Unusual $\beta$-d-Xylosides That Prime Glycosaminoglycans in Animal Cells*

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The biosynthesis of glycosaminoglycans (GAG) takes place while the polysaccharide chains are usually attached to a proteoglycan core protein. Cells also will assemble GAG chains on $\beta$-d-xylosides containing hydrophobic aglycones. In order to evaluate the relationship of the structure of the sugar to priming activity of the glycoside, we synthesized $\beta$-d-xyloside analogs in which the hydroxyls were substituted with hydrogen, fluorine, $\alpha$-methyl, amino, $\alpha$-isopropyl, and $\alpha$-benzyl groups. Epimers at the 2-, 3-, and 4-position of xylose also were made. Their ability to prime GAGs was tested in Chinese hamster ovary cells by measuring $^{35}$SO$_4$ incorporation into polysaccharide chains and by assaying the transfer of galactose to the xylosides by galactosyltransferase I (UDP-$\alpha$-galactoselyxose $\beta$1-4-galactosyltransferase) in vitro. All of the analogs failed to act as primers of GAGs in vivo and as substrates in vitro with the following exceptions. Substitution of 2-OH and 3-OH with $\alpha$-OCH$_3$ groups and uronic acid epimers gives rise to unique saccharide structures that can interact with specific protein ligands (5–8). Although animal cells normally assemble GAG chains on proteoglycan core proteins, they also can use $\beta$-d-xylosides as primers for GAG formation (9–12). These compounds bypass the need for xylosylated core proteins and stimulate GAG synthesis beyond the level that occurs on endogenous proteoglycans, suggesting that xylosylation of proteoglycan core proteins may be rate-limiting. Cells secrete chains generated on the primers in a nearly quantitatively way, since the small hydrophobic aglycones do not suffice to tether the chains to cell membranes. In most cells, xylosides prime chondroitin sulfate efficiently and heparan sulfate only weakly (13). However, the structure of the aglycone can affect the type of GAG chain assembled on a xyloside (13, 14). Xylosides containing two fused aromatic rings will prime a mixture of heparan sulfate and chondroitin sulfate, whereas simpler xylosides prime only chondroitin sulfate (13).

These findings suggested that at least one of the glycosyltransferases involved in heparan sulfate synthesis might require a particular aglyconic structure in order to bind and react with intermediates containing appropriate aglycones at their termini. Subsequent studies showed that the first $\alpha$-GlCNAC transferase ($\alpha$-GlCNAC$\alpha$) that initiates heparan sulfate formation is a likely candidate for this transferase (13). On core proteins containing linkage tetrasaccharides (i.e. natural substrates), $\alpha$-GlCNAC$\alpha$ apparently recognizes specific amino acid sequences consisting of clusters of acidic residues, one or more aromatic residues, or intermediates assembled on adjacent Ser-Gly dipeptides (15, 16).

In this report, we examined the dependence of GAG priming on the $\beta$-xylose residue by chemically modifying the hydroxyl groups. Deoxygenated and alkylated oligosaccharides have been used to probe the specificity of glycosyltransferases in other systems and some of these compounds act as inhibitors in vitro (17–25). Unfortunately, uptake limits their utility as gly-
cosyltransferase inhibitors in vivo, presumably due to the thermodynamic barrier of transferring compounds with multiple hydroxyl groups across the lipophilic interior of cell membranes (26). Xylosides due to their simple structure are taken up efficiently (26) and therefore they serve as a starting point for designing inhibitors that might act in vivo. Our results demonstrate the critical requirement and stereochemistry of all three hydroxyl groups on xylose for priming GAGs. The ability of the derivatives to prime oligosaccharides in cells correlated with their activity as substrates for galactosyltransferase I in vitro. Interestingly, the 4-axial hydrogen can be removed by oxidation or replaced by -CH₃ with retention of priming activity, suggesting that analogs containing reactive groups at this position may act as substrate-based inhibitors in vivo.

