Supporting Information

LC-MS/MS characterization of xyloside-primed glycosaminoglycans with cytotoxic properties reveals structural diversity and novel glycan modifications

Andrea Persson, Alejandro Gomez Toledo, Egor Vorontsov, Waqas Nasir, Daniel Willén, Fredrik Noborn, Ulf Ellervik, Katrin Mani, Jonas Nilsson, and Göran Larson

Tables S1-S4

Figures S1 and S2

^{1}H-NMR (2-Naphthyl-1,3,4,5,6,7,8-d_7) β-D-xylopyranoside

^{13}C-NMR (2-Naphthyl-1,3,4,5,6,7,8-d_7) β-D-xylopyranoside

Table S1. *Proportions of CS/DS and HS of XylNap- and XylNap-d_7-primed GAGs from HCC70 cells and CCD-1095Sk cells.* Proportions of GlcUA in CS/DS (CS/DS_{GlcUA}), IdoUA in CS/DS as alternating or single IdoUA-containing disaccharide units ($CS/DS_{IdoUA\_Alt/single}$), IdoUA in CS/DS in blocks (CS/DS_{IdoUA_ChB}), and HS of XylNap- and XylNap-d_7-primed GAGs from HCC70 cells and CCD-1095Sk cells after chondroitinase ABC, chondroitinase AC-I and -II, chondroitinase B, and heparinase degradation, respectively. The data are the means of two individual experiments.

GAGs	CS/DS_{GlcUA} (%)*	$CS/DS_{IdoUA\_Alt/single}$ (%)*	CS/DS_{IdoUA_ChB} (%)*	HS (%)
XylNap-primed GAGs HCC70	63 (61)	0.69 (2.5)	4.9 (5.1)	31
XylNap-d_7-primed GAGs HCC70	60	2.5 (2.9)	4.1	33
XylNap-primed GAGs CCD-1095Sk	57	15	27	1.8
XylNap-d_7-primed GAGs CCD-1095Sk	57	15	26	2.3

* The values in brackets are shown in Fig 3A; these were calculated using the values after chondroitinase ABC degradation in those cases where the proportions after chondroitinase AC-I and -II degradation exceeded the proportions after chondroitinase ABC degradation (see Table S2).

Table S2. *Disaccharide composition of XylNap- and XylNap-d_7-primed CS/DS from HCC70 cells and CCD-1095Sk cells.* CS/DS disaccharide composition (% of total chondroitinase ABC-degraded GAGs) of XylNap- and XylNap-d_7-primed GAGs from HCC70 cells and CCD-1095Sk cells after chondroitinase ABC/chondroitinase AC-I and -II/chondroitinase B degradation. The data are the means of two individual experiments.

GAGs	ΔUA,2S-GalNAc,4S (%)	ΔUA-GalNAc,4S,6S (%)	ΔUA,2S-GalNAc,6S (%)	ΔUA-GalNAc,4S (%)	ΔUA-GalNAc,6S (%)	ΔUA-GalNAc (%)
XylNap-primed GAGs HCC70	-	5.1/2.3/1.3	0.31/-/-	30/18/6.0	63/70*/-	1.6/2.5*/-
XylNap-d_7-primed GAGs HCC70	-	5.3/2.4/1.3	0.32/-/-	28/16/4.8	66/66/-	0.68/1.4*/-
XylNap-primed GAGs CCD-1095Sk	3.9/-/3.7	0.92/0.24/0.27	5.2/< 0.1/-	59/16/25	30/26/-	0.98/1.7*/-
XylNap-d_7-primed GAGs CCD-1095Sk	3.9/-/3.5	1.1/0.22/0.27	5.3/< 0.1/-	59/16/24	30/26/-	0.94/1.8*/-

*Differences in disaccharide proportion between chondroitinase ABC and chondroitinase AC-I and –II degradation; in Fig. 3B and C, these values are represented by the values after chondroitinase ABC degradation.

Table S3. *Proportions of CS/DS and HS of XylNapOH-primed GAGs from HCC70 cells and CCD-1095Sk cells before and after sialyltransferase inhibition.* The data are the means of three individual experiments.

