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# Note

## Synthesis of β-estradiol β-D-xylopyranosides, primers of heparan sulfate in Chinese hamster ovary cells

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The biosynthesis of heparan sulfate, heparin, dermatan sulfate and chondroitin sulfate in animal cells initiates by the transfer of D-Xyl units from UDP-D-Xyl to specific L-serine residues in proteoglycan core proteins[1]. The xylosylated core protein then serves as an acceptor for successive transfer of two D-Gal units and D-GlcA from the corresponding UDP-sugars to form the core-protein linkage tetrasaccharide, Glc*p*A(β1-3)Gal*p*(β1-3)Gal*p*(β1-4)Xyl*p*(β1 → 3)-Ser. Formation of heparan sulfate and heparin involves the assembly of repeating disaccharides of alternating D-GlcNAc and D-GlcA units on the nonreducing end of the chain. Chondroitin sulfate and dermatan sulfate chains form in an analogous manner, but consist of repeating disaccharide units of D-GalNAc and D-GlcA. After polymer formation, a number of enzymatically catalyzed modifications occur that include addition of sulfate residues and, in the case of dermatan sulfate, heparan sulfate and heparin, epimerization of a portion of D-GlcA residues to L-IdoA.

Animal cells also can use β-D-xylopyranosides as primers for glycosaminoglycan formation[2–4]. These compounds serve as D-Gal acceptors and bypass the need for xylosylated core proteins. Thus, β-D-xylopyranosides stimulate glycosaminoglycan synthesis, even in a cell mutant defective in xylosyltransferase[5]. In normal cells, β-D-xylopyranosides compete with xylosylated core proteins for the enzymes and substrates required for glycosaminoglycan formation, and thus act as competitive inhibitors of proteoglycan synthesis.

In a recent paper[5] we showed that 1,3,5(10)-estratrien-3,17β-diol (β-estradiol) 3-β-D-xylopyranoside (**3**) is a better primer of heparan sulfate than the β-D-xylopyranosides of aglycons such as 4-methylumbelliferone and 4-nitrophenol. We now report the synthesis of β-D-xylopyranosides containing D-Xyl linked to the 3-,

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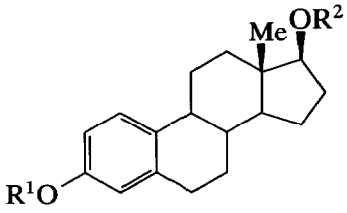


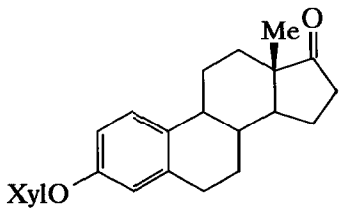


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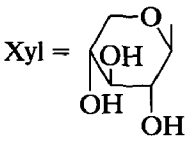
| | $R^1$ | $R^2$ |
|---|---|---|
| 1 | H | H |
| 2 | $Ac_3Xyl$ | H |
| 3 | Xyl | H |
| 4 | Xyl | Xyl |
| 5 | Bz | H |
| 6 | H | Xyl |




$Ac_3Xyl$ = 2,3,4-tri-*O*-acetyl derivative

17-, and 3,17-positions of β-estradiol and the 3-position of estrone (see formula chart). The two estradiol monoxylosides (compounds **3** and **6**) and the dixyloside (compound **4**) all prime heparan sulfate.

Compounds **3**, **4**, **6**, and **7** were synthesized by condensation of 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide[6] with unsubstituted β-estradiol and estrone. 1,3,5(10)-Estratrien-3,17β-diol 3,17-di-β-D-xylopyranoside (**4**) was obtained in three steps from β-estradiol (**1**). Reaction of **1** with tri-*O*-acetyl-α-D-xylopyranosyl bromide in the presence of sodium hydroxide[7] provided exclusively the 3-*O*-substituted derivative **2** in modest yield (45%). Under these reaction conditions, protection of HO-17 was not necessary since the aromatic hydroxyl of β-estradiol is much more reactive than the secondary hydroxyl at C-17. The $^1H$ NMR spectrum of **2** showed signals for three acetate groups at δ 2.02, 2.01, and 2.00, and for H-1′β at δ 5.40 (d, $J_{1',2'}$ 7.3 Hz), and resonances at δ 4.0 and 3.7 attributable to H-5′*eq* and H-5′*ax*, respectively *. The presence of a doublet at δ 4.50 ($J_{OH,17}$ 4.72 Hz) which was eliminated by $D_2O$ exchange indicated that HO-17 of β-estradiol was not substituted.