**EXPERIMENTAL PROCEDURES**

Cell Culture—Chinese hamster ovary cells (CHO-K1), were obtained from the American Type Culture Collection (CCL-61; Rockville, MD). The growth and characteristics of GAG-deficient CHO mutants pgSA-745 (xylosyltransferase-deficient) and pgB-761 (galactosyltransferase I-deficient) have been described (27, 28). Wild-type and mutant cells were grown in F-12 medium (29) supplemented with 7.5% fetal bovine serum (Hyclone, Salt Lake City, UT), penicillin G (100 units/ml), and streptomycin sulfate (100 μg/ml) under an atmosphere of 5% CO₂ in air at 37°C with a Bruker WH-400 spectrometer equipped with an Aspect-3000 computer. Chemical shifts (δ) were measured relative to Me₄Si signal. The 13CNMR chemical shifts of the new compounds are given in Table 1. Reaction products were analyzed by TLC on Silica Gel 60-F₂₅₄ (Merck) with detection by UV absorption and/or charring with 5% (v/v) sulfuric acid in methanol. Column chromatography was performed on Silica Gel 60 Å (E. Merck, 63–200 μm). Elemental analyses were performed by the Robertson Microlit Laboratories, Inc. (Madison, NJ).

**Table I**

| Compound | δ (ppm) | 13C Chemical shifts (δ) for selected β-β-xylosyl derivatives (ppm) |
|----------|---------|---------------------------------------------------------------|
| 2-OMe | 80.47 | 69.24, 70.28, 71.22, 71.95, 63.46, 59.80 |
| 2-O-isopropyl | 74.98 | 69.99, 100.34, 72.72, 72.4, 62.51 |
| 2-Deoxy | 69.28 | 97.27, 32.61, 69.2, 69.82, 68.06 |
| 3-OMe | 84.81 | 102.15, 69.09, 70.92, 73.38, 65.02, 60.40 |
| 3-O-isopropyl | 80.97 | 102.42, 70.92, 73.64, 65.15 |
| 3-Deoxy | 69.23 | 98.62, 65.49, 30.77, 66.90, 64.67 |
| 1,2-Ethane | 84.50 | 102.22, 71.02, 72.95, 73.65, 62.29, 69.26, 61.92 |
| 3-Deoxy-3-amino | 72.98 | 102.20, 69.60, 59.16, 69.85, 66.85 |
| 4-Deoxy | 75.13 | 101.90, 70.63, 70.89, 71.36, 61.12 |
| 4-Methyl | 72.13 | 100.52, 71.28, 69.82, 69.27, 68.02 |

Calculated: C 60.0 H 6.67
Found: C 59.89 H 6.69

Benzyl-4-deoxy-β-β-xyloside[5]–13 (280 mg, 1 mmol) and p-toluenesulfonfyl chloride (1.5 mmol) were stirred in pyridine (2 ml) at room temperature. After 16 h, ice-cold saturated NaHCO₃ was added and the mixture was diluted with CH₂Cl₂ (50 ml) at 10°C in the presence of freshly prepared dry sodium carbonate. The mixture was concentrated and mixed with CH₃CN (30 ml) containing 1.0 μm acetic acid (2 ml), the mixture was concentrated and partitioned between water (5 ml) and ethyl acetate (50 ml).

The residue was dissolved in 1 ml of water, and the radioactivity was incorporated UDP-Gal and other degradation products were eluted from the cartridges with 10% (v/v) methanol in water (10 ml) and the supernatant was diluted with water (50 ml) and loaded on reverse-phase C₁₈ Sep-Pak cartridges (Waters Associates), which had been prewashed with methanol and water. Unincorporated UDP-Gal and other degradation products were eluted from the cartridges with 10% (v/v) methanol in water (10 ml) and the product was eluted with methanol (5 ml). After lyophilization, the residue was dissolved in 1 ml of water, and the radioactivity was measured by liquid scintillation spectrometry using Ultima Gold XR scintillant (Packard). Product formation was linear with time for 2 h at the protein concentrations used.
matography on a short silica gel column by stepwise elution with hexanes/ethanol acetate (1:1, v/v) to yield S (40 mg, 33%) (m.p. 92–93 °C). 1H NMR (CDCl3) δ 7.38 (m, 5H, Ph), 4.30 (d, J = 6.86 Hz, 1H, H-1), 4.00 (m, 1H), 3.70 (m, 1H), 3.50 (m, 1H), 3.40 (m, 1H), 2.75 (d, 1H, OH), 2.60 (t, 1H, OH), 2.95 (m, 1H, H-4eq), 1.75 (m, 1H, H-4ax). 13C NMR, see Table I.

