ 8.00 Hz, H-1)), 5.16 (dd, 1 H, $J_{1',2'}$ 7.7, $J_{2',3'}$ 9.2 Hz, H′-2), 4.82 (dd, 1 H, $J_{2',3'}$ 9.2, $J_{3',4'}$ 3.4 Hz, H′-3), 4.48 (d, 1 H, $J_{1',2'}$ 7.7 Hz, H′-1), 2.15, 2.08, 1.99, 1.90 (4s, 12 H, 4 OAc). Anal. Calcd for $C_{51}H_{48}O_{18}$: C, 64.55; H, 5.10 Found: C, 64.28; H, 5.05.

2-Naphthyl O-(β-D-galactopyranosyl)-(1 → 3)-β-D-galactopyranoside (**7**).—Compound **6** (0.15 g, 0.2 mmol) was treated with NaOMe and purified by passing through a C_{18} Sep-Pak cartridge to give **7** (0.068 g, 90%), $[\alpha]_D$ −5°(*c* 0.5, H_2O–MeOH 1:1). ^{1}H-NMR data (Me_2SO-d_6): δ, 7.88–7.21 (m, 7 H, aromatic), 5.11 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 4.28 (d, 1 H, $J_{1',2'}$ 7.61 Hz, H′-1). ^{13}C-NMR data (Me_2SO-d_6): δ 105.22 (C-1), 100.4 (C′-1), 83.12 (C-3), 75.30 (C-5, C′-5), 73.08 (C′-3), 71.22 (C′-2), 69.45 (C-2),

68.05 (C′-4), 67.42 (C-4), 60.36 and 60.21 (C′-6 and C-6). Anal. Calcd for $C_{22}H_{28}O_{11}$: C, 56.40; H, 6.02 Found: C, 56.04; H, 5.92.

Methyl (phenyl 2,3,4-tri-O-acetyl-1-thio-β-D-glucopyranosid)uronate (**8**).—A mixture of methyl (1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranosyl)uronate [32] (2 g, 5.3 mmol), phenylthiotrimethyl silane (3 mL, 15.8 mmol), and trimethylsilyl trifluoromethanesulfonate (2.5 mL, 12.9 mmol) in 1,2-dichloroethane (50 mL) was stirred at 55°C for 24 h. The reaction mixture was cooled to room temperature and washed with cold saturated aq $NaHCO_3$ and water. The sample was dried with Na_2SO_4 and concentrated. The dark brown residue was applied to a column of silica gel and eluted from the column using pure hexanes and hexanes–ethyl acetate (1:1, v/v). The fractions corresponding to product were combined, concentrated to dryness, dissolved in ethanol and decolorized by boiling with charcoal. The blocked glucuronide was recrystalized from the same solvent. Yield, 1.4 g (61%), mp 115–116°C, R_f (hexanes–ethyl acetate, 1:1, v/v) = 0.37, $[\alpha]_D$ +4.5° (*c* 1, $CHCl_3$) (lit. [33]. mp 118–120°C, $[\alpha]_D$ −22°); ^{1}H-NMR data ($CDCl_3$): δ 7.52–7.30 (m, 5 H, arom.), 5.27 (dd, 1 H, $J_{3,4}$ 9.9, $J_{4,5}$ 8.4 Hz, H-4), 5.18 (t, 1 H, $J_{2,3} = J_{3,4} = 9.8$ Hz, H-3), 4.97 (dd, 1 H, $J_{2,3}$ 9.9, $J_{1,2}$ 10.0 Hz, H-2,), 4.74 (d, 1 H, $J_{1,2}$ 10.0 Hz, H-1), 4.05 (d, 1 H, $J_{4,5}$ 9.9 Hz, H-5), 3.76 (s, 3 H, -COOMe), 2.09, 2.02, 2.00 (3s, 9 H, 3 OAc). Anal. Calcd for $C_{19}H_{22}SO_9$: C, 53.51; H, 5.20; S, 7.52. Found: C, 53.33; H, 5.09; S, 7.20.