Reaction of **2** with 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide to produce **4** was promoted by silver silicate[8]. Deprotection of the intermediate under mild conditions ($MeOH–Et_3N–H_2O$) was slow due to low solubility, but gave **4** in 50% yield. Attempted synthesis of **4** by coupling β-estradiol with two equivalents of 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide in the presence of silver silicate followed by deprotection met with little success; a complex mixture of compounds arose. The $^1H$ NMR spectrum of **4**, with its two β-linked D-xylopyranose residues,

* Single (′) and double primed (″) numbers refer to the sugar moieties at the 3 and 17 positions, respectively, of the steroids.

exhibited characteristic doublets at $\delta$ 4.75 ($J_{1',2'}$ 7.2 Hz) for H-1′ and $\delta$ 4.20 ($J_{1'',2''}$ 7.5 Hz) for H-1″. The $^{13}C$ NMR of **4** showed two anomeric carbon signals, at $\delta$ 104.0 (C-1′) and 101.3 (C-1″).

Deacetylation of **2** afforded **3** quantitatively. The $^1H$ NMR spectrum of **3** agreed with the proposed structure. The resonance for H-1′ at $\delta$ 4.75 (d, $J_{1',2'}$ 7.2 Hz) indicated the $\beta$ linkage.

The synthesis of **6** was achieved by starting with commercially available $\beta$-estradiol 3-benzoate (**5**). Reaction of **5** with 2,3,4-tri-*O*-acetyl-$\alpha$-D-xylopyranosyl bromide catalyzed by silver silicate, followed by deprotection, furnished **6** in 74% overall yield. The structure of **6** was evident from the $^1H$ and $^{13}C$ NMR spectra, and from its exact mass determined by high resolution mass spectrometry.

Reaction of estrone with 2,3,4-tri-*O*-acetyl-$\alpha$-D-xylopyranosyl bromide in aqueous sodium hydroxide and acetone, followed by deacetylation, furnished 1,3,5(10)-estratrien-3-ol-17-one 3-$\beta$-D-xylopyranoside (**7**) in 56% yield. The structure of **7** was evident from its $^1H$ and $^{13}$ NMR spectra. The resonance for H-1′ at $\delta$ 4.8 (d, $J_{1',2'}$ 7.2 Hz) indicated the $\beta$ linkage.

Compound **3** proved previously to be a good primer of heparan sulfate[5]. Compounds **3**, **4**, **6**, and **7** also prime heparan sulfate when fed to cells[9]. Preliminary data indicate that the position of the D-Xyl residue on the $\beta$-estradiol skeleton does not change the maximal amount of heparan sulfate made, nor does oxidation of HO-17 to a ketone.

## EXPERIMENTAL

*General methods.*—Uncorrected melting points were determined with a Fisher–Johns melting point apparatus. Optical rotations of 1% solutions in $Me_2SO$ were measured at 20–25°C with a Perkin–Elmer Model 141 polarimeter. $^1H$ NMR (400 MHz) spectra were recorded at 24°C with a Bruker WH-400 spectrometer equipped with an Aspect-300 computer. $^{13}C$ NMR (75.4 MHz) spectra were recorded at 24°C on a Varian Gemini-300 instrument. Chemical shifts ($\delta$) were measured relative to the signal for $Me_4Si$. Coupling constants are given in hertz. High resolution mass spectra were obtained with a Finnigan-MAT 900 system. Reaction products were analyzed by TLC on Silica Gel 60-$F_{254}$ (Merck) with detection by charring with 5% (v/v) $H_2SO_4$ in MeOH. Column chromatography was performed on silica gel 60 Å (Merck, 63–200 $\mu$m) and flash chromatography on silica gel (Merck, 40–63 $\mu$m). Elemental analyses were performed by the Robertson Microlit Laboratories, Inc., (Madison, NJ).

All deacetylations were done under mild conditions using 2:1:1 MeOH–$Et_3N$–$H_2O$ at room temperature.