C12H16O4
Calculated: C 64.29 H 7.14
Found: C 64.29 H 7.14

Benzyl-4-O-methyl-β-D-xylopyranoside [17]—This was prepared as described (34) (m.p. 77–78 °C).

Benzyl-β-D-threo-pentopyranos-4-ulose [12]—13 (0.56 g, 2 mmol) was added to a mixture of anhydrous Me2SO (40 ml) and acetic anhydride (5 ml) and the mixture was stirred at 40 °C under argon for 3 days (35). The brown syrup after lyophilization was treated with Amberlite cation exchange resin in aqueous methanol at room temperature for 1 h to remove the isopropylidene protecting group. The product was purified by a silica gel column to give a colorless syrup. Although the product after purification showed only one spot on TLC, its 1H NMR spectrum was complex. The 13C NMR spectrum showed one peak at 175 ppm due to C 5 and the infrared spectrum showed two absorption peaks at 3400 cm–1 and 3300 cm–1. The compound gave only one peak at 175 ppm due to C 5 in the 13C NMR spectrum, indicating the absence of the isopropylidene group. The product was purified on a short silica gel column to give a colorless syrup (20 mg). The stereochemistry at C-4 was assigned by a combination of COESY and NOE techniques. A 50% NOE between the 4-methyl and the equatorial H-5 was assigned by a combination of COESY and NOE techniques. A 50% NOE between the 4-methyl and the equatorial H-5 was assigned by a combination of COESY and NOE techniques. The NOE between the 4-methyl and the equatorial H-5 was assigned by a combination of COESY and NOE techniques. The NOE between the 4-methyl and the equatorial H-5 was assigned by a combination of COESY and NOE techniques.

Benzyl-2-O- and 3-O-isopropyl-β-D-xylopyranoside (30 mg) was reacted with methyl magnesium bromide in tetrahydrofuran at 4 °C. Excess reagent was destroyed with acetone and the sample was filtered. Dichloromethane (10 ml) was added to the filtrate, and the mixture was washed with 0.5 n NaCl (2 ml), dried with Na2SO4, and concentrated. Excess benzyl alcohol was removed by stirring for 1 h at room temperature. After 15 min, nitrogen was passed through the mixture to remove the hydrogen chloride gas. The crude reaction mixture was dehydrated with sodium methoxide in methanol, neutralized with IR-120 (H+ form) in aqueous methanol at 4°C saturated with hydrogen chloride gas in a React-Vial. The mixture was added to a mixture of anhydrous Me2SO (40 ml) and acetic anhydride (5 ml). The organic layer was dried over anhydrous Na2SO4 and concentrated. Excess benzyl alcohol was removed by stirring for 1 h with 20 mg of Amberlite cation exchange resin-118H (hydrogen form) in methanol/water (9:1, v/v). The sample was filtered, concentrated, and crystallized (isopropyl ether), yielding benzyl-2-O-methyl-β-D-xylopyranoside (220 mg, 67%) (m.p. 86–87 °C (isopropyl alcohol)). 1H NMR (CDCl3) δ 7.38 (m, 5H, Ph), 4.65 (d, J = 4.84 Hz, 1H, H-1), 4.20 (dd, 1H), 3.70 (m, 2H), 3.51 (s, 3H), 3.45 (m, 1H), 3.25 (m, 2H), 3.15 (m, 1H).