O-*[Methyl (2,3,4-tri-O-acetyl-β-D-glucopyranosyl)-uronate]-(1 → 3)-1-O-acetyl-2,4,6-tri-O-benzoyl-β-D-galactopyranose* (**9**).—A mixture of **8** (0.5 g, 1.2 mmol) and 1-*O*-acetyl-2,4,6-tri-*O*-benzoyl-β-D-galactopyranose (0.42 g, 0.8 mmol) was treated with NIS (0.45 g, 2 mmol) in the presence of 0.15 M TFMS (as described for **5**) to give **9** (0.3 g, 45%), $[\alpha]_D$ +20° (*c* 0.7, $CHCl_3$). ^{1}H-NMR data ($CDCl_3$): δ 8.20–7.40 (m, 15 H, aromatic), 6.04 (d, 1 H, $J_{3,4}$ 3.4 Hz, H-4), 6.00 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 5.25 (t, 1 H, $J_{3',4'} = J_{4',5'} = 9.7$ Hz, H′-4), 5.12 (t, 1 H, $J_{2',3'} = J_{3',4'} = 9.7$ Hz, H′-3), 4.92 (dd, 1 H, $J_{2',3'}$ 9.5 Hz, $J_{1',2'}$ 7.9 Hz, H′-2), 4.70 (d, $J_{1',2'}$ 7.9 Hz, H′-1), 4.41 (d, 2 H, H-6), 4.28 (m, 1 H, H-3), 4.02 (m, 2 H, H-3 and H′-5), 3.77 (s, 3 H, COOMe), 2.02, 1.96, 1.92, 1.68 (4s, 12 H, 4OAc).

Anal. Calcd for $C_{42}H_{42}O_{19}$: C, 59.29; H, 4.98. Found: C, 59.11; H, 4.96.

2-Naphthyl O-[methyl (2,3,4-tri-O-acetyl-β-D-glucopyranosyl)uronate]-(1 → 3)-2,4,6-tri-O-benzoyl-β-D-galactopyranoside (**10**).—Compound **9** (0.25 g, 0.3 mmol) was first converted to *O*-[methyl (2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)uronate]-(1 → 3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl bromide (**9a**) as described for converting **5** to **5a**. It was coupled to 2-naphthol (0.070 g, 0.5 mmol) in presence of NaH (11 mg, 0.5 mmol) to give **10** (0.14 g, 52%); $[\alpha]_D$ +24° (*c* 0.7, chloroform). ^{1}H-NMR data ($CDCl_3$): δ 7.95–7.20 (m, 22 H, arom.), 6.04 (d, 1 H, $J_{3,4}$ 3.2 Hz, H-4), 5.85 (dd, 1 H, $J_{1,2}$ 8.1, $J_{2,3}$ 9.7 Hz, H-2), 5.52 (d, $J_{1,2}$ 8.1 Hz, H-1), 5.25 (t, 1 H, $J_{3',4'} = J_{4',5'} = 9.7$ Hz, H′-4), 5.10 (t, 1 H, $J_{2',3'} = J_{3',4'} = 9.7$ Hz, H′-3), 4.92 (dd, 1 H, $J_{1',2'}$ 7.7, $J_{2',3'}$ 9.5 Hz, H′-2), 4.86 (d, 1 H, $J_{1',2'}$ 7.7 Hz, H′-1,), 4.28 (m, 1 H, H-3), 4.02 (m, 1 H, H-5), 4.00 (d, 1 H, $J_{4',5'}$ 9.7 Hz, H′-5), 3.98 (1 H, H-3), 3.77 (s, 3 H, -COOMe), 1.96, 1.92, 1.68 (3s, 9 H, $-COCH_3$). Anal. Calcd for $C_{50}H_{46}O_{19}$: C, 64.23; H, 4.96. Found: C, 64.11; H, 5.02.