GAGs	CS/DS (%)	HS (%)
XylNapOH-primed GAGs HCC70	84	16
XylNapOH-primed GAGs -Neu5Ac HCC70	75	25
XylNapOH-primed GAGs CCD-1095Sk	97	2.8
XylNapOH-primed GAGs -Neu5Ac CCD-1095Sk	96	4.5

Table S4. *Disaccharide composition of XylNapOH-primed CS/DS from HCC70 cells and CCD-1095Sk cells before and after sialyltransferase inhibition.* CS/DS disaccharide composition (% of total chondroitinase ABC-degraded GAGs) of XylNapOH-primed GAGs from HCC70 cells and CCD-1095Sk cells before and after sialyltransferase inhibition, after chondroitinase ABC degradation. The data are the means of three individual experiments.

GAGs	ΔUA,2S-GalNAc,4S (%)	ΔUA-GalNAc,4S,6S (%)	ΔUA,2S-GalNAc,6S (%)	ΔUA-GalNAc,4S (%)	ΔUA-GalNAc,6S (%)	ΔUA-GalNAc (%)
XylNapOH-primed GAGs HCC70	-	3.2	0.28	21	75	0.88
XylNapOH-primed GAGs -Neu5Ac HCC70	-	4.1	0.40	20	75	0.81
XylNapOH-primed GAGs CCD-1095Sk	3.0	0.93	3.9	62	29	1.6
XylNapOH-primed GAGs -Neu5Ac CCD-1095Sk	2.3	0.94	3.8	58	33	1.9