C13H16O3
Calculated: C 61.42 H 7.10
Found: C 61.88 H 7.29

Benzyl-2-O-benzyl-β-D-xylopyranoside [16]—14 (140 mg, 0.5 mmol), sodium hydride (1 mmol), and benzyl bromide (0.5 mmol) in dimethylformamide (4 ml) were stirred at room temperature for 3 h. Methanol (5 ml) was added and the mixture was concentrated in vacuo. The residue was partitioned between water and dichloromethane, and the organic layer was concentrated. The isopropylidene ring was removed as described above and the residue was purified by flash chromatography on silica gel using hexane/ethyl acetate (7:3, v/v). Benzyl-2-O-benzyl-β-D-xylopyranoside was obtained as a pure amorphous solid (180 mg). 1H NMR (CDCl3) δ 7.38 (m, 1OH, Ph), 4.69 (d, J = 4.92 Hz, 1H, H-1), 4.10 (dd, 1H), 3.70 (m, 2H), 3.45 (m, 2H), 3.0 (m, 2H).

C19H20O5
Calculated: C 68.87 H 6.40
Found: C 68.87 H 6.40

Benzyl-2-O-ribopyranoside [3]—O-Ribose (1.5 g, 10 mmol) was heated to 70 °C with 2% HCl in benzyl alcohol as described previously (41). The pure material was crystallized twice from ethyl acetate (1 g, 40%). 1H NMR (Me2SO-d6) δ 7.27–7.35 (m, 5H, Ph), 4.98, 4.96, 4.86 (s, 3H, D2O, exchangeable hydroxyl protons), 4.65 (d, J = 5.5 Hz, 1H, H-1). 13C NMR (CDCl3) δ 138.0, 128.45, 127.8, 127.7 (aromatic), 100.1 (C-1), 70.8 (C-2), 69.0 (C-3), 68.5(C-4), 63.9 (C-5).

Benzyl-2-O- and 3-O-isopropyl-β-D-xylosides [20 and 21]—Lithium aluminum hydride (1 g) was added to benzyl-2-O-isopropylidene 4-O-tolyloxyl-β-D-xylopyranoside (434 mg, 1 mmol) in anhydrous ether (20 ml) and dry toluene (30 ml) and the mixture was refluxed for 16 h with stirring. After cooling the sample, it was processed as described for 5. The residue was partitioned between water and dichloromethane, and the organic layer was dried (Na2SO4) and concentrated. The material was eluted from a column of silica gel (40 g) with hexane/ethyl acetate (4:3, v/v) to give 20 (200 mg, 46%) (m.p 120–121 °C (ethyl acetate/hexane)). 1H NMR (CDCl3) δ 6.31, 6.24, 6.15 (d, J = 3.0 Hz, H-1), 3.65 (m, 1H), 3.45 (m, 1H), 3.30 (m, 1H), 1.21 (q, 6H).

C19H20O5
Calculated: C 61.88 H 7.29
Found: C 61.86 H 7.29

Further elution of the column gave 21 (210 mg, 50%) (m.p. 112–114 °C (ethyl acetate/hexane)). 1H NMR (CDCl3) δ 7.38 (m, 5H, Ph), 4.67 (d, J = 3.97 Hz, 1H, H-1), 4.20 (dd, 1H), 4.20 (dd, 1H), 3.90 (m, 1H), 3.70 (m, 2H), 3.55 (dd, 1H), 3.40 (m, 2H), 3.15 (d, 1H), 1.16 (q, 6H).

C19H20O5
Calculated: C 63.83 H 7.80
Found: C 63.46 H 8.07

Benzyl-3-deoxy-β-D-erythro-pentopyranoside [6]—10 (444 mg, 2 mmol) and lithium aluminum hydride were stirred for 2 h in anhydrous ether at room temperature. After drying the sample, the residue was purified by flash chromatography (hexane/ethyl acetate, 3:1, v/v) to give the pure product as a colorless syrup (403 mg, 90%). αL = 118° (methanol), literature [α]20D = 121° (33). 1H NMR (CDCl3) δ 7.38 (m, 5H, Ph), 4.75 (d, J = 5.15 Hz, 1H, H-1), 4.05 (dd, 1H), 3.90 (bm, 1H), 3.70 (m, 1H), 3.65 (m, 1H), 3.15 (s, 2H D2O exchangeable), 2.15 (m, 1H, H-2ax), 1.90 (m, 1H, H-2eq).