2-Naphthyl O-(β-D-glucopyranosyluronic acid)-(1 → 3)-β-D-galactopyranoside (**11**).—Aqueous NaOH (1 M, 0.5 mL) was added to a solution of **10** (0.12 g, 0.1 mmol) in

tetrahydrofuran (2 mL) at room temperature with stirring. The mixture was stirred overnight, neutralized with AcOH and concentrated to dryness. The residue was separated on a Sep-Pak C_{18} cartridge to give **11** (0.054 g, 87%); $[\alpha]_D$ −30°(*c* 0.7, H_2O–MeOH 1:1). ^{1}H-NMR data (Me_2SO-d_6): δ 7.95–7.50 (m, 7 H, arom.), 5.35 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.46 (d, 1 H, $J_{1',2'}$ 7.1 Hz, H'-1). ^{13}C-NMR data (Me_2SO-d_6): δ, 170.95 (C=O), 126–135 (aromatic), 104.46 (C-1), 102.17 (C'-1), 83.27 (C-3), 75.70 (C'-5), 75.21 (C'-3), 75.07 (C-5), 73.68 (C'-2), 71.60 (C'-4), 69.60 (C-2), 67.44 (C-4), 60.36 (C-6). Anal. Calcd for $C_{22}H_{26}O_{12}$: C, 54.77; H, 5.43. Found: C, 54.37; H, 5.40.

Cell culture and priming studies.—A Chinese hamster ovary cell mutant defective in xylosyltransferase, *pgs*A-745, was described previously [34]. Cells were grown in F12 medium supplemented with 10% (v/v) fetal bovine serum under an atmosphere of 5% CO_2/95% air and 100% relative humidity. The cells were subcultured every 3–4 days with trypsin as described [34]. The glycosides were dissolved in Me_2SO and added to growth medium before incubation with cells. The final concentration of Me_2SO was 0.5% (v/v) in all experiments. Approximately 2.5×10^4 cells were added to individual wells of a 24-well plate and after 24 h the medium was removed and fresh F12 medium was added with 10% dialyzed fetal bovine serum, 100 U/mL penicillin, $H_2{}^{35}SO_4$ (25 μCi/mL), and one of the disaccharides at the concentration indicated in Fig. 3. After incubating the cells at 37°C for 24 h, [^{35}S]GAG was isolated from the cells plus the growth medium as described [8].

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References

[1] L. Zhang and J.D. Esko, *J. Biol. Chem.*, 269 (1994) 19295–19299.
[2] L. Rodén, *Structure and metabolism of connective tissue proteoglycans*, in W.J. Lennarz (Ed.), *The Biochemistry of Glycoproteins and Proteoglycans*, Plenum, New York, 1980, pp 267–271.
[3] M. Okayama, K. Kimata, and S. Suzuki, *J. Biochem. (Tokyo)*, 74 (1973) 1069–1073.
[4] H.C. Robinson, M.J. Brett, P.J. Tralaggan, D.A. Lowther, and M. Okayama, *Biochem. J.*, 148 (1975) 25–34.
[5] M. Sobue, H. Habuchi, K. Ito, H. Yonekura, K. Oguri, K. Sakurai, S. Kamohara, Y. Ueno, R. Noyori, and S. Suzuki, *Biochem. J.*, 241 (1987) 591–601.
[6] S.O. Kolset, K. Sakurai, I. Ivhed, A. Øvervatn, and S. Suzuki, *Biochem. J.*, 265 (1990) 637–645.
[7] F.N. Lugemwa and J.D. Esko, *J. Biol. Chem.*, 266 (1991) 6674–6677.
[8] T.A. Fritz, F.N. Lugemwa, A.K. Sarkar, and J.D. Esko, *J. Biol. Chem.*, 269 (1994) 300–307.
[9] J.A. Robinson and H.C. Robinson, *Biochem. J.*, 227 (1985) 805–814.
[10] H.H. Freeze, D. Sampath, and A. Varki, *J. Biol. Chem.*, 268 (1993) 1618–1627.
[11] J.R. Etchison, G. Srikrishna, and H.H. Freeze, *J. Biol. Chem.*, 270 (1995) 756–764.

