Supplementary Material

Attenuation of Tumor Growth by Formation of Antiproliferative Glycosaminoglycans Correlates with Low Acetylation of Histone H3

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Running Title: Xyloside inhibition of tumor growth
Figure S1. Structure of a proteoglycan (PG) (1). PGs are composed of glycosaminoglycans linked to a core protein by the linker tetrasaccharide.
Figure S2. Synthesis of labeled xylosides (See also Supplemental Methods). A, In(OTf)$_3$, ICl, MeCN, MS3Å. B, NaOMe, MeOH. C, $^{3}$H$_2$, Pd/C.
Supplemental Methods

Chemistry

2-Naphthyl-2,3,4-tetra-O-acetyl-\(\beta\)-D-xylopyranoside (1) (2), and 2-(6-Acetoxy-naphthyl)-2,3,4-tetra-O-acetyl-\(\beta\)-D-xylopyranoside (2) (3), were iodinated using In(OTf)\(_3\)/ICl in acetonitrile with molecular sieves present to give compounds 3-4 in excellent yields (4). Compounds 3-4 were then deacetylated using standard Zemplén conditions to give compounds 5-6. Hydrodehalogenation (Pd/C in methanol with triethylamine) were tested using H\(_2\) and then repeated using tritium gas to give the radioactively labeled compounds 7-8 (Active Biotech AB, Scheelevägen 22, P.O. Box 724, SE-220 07 Lund, Sweden).\(^1\)H-NMR spectra were recorded with a Bruker Avance II 400 MHz. \(^1\)H-NMR spectra were assigned using 2D-methods (COSY). Chemical shifts are given in ppm downfield from the signal for Me\(_4\)Si, with reference to residual CHCl\(_3\), MeOH or DMSO. Reactions were monitored by TLC using alumina plates coated with silica gel and visualized using either UV light or by charring with \(\text{para}\)-anisaldehyde. Preparative chromatography was performed with silica gel (35-70 μm, 60 Å). CH\(_2\)Cl\(_2\) was dried on Al\(_2\)O\(_3\), MeCN was distilled from CaH. Known and commercially available compounds were in agreement with previously published data (e.g. NMR). Radioactive labeling was performed at Active Biotech AB, Scheelevägen 22, P.O. Box 724, SE-220 07 Lund, Sweden.

2-Naphthyl 2,3,4-tetra-\(O\)-acetyl-\(\beta\)-D-xylopyranoside (1): [\(\alpha\)]\(_{D}\)\(^{22}\) -27 (c 1.0, CHCl\(_3\)). \(^1\)H-NMR (CDCl\(_3\)): \(\delta\) 7.73-7.80 (m, 3H, H-4’, H-5’, H-8’), 7.36-7.48 (m, 3H, H-1’, H-6’, H-7’), 7.18 (dd, 1H, \(J\) 8.9, 2.5 Hz, H-3’), 5.34 (d, 1H, \(J\) 5.7 Hz, H-1), 5.22-5.29 (m, 2H, H-2, H-3), 5.05 (dt, 1H, \(J\) 7.4, 4.6 Hz, H-4), 4.28 (dd, 1H, \(J\) 12.1, 4.6 Hz, H-5), 3.60 (dd, 1H, \(J\) 12.1, 7.4 Hz, H-5), 2.11, 2.10, 2.10 (s, 3H each, OAc). \(^13\)C-NMR (CDCl\(_3\)): \(\delta\) 170.1, 170.0, 169.6, 154.5,
2-(1-Iodo-naphthyl) 2,3,4-tetra-O-acetyl-β-D-xylopyranoside (3): Compound 1 (100 mg, 0.25 mmol) and In(OTf)$_3$ (73 mg, 0.13 mmol) and molecular sieves 3 Å (1 g) were dissolved in MeCN (5 mL). ICl (1 M in CH$_2$Cl$_2$, 0.280 mL, 0.28 mmol) was added dropwise and the mixture was stirred for 60 min at room temperature. Triethylamine (1.5 mL) followed by Na$_2$S$_2$O$_3$ (1.5 g) were added and the mixture was concentrated and chromatographed (SiO$_2$, 2:1→1:1 heptane/EtOAc) to give 3 (121 mg, 92%). [α]$_D^{24}$ -86 (c 0.34, CHCl$_3$). $^1$H-NMR (CDCl$_3$): δ 8.16 (d, 1H, $J$ 8.6 Hz, H-8$'$), 7.81 (d, 1H, $J$ 8.9 Hz, H-4$'$), 7.76 (d, 1H, $J$ 8.0 Hz, H-5$'$), 7.56 (ddd, 1H, $J$ 8.6, 6.9, 1.3 Hz, H-7$'$), 7.44 (ddd, 1H, $J$ 8.1, 6.9, 1.1 Hz, H-6$'$), 7.34 (d, 1H, $J$ 9.0, H-3$'$), 5.33-5.37 (m, 2H, H-1, H-2), 5.25 (t, 1H, $J$ 7.1 Hz, H-3), 5.05 (dt, 1H, $J$ 6.9 Hz, 4.4 Hz, H-4), 4.36 (dd, 1H, $J$ 12.2, 4.3 Hz, H-5), 3.62 (dd, 1H, $J$ 12.2, 6.9 Hz, H-5), 2.15, 2.14, 2.11 (s, 3H each, OAc). $^{13}$C-NMR (CDCl$_3$): δ 170.1, 170.0, 169.5, 154.5, 135.6, 131.9, 131.0, 130.6, 128.4, 128.3, 125.5, 116.6, 99.6, 90.0, 70.3, 69.8, 68.4, 61.9, 21.3, 21.1, 21.0. HRMS calcd. for C$_{21}$H$_{22}$O$_8$Na (M+Na): 425.1212; found: 425.1198.