C19H20O5
Calculated: C 63.83 H 7.80
Found: C 63.66 H 7.95

Benzyl-3-deoxy-β-D-erythro-pentopyranoside [8]—10 (34) (1.1 g, 5 mmol), 1 g of activated powdered 4Å molecular sieves, KH2PO4 (3 g), and sodium fluoride (3 g) in freshly distilled dry ethanol 1,2-diol (20 ml) were gently refluxed for 3 h under argon. When cool, the solution was poured into a
Benzyl-3,4-anhydro-\(\beta\)-D-xylopyranoside (10)—This was prepared as described previously (34).

Benzyl-3,4-anhydro-\(\alpha\)-L-arabinopyranoside [41]—A mixture of benzyl-2,3-di-O-benzoyl-\(\beta\)-D-xylopyranose (35) (219 mg, 0.5 mmol) and \(p\)-toluenesulfonyl chloride (191 mg, 1.0 mmol) in anhydrous pyridine (5 ml) were stirred for 16 h at room temperature. Ice-water (10 ml) was added and the mixture was extracted with dichloromethane (3 × 15 ml). The organic layer was dried (\(Na_2SO_4\)) and concentrated in vacuo to give a syrup (280 mg). This material was treated without purification with triethylamine/methanol/water (1:2:1, v/v) (10 ml) at 50°C. After 16 h, the mixture was concentrated and partitioned between 5% \(NaHCO_3\) (2 ml) and dichloromethane (7 ml). The aqueous layer was back extracted with dichloromethane (2 × 5 ml) and the combined organic layers were dried (80 mg). Crystallization from isopropyl ether gave pure 11. \(^1\)H NMR (CDCl\(_3\)) \(\delta\) 7.38 (m, 5H, Ph), 4.24 (dd, 1H), 4.15 (d, J = 6.86 Hz, 1H, H-1), 3.90 (dd, 1H), 3.75 (m, 1H), 3.21 (t, 1H, CH\(_2\)), 3.13 (dd, 1H), 2.97 (t, 1H, CH\(_2\)), 2.5 (m, 1H, H-3).

RESULTS AND DISCUSSION

Animal cells initiate the assembly of GAG chains by transferring a xylose residue to specific serine residues within a proteoglycan core protein. However, the requirement for xylosylated core proteins can be bypassed by synthetic \(\beta\)-D-xylosides containing hydrophobic aglycones (e.g., benzyl, \(p\)-nitrophenyl-, naphthyl-, or 4-methylumbelliferyl-), which allow the sugar to enter the cells by passive diffusion (13, 26). Providing the compounds in the growth medium of cultured cells results in synthesis and secretion of GAG chains on the xyloside primers. To examine the role of the hydroxyl groups of the xylose residue for priming GAG chains, we synthesized derivatives with altered hydroxyl functionalities (Fig. 1). The ability of these analogs to initiate GAGs was tested in a CHO cell mutant, pgsA-745, which does not express xylosyltransferase (27, 28). This mutant makes about 1–2% of the GAGs synthesized by wild-type CHO cells, but the addition of as little as 10 \(\mu\)M \(\beta\)-D-xyloside restores GAG synthesis to supranormal levels.