*1,3,5(10)-Estratrien-3,17β-diol 3-β-D-xylopyranoside* (**3**).—$\beta$-Estradiol (**1**, 1.36 g, 5 mmol) was dissolved in 10 mL of 1:1 (v/v) aq M NaOH–acetone. A solution of 2,3,4-tri-*O*-acetyl-$\alpha$-D-xylopyranosyl bromide[6] (1.70 gm, 5 mmol) in acetone (5 mL) was added to the resulting light yellow solution. The mixture became heteroge-

neous after a few seconds and was stirred in a well closed flask. After 16 h, the mixture was filtered and concentrated. The residue was washed several times with cold water and dried. Examination by TLC (2:3 (v/v) EtOAc–hexane), showed a major product at $R_f$ 0.35 and starting material near the solvent front. Column chromatography (1:20 to 1:1 (v/v), stepwise, EtOAc–hexane) gave pure **2** (1.2 g, 45%); mp 252–253°C (hexane–EtOAc); $[\alpha]_D$ +44°; $^1$H NMR ($Me_2SO$-$d_6$): $\delta$ 7.2 (d, 1 H, aromatic), 6.75 (dd, 1 H, aromatic), 6.65 (d, 1 H, aromatic) 5.40 (d, 1 H, $J_{1',2'}$ 7.3, H-1'), 5.30 (t, 1 H, $J_{3',4'} = J_{3',2'} = 9.0$, H-3'), 5.0 (t, 1 H, H-2'), 4.95 (m, 1 H, H-4'), 4.50 (d, 1 H, $J_{OH,17}$ 4.72, O*H*-17), 4.0 (dd, 1 H, $J_{5'eq,4'}$ 5.3, $J_{5'eq,5'ax}$ 11.3, H-5'*eq*), 3.70 (dd, 1 H, $J_{5'ax,4'}$ 9.62, H-5'*ax*), 3.55 (m, 1 H, H-17), 3.32 (d, 1 H, *J* 9.6, H-8), 2.3 (m, 1 H), 2.1 (m, 1 H); 2.02, 2.01, and 2.00 (3 s, C*H*$_3$CO), 1.95–1.0 (m, C*H*$_2$), and 0.65 (s, C*H*$_3$); $^{13}$C NMR ($Me_2SO$-$d_6$): $\delta$ 169.4 (2 *C*=O), 169.0 (*C*=O), 154.1 (C-3), 137.7, 134.7, 126.2, 116.5, 113.9 (5 C, aromatic), 97.9 (C-1'), 79.9, 71.0, 70.4, 68.3, 61.4, 49.4, 43.5, 42.7, 36.5, 29.8, 29.0, 26.6, 25.9, 22.7, 20.3, and 11.14 (*C*H$_3$); HRMS (FAB$^+$): *m/z* calcd for $C_{29}H_{39}O_9$ (M + 1) 531.259, found 531.257. Anal. Calcd for $C_{29}H_{38}O_9$: C, 65.66; H, 7.17. Found: C, 65.43; H, 7.26.

A portion of the material was deacetylated to furnish **3**; mp 239–240°C (EtOH-$H_2O$); $[\alpha]_D$ +64°; $^1$H NMR ($Me_2SO$-$d_6$–$D_2O$): $\delta$ 7.2 (d, 1 H, aromatic), 6.75 (dd, 1 H, aromatic), 6.65 (d, 1 H, aromatic), 4.75 (d, 1 H, $J_{1',2'}$ 7.2, H-1'), 3.75 (dd, 1 H, H-5'*eq*), 3.5 (m, 6 H), 3.35 (m, 1 H), 3.20 (m, 1 H), 2.3–1.0 (m, C*H*$_2$), and 0.65 (s, C*H*$_3$); $^{13}$C NMR ($Me_2SO$-$d_6$): $\delta$ 154.8 (C-3), 137.0, 130.2, 126.0, 114.8, 112.6, (5 C, aromatic), 104.3, (C-1'), 87.2, 76.7, 73.4, 69.5, 65.6, 49.2, 43.3, 42.9, 38.4, 36.9, 29.0, 28.5, 26.7, 25.9, 25.9, 22.5, and 11.3 ($CH_3$); HRMS (FAB$^+$): *m/z* calcd for $C_{23}H_{33}O_6$ (M + 1) 405.228, found 405.228. Anal. Calcd for $C_{23}H_{32}O_6$: C, 68.32; H, 7.92. Found: C, 68.27; H, 8.10.