2-(1-Iodo-naphthyl) β-D-xylopyranoside (5): Compound 3 (121 mg, 0.23 mmol) was dissolved in methanolic NaOMe (0.05 M, 10 mL) and stirred for 60 min. The mixture was acidified with HOAc and concentrated. The residue was chromatographed (SiO$_2$, 10:1 CH$_2$Cl$_2$/MeOH) to give 7 (89 mg, 96%). [α]$_D^{24}$ -34 (c 0.28, MeOH). $^1$H-NMR (MeOH-$d_4$): δ 8.14 (dd, 1H, $J$ 8.6, 0.9 Hz, H-8$'$), 7.87 (d, 1H, $J$ 9.0 Hz, H-4$'$), 7.80 (dt, 1H, $J$ 8.1, 0.6 Hz, H-5$'$), 7.55 (ddd, 1H, $J$ 8.6, 6.7, 1.3 Hz, H-7$'$), 7.43 (ddd, 1H, $J$ 8.1, 6.9, 1.1 Hz, H-6$'$), 7.40 (d, 1H, $J$ 9.0 Hz, H-3$'$), 5.14 (d, 1H, $J$ 7.2 Hz, H-1), 3.96 (dd, 1H, $J$ 11.4, 5.2 Hz, H-5), 3.60-3.67 (m, 2H, H-2, H-4), 3.48 (t, 1H, $J$ 8.8 Hz, H-3), 3.39 (dd, 1H, $J$ 11.4, 9.7 Hz, H-5). $^{13}$C-NMR (MeOH-$d_4$): δ 156.3, 136.8, 132.5, 132.1, 131.4, 129.4, 129.0, 126.1, 117.5, 103.7, 90.0, 77.7, 74.8, 71.0, 67.1. HRMS calcd. for C$_{15}$H$_{15}$O$_5$INa (M+Na): 424.9862; found: 424.9873.
2-(6-Acetoxy-1-iodo-naphthyl)-2,3,4-tetra-O-acetyl-β-D-xylopyranoside (4): Compound 2 (98 mg, 0.21 mmol) and In(O Tf)₃ (63 mg, 0.11 mmol) and molecular sieves 3 Å (1 g) were dissolved in MeCN (5 mL). ICl (1 M in CH₂Cl₂, 0.240 mL, 0.24 mmol) was added dropwise and the mixture was stirred for 60 min at room temperature. Triethylamine (1.5 mL) followed by Na₂S₂O₃ (1.5 g) were added and the mixture was concentrated and chromatographed (SiO₂, 1:1 heptane/EtOAc) to give 4 (89 mg, 72%). [α]D²⁴⁻⁷₂ (c 0.38, CHCl₃). ¹H-NMR (CDCl₃): δ 8.19 (d, 1H, J9.2 Hz, H-8''), 7.75 (d, 1H, J8.9 Hz, H-4''), 7.52 (d, 1H, J2.3 Hz, H-5''), 7.33 (d, 1H, J9.0 Hz, H-3''), 7.30 (dd, 1H, J9.2, 2.4 Hz, H-7''), 5.31-5.37 (m, 2H, H-1, H-2), 5.25 (dd, 1H, J7.1, 7.0 Hz, H-3), 5.02-5.07 (m, 1H, H-4), 4.35 (dd, 1H, J12.2, 4.3 Hz, H-5), 3.61 (dd, 1H, J7.1, 6.8 Hz, H-5), 2.36 (s, 3H, Ar-OAc), 2.14, 2.14, 2.11 (s, 3H each, OAc). ¹³C-NMR (CDCl₃): δ 170.1, 170.0, 169.6, 169.5, 154.5, 148.2, 133.6, 130.9, 130.2, 123.5, 118.8, 117.3, 99.6, 89.8, 70.2, 69.8, 68.4, 61.9, 21.34, 21.27, 21.1, 21.0. HRMS calcd. for C₂₃H₂₃O₁₀INa (M+Na): 609.0234; found: 609.0224.

2-(6-Hydroxy-1-iodo-naphthyl)-β-D-xylopyranoside (6): Compound 4 (85 mg, 0.14 mmol) was dissolved in methanolic NaOMe (0.05 M, 5 mL) and stirred for 60 min. The mixture was acidified with HOAc and concentrated. The residue was chromatographed (SiO₂, 10:1 CH₂Cl₂/MeOH) to give 6 (48 mg, 82%). [α]D²⁴⁻₃₈ (c 0.26, MeOH). ¹H-NMR (MeOH-d₄): δ 8.00 (d, 1H, J9.1 Hz, H-8''), 7.64 (d, 1H, J8.9 Hz, H-4''), 7.29 (d, 1H, J9.0 Hz, H-3''), 7.12 (dd, 1H, J9.1, 2.5 Hz, H-7''), 7.08 (d, 1H, J2.5 Hz, H-5''), 5.04 (d, 1H, J7.3 Hz, H-1), 3.95 (dd, 1H, J11.5, 5.3 Hz, H-5), 3.59-3.65 (m, 2H, H-2, H-4), 3.48 (dd, 1H, J8.8, 8.7 Hz, H-3), 3.35 (dd, 1H, J11.5, 9.7 Hz, H-5). ¹³C-NMR (MeOH-d₄): δ 156.1, 154.2, 134.1, 133.3, 131.3, 129.5, 121.1, 118.3, 110.3, 104.1, 90.4, 77.6, 74.8, 71.0, 67.0. HRMS calcd. for C₁₅H₁₅O₆INa (M+Na): 440.9811; found: 440.9802.
Supplemental References