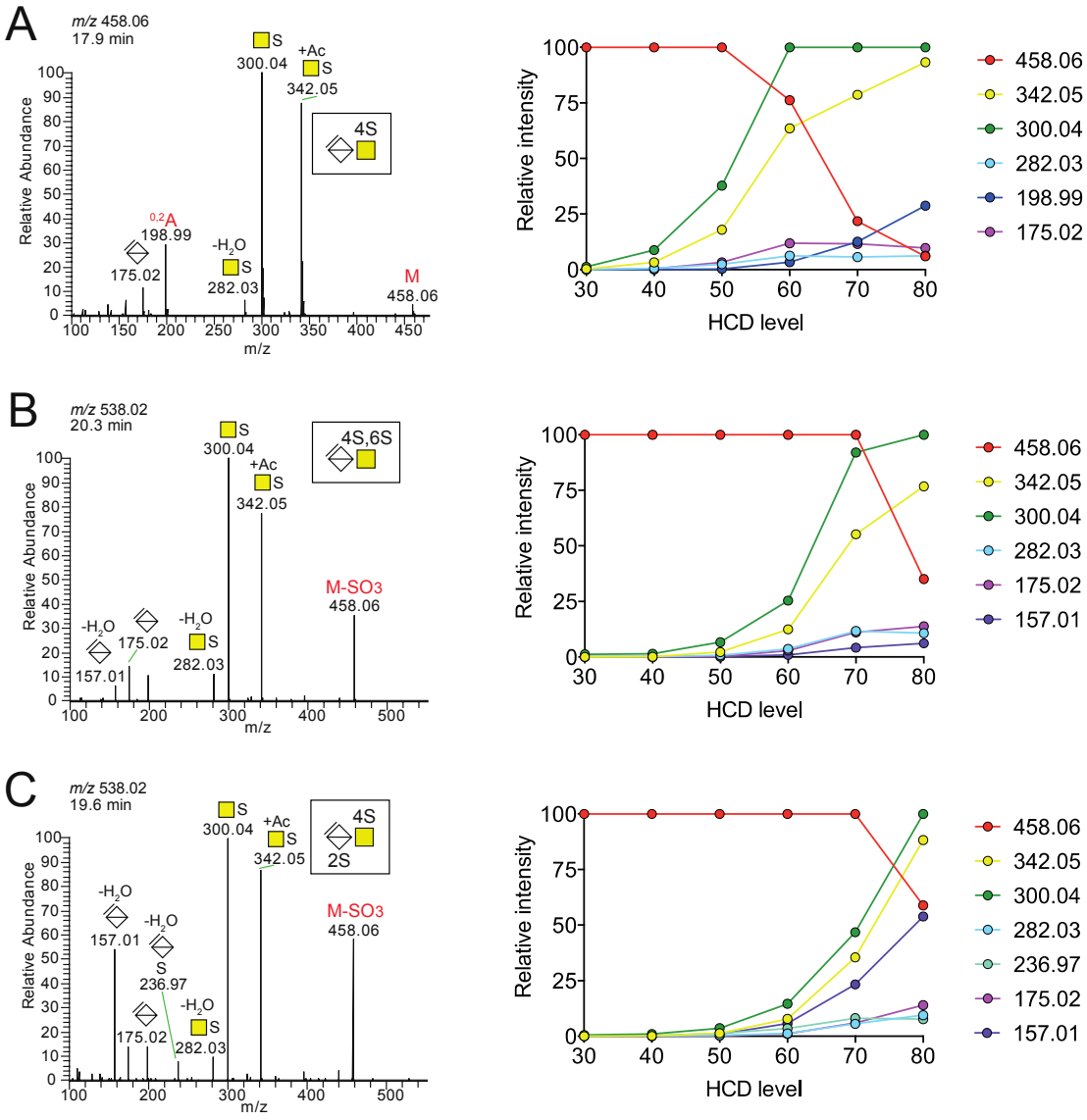


Figure S1. *MS/MS fragmentation of disaccharide standards recorded at different HCD levels.* ΔUA-GalNAc,4S (A), ΔUA-GalNAc,4S,6S (B), and ΔUA,2S-GalNAc,4S (C) were subjected to HCD fragmentation at normalized collision energies of 30-80% at 10% intervals. The HCD-MS^2 spectra at the 80% level are displayed in the left panel (also displayed in Fig. 4B, D, and E, respectively) and the fragment ion intensity profiles are displayed in the right panel.