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**FIG. 1.** Xyloside analogs.

| Structure | Formula |
|-----------|---------|
| C\(_{13}\)H\(_{18}\)O\(_{5}\) | Calculated: C 61.42 H 7.09 Found: C 61.85 H 7.30 |

**TABLE 1.**

| Xyloside Analog | Mass (M) | Synthetic Method |
|----------------|---------|-----------------|
| Benzyl-3-0-methyl-\(\beta\)-D-xyloside (18)—A solution of 10 (444 mg, 2 mmol) in methanol (5 ml), isopropyl ether (3 ml), \(Al_2O_3\) (0.5 g) under nitrogen was treated with sodium methoxide (4\(\times\)m in methanol) at gentle reflux for 16 h. After cooling to room temperature, the reaction was diluted with methanol (20 ml), neutralized with \(1\%\) HCl, filtered, and concentrated. The residue was extracted with dichloromethane (10 ml). The combined extracts were dried (\(Na_2SO_4\)) and concentrated in vacuo to afford 17 as a colorless solid (490 mg, 96%). The pure product was recrystallized from isopropyl ether (m.p. 119–120°C). \(\beta\)-D-Xyloside restores GAG synthesis to supranormal levels.

**TABLE 2.**

| Compound | Mass (M) | Synthetic Method |
|----------|---------|-----------------|
| C\(_{13}\)H\(_{18}\)O\(_{5}\) | Calculated: C 61.42 H 7.09 Found: C 61.85 H 7.30 |


\[ \text{Galactosyltransferase I activity.} \]

The rate of galactose transfer to various substrates was measured by incubating each compound at the indicated concentration with \( \sim 100 \mu \text{g} \) of cell extract for 1 h. The products were collected by C18 reversed-phase chromatography and counted ("Experimental Procedures"). Benzyl-\( \beta \)-D-xyloside \( [1] \), 4-keto analog \( [12] \), 3-O-methyl derivative \( [18] \), \( \Delta \), 2-O-methyl derivative \( [15] \), \( \text{3-deoxy-3-fluoro derivative} \) \( [8] \), 4-O-methyl derivative \( [17] \).

(28). The low background level of GAG biosynthesis on proteoglycan core proteins makes this strain ideal for testing the ability of compounds to prime GAGs.

**\( \beta \)-D-Xylosides act as primers because they serve as substrates for galactosyltransferase I (UDP-\( \alpha \)-galactosexylose-\( \beta \)-1-4-galactosyltransferase), generating Gal\( \beta \)1-4Xyl-OR as the product (42). This intermediate acts as a substrate for subsequent sugar additions, giving rise to normal oligosaccharide intermediates and eventually to mature GAG chains. One would predict that modifying the 4-OH of xylosides should produce molecules that cannot initiate GAG biosynthesis since it would no longer serve as a substrate for galactosyltransferase I. As predicted, the 4-deoxygenated analog \( [5] \) did not prime GAGs when fed to cells (Table II). Similarly, introducing an O-methyl group at the 4-position \( [17] \) or changing its stereoisomer from equatorial to axial (benzyl-\( \alpha \)-L-arabinopyranoside, \( [2] \)) also abolished priming. The 4-deoxygenated, 4-O-methyl, and 4-epimeric analogs also were not substrates for galactosyltransferase I in vitro (Fig. 2).

**TABLE II**

| Xyloside analog | 0.01 mM | 0.1 mM | 1 mM |
|-----------------|---------|--------|------|
| Benzyl-\( \beta \)-D-xylopyranoside \( [1] \) | 150 140 130 | 0.2 0.2 0.2 | 0.3 0.3 0.2 |
| 4-Deoxy \( [5] \) | 0.2 0.3 0.3 | 0.3 0.3 0.2 |
| 4-O-Methyl \( [17] \) | 0.3 0.3 0.2 |
| 4-Epimer \( [2] \) | 0.3 0.3 0.2 |
| 2-Deoxy \( [9] \) | 0.3 0.3 0.2 |
| 2-O-Methyl \( [15] \) | 0.6 10 150 |
| 2-O-Isopropyl \( [20] \) | 0.2 0.3 0.5 |
| 2-O-Benzyl \( [16] \) | 0.2 0.3 0.3 |
| 2-Epimer \( [4] \) | 0.2 0.3 0.3 |
| 3-Deoxy \( [6] \) | 0.5 0.4 0.4 |
| 3-O-Methyl \( [18] \) | 0.4 0.4 0.4 |
| 3-Deoxy-3-fluoro \( [8] \) | 0.5 120 160 |
| 3-Deoxy-3-amino \( [7] \) | 0.3 0.4 0.3 |
| 3-O-Isopropyl \( [21] \) | 0.3 0.3 0.4 |
| 3-Epimer \( [3] \) | 0.2 0.3 0.3 |
| 3,3-Anhydro \( [10] \) | 0.2 0.2 0.3 |
| 3,4-Anhydro \( [11] \) | 0.2 0.2 0.3 |