*1,3,5(10)-Estratrien-3,17β-diol 3,17-di-β-*D*-xylopyranoside* (**4**).—1,3,5(10)-Estratrien-3,17β-diol 3-(tri-*O*-acetyl-D-xylopyranoside) (**2**, 265 mg, 0.5 mmol) was stirred in dry $CH_2Cl_2$ (10 mL) together with freshly prepared dry silver silicate[8] (1.5 g) and activated powdered 4A molecular sieves (1 g) for 30 min in the dark. The mixture was cooled to 10°C, and 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide (255 mg, 0.75 mmol) in dry $CH_2Cl_2$ (5 mL) was added. Stirring was continued at room temperature in the dark. After 1 h, the mixture was diluted with $CH_2Cl_2$ (15 mL) and filtered through Celite. The filtrate was washed with water, dried ($MgSO_4$), and concentrated. The resulting colorless syrup was treated with deacetylating solution (20 mL) for 60 h with stirring. Concentration of the reaction mixture yielded a glassy material that was dried in vacuo and subjected to flash-column chromatography on silica gel (EtOAc to MeOH, stepwise) to give 133 mg (50%) of the title compound as an amorphous solid; $[\alpha]_D$ +9°; $^1$H NMR ($Me_2SO$-$d_6$): $\delta$ 7.2 (d, 1 H, aromatic), 6.75 (dd, 1 H, aromatic), 6.65 (d, 1 H, aromatic), 4.75 (d, 1 H, $J_{1',2'}$ 7.2, H-1'), 4.2 (d, 1 H, $J_{1'',2''}$ 7.5, H-1''), 3.7 (br m, 3 H), 2.3–1.0 (m, C*H*$_2$), and 0.8 (s, C*H*$_3$); $^{13}$C NMR ($Me_2SO$-$d_6$): $\delta$ 154.9 (C-3), 137.2, 133.6, 126.0, 116.4, 113.9, (5 C, aromatic), 104.0 (C-1'), 101.3 (C-1''), 87, 76.7,

76.4, 73.4, 73.0, 69.6, 69.3, 65.6, 49.2, 43.3, 42.9, 38.2, 36.8, 29.1, 28.5, 26.6, 25.8, 22.5, and 11.3 ($CH_3$); HRMS ($FAB^+$): $m/z$ calcd for $C_{28}H_{41}O_{10}$ (M + 1) 537.270, found 537.268. Anal. Calcd for $C_{28}H_{40}O_{10} \cdot 1.5H_2O$: C, 59.67; H, 7.69. Found: C, 59.96; H, 7.25.

*1,3,5(10)-Estratrien-3,17β-diol 17-β-D-xylopyranoside* (**6**).—β-Estradiol 3-benzoate (**5**) (Aldrich Chemical Co. Inc., Milwaukee, WI) (377 mg, 1.0 mmol) was stirred in dry $CH_2Cl_2$ (15 mL) with freshly prepared, dry silver silicate (1.5 g) and activated powdered 4A molecular sieves (1.5 g) for 20 min in the dark. The mixture was cooled to 10°C and tri-*O*-acetyl-α-D-xylopyranosyl bromide (340 mg, 1 mmol) in dry $CH_2Cl$ (5 mL) was added. Stirring was continued for 10 min, then the mixture was diluted with $CH_2Cl_2$ (20 mL) and filtered through Celite. The colorless filtrate was washed with water, dried ($MgSO_4$), and concentrated. The resulting syrup was deacetylated (24 h), reconcentrated, and partitioned between EtOAc and water. The organic layer was successively washed with water and 10% $NaHCO_3$, and concentrated. Column chromatography (hexane to EtOAc, stepwise) gave pure **6** (300 mg, 74%); mp 201–202°C (EtOH–water); $[\alpha]_D$ −1°; $^1H$ NMR ($Me_2SO$-$d_6$): δ 9.0 (s, 1 H, $D_2O$ exchangeable, 3-O*H*), 7.1 (d, 1 H, aromatic), 6.55 (dd, 1 H, aromatic), 6.40 (d, 1 H, aromatic), 4.95 (d, 3 H, $D_2O$ exchangeable, O*H*), 4.15 (d, 1 H, $J_{1''2''}$ 7.5, H-1''), 3.7 (m, 2 H), 3.35 (m, 1 H), 3.25 (m, 1 H), and 3.15–2.90 (m, 5 H); $^{13}C$ NMR ($Me_2SO$-$d_6$): δ 155.0 (C-3), 137.3, 133.7, 126.0, 116.4, 113.9, 101.4, (C-1''), 80.0, 76.4, 73.0, 60.3, 65.6, 49.5, 43.5, 42.7, 38.4, 36.5, 29.4, 29.1, 26.8, 26.0, 22.7, and 11.2 ($CH_3$); HRMS ($FAB^+$): $m/z$ calcd for $C_{23}H_{33}O_6$ (M + 1) 405.229, found 405.228. Anal. Calcd for $C_{23}H_{32}O_6$: C, 68.32; H, 7.92. Found: C, 68.32; H, 7.96.