[12] A. Manzi, P.V. Salimath, R.C. Spiro, P.A. Keifer, and H.H. Freeze, *J. Biol. Chem.*, 270 (1995) 9154–9163.
[13] P.V. Salimath, R.C. Spiro, and H.H. Freeze, *J. Biol. Chem.*, 270 (1995) 9164–9168.
[14] S. Shibata, K. Takagaki, T. Nakamura, J. Izumi, K. Kojima, I. Kato, and M. Endo, *J. Biol. Chem.*, 270 (1995) 13794–13798.
[15] J. Izumi, K. Takagaki, T. Nakamura, S. Shibata, K. Kojima, I. Kato, and M. Endo, *J. Biochem. (Tokyo)*, 116 (1994) 524–529.
[16] T. Nakamura, J. Izumi, K. Takagaki, S. Shibata, K. Kojima, I. Kato, and M. Endo, *Biochem. J.*, 304 (1994) 731–736.
[17] R. Kovác, B. Taylor, and C.P.J. Glaudemans, *J. Org. Chem.*, 50 (1985) 5323–5333.
[18] H.M. Flowers, *Carbohydr. Res.*, 4 (1967) 312–317.
[19] B. Erbing, B. Lindberg, and T. Norberg, *Acta Chem. Scand.*, 32 (1978) 308–300.
[20] P.J. Garegg, B. Lindberg, and T. Norberg, *Acta Chem. Scand.*, 33 (1979) 449–452.
[21] L. Lindberg, L. Rodén, and B.-G. Silvander, *Carbohydr. Res.*, 2 (1966) 43–40.
[22] S. Rio, J.-M. Bean, and J.-C. Jacquinet, *Carbohydr. Res.*, 219 (1991) 71–90.
[23] M. Zsiska and B. Meyer, *Carbohydr. Res.*, 215 (1991) 279–292.
[24] G.H. Veeneman, S.H. van Leeuwen, and J.H. van Boom, *Tetrahedron Lett.*, 31 (1990) 1331–1334.
[25] R.R. Schmidt, *Angew. Chem., Int. Ed. Engl.*, 25 (1986) 212–235.
[26] S. David and S. Hanessian, *Tetrahedron*, 41 (1985) 643–663.
[27] M. Bertolini and C.P.J. Glaudemans, *Carbohydr. Res.*, 15 (1970) 263–270.
[28] M. Nilsson, J. Westamn, and C.-M. Svahn, *J. Carbohydr. Chem.*, 12 (1993) 23–37.
[29] P.H. Amvam-Zollo and P. Sinay, *Carbohydr. Res.*, 150 (1986) 199–212.
[30] A.K. Sarkar and K.L. Matta, *Carbohydr. Res.*, 233 (1992) 245–250.
[31] G. Ekborg, T. Curenton, N. Ramakrishna, and L. Rodén, *J. Carbohydr. Chem.*, 9 (1990) 15–37.
[32] G.N. Bollenback, J.W. Long, D.G. Benjamin, and J.A. Lindquist, *J. Am. Chem. Soc.*, 77 (1955) 3310–3312.
[33] R.J. Ferrier and R.H. Furneaux, *Carbohydr. Res.*, 52 (1976) 63–68.
[34] J.D. Esko, T.E. Stewart, and W.H. Taylor, *Proc. Natl. Acad. Sci. U.S.A.*, 82 (1985) 3197–3201.
[35] A.K. Sarkar, T.A. Fritz, W.H. Taylor, and J.D. Esko, *Proc. Natl. Acad. Sci. U.S.A.*, 92 (1995) 3323–3327.
[36] T.A. Fritz, M.M. Gabb, G. Wei, and J.D. Esko, *J. Biol. Chem.*, 269 (1994) 28809–28814.
[37] O. Hindsgaul, K.J. Kaur, G. Srivastava, M. Blaszczyk-Thurin, S.C. Crawley, L.D. Heerze, and M.M. Palcic, *J. Biol. Chem.*, 266 (1991) 17858–17862.
[38] S.H. Khan, S.C. Crawley, O. Kanie, and O. Hindsgaul, *J. Biol. Chem.*, 268 (1993) 2468–2473.
[39] T.L. Lowary and O. Hindsgaul, *Carbohydr. Res.*, 251 (1994) 33–67.