To explore the effects of modifying the hydroxyls away from the site where galactose is added, we tested 2- and 3-position analogs (Fig. 1). Like the 4-deoxygenated derivative, the 2- and 3-deoxygenated analogs did not prime GAG chains (Table II). Similarly, the epimeric compounds (lyxopyranoside and ribopyranoside) also did not act as primers indicating that both oxygen atoms and the proper stereochemistry of the -OH groups (equatorial) were important. In contrast, the 2-O- and 3-O-methylated analogs primed GAGs (Table II), but they were about 10-fold less potent than the parent compound at 0.1 mM and comparable in activity at 1 mM. Both analogs acted as substrates for galactosyltransferase I, but unlike the parent compound they did not exhibit saturability, even up to 40 mM, the limit of their solubility (Fig. 2). To rule out the possibility that a different enzyme used these analogs as substrates at high concentration (e.g., lactose synthase) (28), we assayed galactose transfer activity in an extract prepared from pgsB-761, a CHO mutant deficient in galactosyltransferase I (28). Extracts from the mutant did not form products from the parent compound, benzyl-\( \beta \)-D-xyloside, or the 2-O- or 3-O-methylated derivatives (Table III). These findings indicated that galactosyltransferase I was responsible for the observed galactosylation activity measured in cell extracts and the priming activity in intact cells. The larger O-isopropyl group at the 2- or 3-position or a benzyl group at the 2-position completely abolished priming activity (Table II). These analogs were poor substrates for galactosyltransferase I as well (data not shown).

Fluorine is a bioisostere of a hydroxyl group and therefore its substitution at either the 2- or 3-position might not alter the activity of xylosides. Substituting F for the hydroxyl group at C3 reduced priming at 0.01 mM, but had no effect at higher concentration (Table II). However, an amino group at the 3-position abolished priming, even at high concentration. At neutral pH, this compound would exist mostly as a charged species, which would limit its passage across cell membranes. Anhydro analogs, where the 2- and 3-hydroxyls or the 3- and 4-hydroxyls were tied together, also did not prime GAGs.

Together, these findings indicated that priming by \( \beta \)-D-xylosides depends on all three hydroxyl groups and correlates with the ability of these compounds to serve as substrates for galactosyltransferase I. The hydroxyl groups may form hydrogen bonds with amino acid residues in the active site of the enzyme. Presumably, O-2 and O-3 act as hydrogen bond acceptors since the O-methylated derivatives had activity, whereas the deoxygenated analogs did not. The nearly normal activity of the fluorinated analog is consistent with this idea as well since fluorine forms strong hydrogen bonds. The somewhat lower priming efficacy of the methylated derivatives may be due to steric hindrance. In contrast, the 4-OH group is most likely a nucleophile in the transferase reaction. The failure of the 4-deoxy and 4-O-methyl derivatives to inhibit synthesis on endogenous pro-
teoglycan core proteins or added primers supports the idea that the 4-OH group also plays a role in binding of substrate to enzyme.