*1,3,5(10)-Estratrien-3-ol-17-one 3-β-D-xylopyranoside* (**7**).—Estrone (540 mg, 2 mmol) was dissolved in 4:5 (v/v) M NaOH–acetone (4.5 mL). To the resulting solution was added a solution of 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide (0.7 g, 2 mmol) in acetone (2 mL). The mixture was stirred in a well closed flask for 6 h, then concentrated and filtered. The precipitate was washed with cold water and dried. Deacetylation (12 h) followed by flash-column chromatography on silica gel (hexane to EtOAc, stepwise) gave 450 mg (56%) of the title compound; mp 241–242°C; lit.[10] mp 230–240°C; $[\alpha]_D$ +105°; lit.[10] $[\alpha]_D$ +102° (*c* 0.355, MeOH); $^1H$ NMR ($Me_2SO$-$d_6$): δ 7.2 (d, 1 H, aromatic), 6.75 (dd, 1 H, aromatic), 6.65 (d, 1 H, aromatic), 5.3, 5.15, 5.05 (all d, 3 H, O*H*), 4.8 (d, 1 H, $J_{1',2'}$ 7.2, H-1'), 3.75 (dd, 1 H, H-5'*eq*), 3.35 (m, 1 H), 3.35 (m, 1 H), and 0.8 (s, $CH_3$); $^{13}C$ NMR ($Me_2SO$-$d_6$): δ 219.6 (*C*=O), 155.1 (C-3), 137.4, 133.2, 126.1, 116.5, 114.0 (5 C, aromatic), 101.31 (C-1'), 76.4 (C-3'), 73.1 (C-2'), 69.3 (C-4'), 65.65 (C-5'), 49.6, 47.3, 43.4, 37.7, 35.3, 31.3, 29.1, 26.0, 25.4, 21.1, and 13.5 ($CH_3$).

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## REFERENCES

1 L. Roden, in W.J. Lennarz (Ed.), *The Biochemistry of Glycoproteins and Proteoglycans*, Plenum Press, New York, 1980, pp 267–371.
2 M. Okayama, K. Kimata, and S. Suzuki, *J. Biochem. (Tokyo)*, 74 (1973) 1069–1073.
3 H.C. Robinson, M.J. Brett, P.J. Tralaggan, D.A. Lowther, and M. Okayama, *Biochem. J.*, 148 (1975) 25–34.
4 J.A. Robinson and H.C. Robinson, *Biochem J.*, 194 (1981) 839–846.
5 F.N. Lugemwa and J.D. Esko, *J. Biol. Chem.*, 226 (1991) 6674–6677.
6 J.K. Dale, *J. Am. Chem. Soc.*, 37 (1915) 2745–2747; M. Bárczai-Martos and F. Korosy, *Nature (London)*, 165 (1950) 369.
7 M. Seidman and K.P. Link, *J. Am. Chem. Soc.*, 72 (1950) 4324.
8 H. Paulsen and O. Lockhoff, *Chem. Ber.*, 114 (1981) 3102–3114.
9 F.N. Lugemwa and J.D. Esko, unpublished results.
10 D.S. Layne, R.S. Labow, P. Alenka, and D.G. Williamson, *Biochemistry*, 15 (1976) 1268–1270.