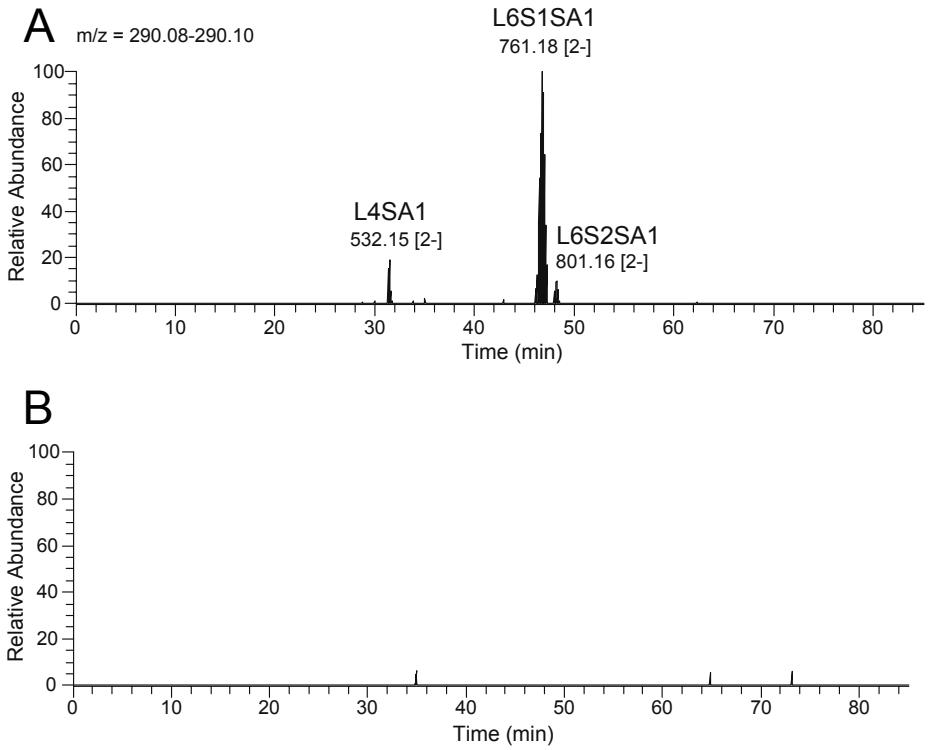
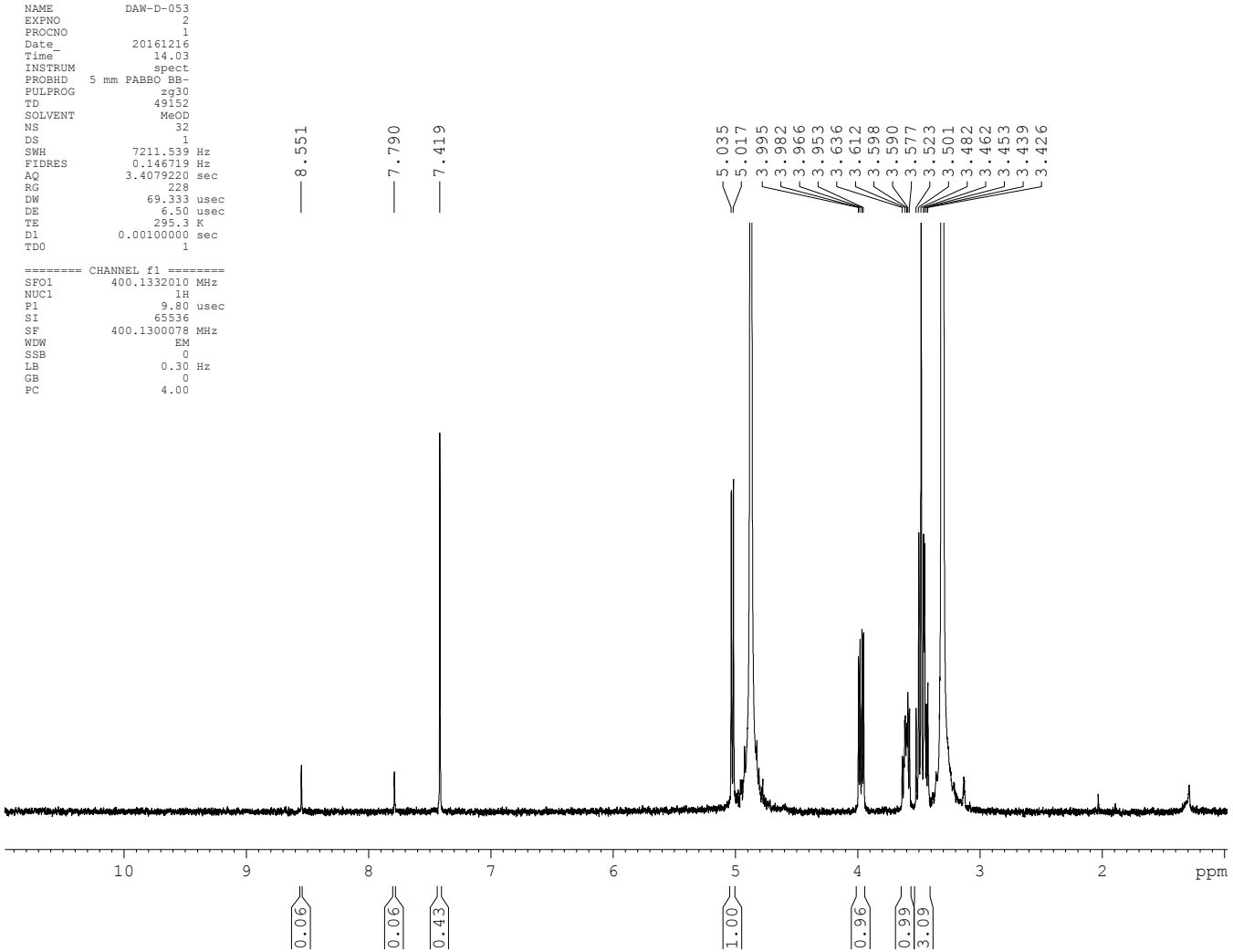


Figure S2. *Inhibition of Neu5Ac modification in the linkage region*. Presence of Neu5Ac in the linkage region of XylNapOH-primed GAGs from HCC70 cells before (A) and after (B) treatment with sialyltransferase inhibitor. Precursor ions for L4SA1 and L6S2SA1 were observed also in the XylNap-primed samples after chondroitinase ABC degradation; however, they did not fragment and were therefore omitted in Fig. 5.

^{1}H-NMR (2-Naphthyl-1,3,4,5,6,7,8-d_7) β-D-xylopyranoside



^{13}C-NMR (2-Naphthyl-1,3,4,5,6,7,8-d_7) β-D-xylopyranoside