Unusual Glycosaminoglycan Primers—Based on the lack of priming activity of xylosides modified at the C-4 hydroxyl group, one would predict that oxidation of this carbon would also abolish activity. Thus, we were surprised to find that the 4-keto derivative (benzyl-β-D-threo-pentopyranos-4-uloseside [12]) primed GAGs almost as well as benzyl-β-D-xyloside (Fig. 3). The compound also had activity as a substrate for galactosyltransferase I (Fig. 2). Thin layer chromatography of the compound in three different solvents gave only one spot in each system. Its 13C NMR spectrum and an infrared absorption peak at 1725 cm\(^{-1}\) indicated the presence of the carbonyl group, and reduction yielded α-xyloside and l-arabinoside quantitatively. These analytical data suggested that the priming activity was not due to contaminating xyloside.

The ability of the 4-keto xyloside analog to prime was not limited to the xylosyltransferase mutant since assay of IldID cells (43) gave comparable results (data not shown). One possible explanation was that the compound underwent enzymatic reduction, possibly by an aldo-keto reductase (44, 45). Another possibility was that priming occurred on a hydrated form of the analog in which water was reversibly added across the carbonyl to form a transient diol. If the equatorial hydroxyl group was available for adding galactose and the presence of an axial hydroxyl group did not interfere with the reaction, then the resulting galactosylated compound might be stable long enough for the next sugar to add, thus initiating the assembly of GAG chains. Although we have not formally tested this possibility, we examined the behavior of benzyl-4-methyl-β-D-xyloside [22], which contains a methyl group instead of the axial hydroxyl group of the 4-keto xyloside analog. It nevertheless primed polysaccharide chains suggesting that galactosyltransferase I tolerates substituents at C-4 axial position.

In summary, we have shown that all of the hydroxyl groups on a xyloside are important for priming GAG chains in animal cells. Their role presumably reflects their ability to participate as hydrogen bond acceptors (2-OH and 3-OH) or donors (4-OH). Recent studies have shown that β-D-xylosides prime unusual oligosaccharides in addition to GAGs. These include Neu5Ac2→3Galβ1→4Xyl-R, GlcClAβ1→4Xyl-R, 3-O-SO\(_2\)GluUAβ1→4Xyl-R, Xylβ1→4Xyl-R, and GalNACα1→4GlcUAβ1→3Galβ1→4Xyl-R (46–50). Priming reactions in general utilize UDP-sugar precursors required by the various bio-synthetic enzymes and divert the formation of oligosaccharide chains from endogenous glycoproteins and glycolipids. Thus, xyloside primers also act as inhibitors of glycoconjugate formation. Some of the analogs described in this report may prime subsets of oligosaccharides with different efficiencies, due to differences in binding to the various transferases.

**Table III**

| Cells          | Benzyl-β-D-xyloside | Benzyl-2-O-methyl-β-D-xyloside | Benzyl-3-O-methyl-β-D-xyloside |
|---------------|---------------------|-------------------------------|-------------------------------|
| Wild-type CHO | 98                  | 15                            | 17                            |
| Mutant pgsB-761 | 5                   | 2                             | 1                             |

**Fig. 3.** Priming of glycosaminoglycans by the unusual β-D-xylosides. Multiple 60-mm diameter tissue culture dishes were seeded with ~5 × 10^5 cells in complete growth medium. One day later, the cells were treated for 6 h with the indicated concentrations of β-D-xyloside in sulfate-deficient medium containing ^35SO\(_4\)\(^{-}\) (10 μCi/ml). The amount of radioactive GAGs was measured in the cells plus medium ("Experimental Procedures"). ◊, benzyl-β-D-xyloside [1]; ○, 4-keto analog [12]; □, 4-methyl, α-analog [22].

Thus, the derivatives may provide a way to target priming reactions in cells and to achieve greater specificity for inhibiting glycosylation.

One of the intriguing findings of this study is that galactosyltransferase I will act on compounds containing an axial substitution of methyl for hydrogen at the C-4 position. This observation suggests that the active site of the enzyme has a certain amount of flexibility, which may make it possible to replace the axial hydrogen with bulkier substituents or reactive groups that might covalently bind to the enzyme and inhibit its activity. Axial substitutions in other positions might be tolerated as well. These observations suggest the possibility of novel inhibitors, which would provide powerful agents for studying the function of GAGs in cells and tissues.

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