

Supplementary Material

S1. Independent confirmations of the linkage arrangements

Sequential assignment of the central GlcA and GlcNAc rings within K5 oligosaccharides was achieved using the progressive chemical shift changes across the series from K5₂ to K5₆ as well as clear nuclear Overhauser enhancements (NOEs) across the glycosidic linkages (e.g., in the NOESY spectrum of K5₆, three inter-residue NOEs from protons in ring III to the H-1 proton of ring IV are clearly visible; see Fig. 4). Acetamido sidechain H-Me chemical shifts were assigned using NOEs to the corresponding GlcNAc N-H protons.

¹³C chemical shift changes induced by transfer of K5₄ from 5% D₂O to 100 % D₂O were also measured (see Table 1). These deuterium isotope-effects, resulting from the exchange of hydroxyl hydrogen atoms for deuterium, are considerably greater at (unsaturated) ¹³C-atoms bonded to hydroxyl oxygen atoms (-0.106 to -0.190ppm) compared with ¹³C-atoms bonded to glycosidic linkage oxygen atoms (+0.012 to -0.050 ppm).

One-bond ¹J_{H,C} and ¹J_{H,N} coupling constants within K5₆ were directly measured from [¹H, ¹³C]-HSQC and [¹H, ¹⁵N]-HSQC spectra recorded with heteronuclear broadband decoupling disabled during acquisition (see Table S1). All ¹J_{H,C} values are typical of saccharide rings and the ¹J_{H-1,C-1} values, which are characteristic for α- and β-geometries, further corroborate the linkage arrangements [Gettins and Horne, 1992, Yates *et al.*, 2000]. The ¹J_{H,N} values are typical of amide groups.

S2 α : β anomer ratio in K5 oligosaccharides

The α : β anomer ratio for K5 oligosaccharides was determined to be 62 ± 1 % α -anomer at pH 6.0 and 24.4°C (irrespective of oligosaccharide length) by comparison of I_α and I_β [H-Me¹,C-Me¹] peak volumes in the [¹H, ¹³C]-HSQC spectra, i.e., the ratio is barely different from that of GlcNAc monosaccharide (60 ± 1 % [Blundell *et al.*, 2006]). Differences in chemical shift between α - and β -anomer forms of K5 oligosaccharides were seen throughout the adjacent ring (i.e., ring II), but no further (as is typically the case).

S3. Conformation of the Δ 4,5-unsaturated GlcA ring

The conformation of the Δ 4,5-unsaturated GlcA ring in solution is expected to be a ¹H₂ or ²H₁ half-chair form, or a mixture of these forms [Mikhailov *et al.*, 1996, Mikhailov *et al.*, 1997]. The ¹H₂ form is characterised by near-equal ³J_{1,2} and ³J_{2,3} ¹H coupling constants of about 4 Hz, whereas the ²H₁ form shows ³J_{1,2} and ³J_{2,3} coupling constants greater than 6 Hz. Since the Δ 4,5-GlcA ring in K5₄ (ring IV) has ³J_{1,2} and ³J_{H2,H3} coupling constants of 6.4 and 5.7 Hz (see Table 2), respectively, it is evident that the unsaturated GlcA ring predominantly adopts the ²H₁ conformation in solution. This is in contrast to reports of the conformation of a 2-O-sulphated Δ 4,5-GlcA ring in heparin tetra- and hexasaccharides, which were predominantly in the ¹H₂ conformations [Mikhailov *et al.*, 1996, Mikhailov *et al.*, 1997]; this difference in ring geometry is presumably due to the bulky 2-O-sulphate group being better sterically accommodated in the ¹H₂ conformation.

S4. Amide nitrogen temperature coefficients

The amide nitrogen temperature coefficients are also reported here ($\Delta\delta_N/\Delta T$, Table 4), but currently these cannot be related to a simple physical model [Blundell and Almond, 2007].

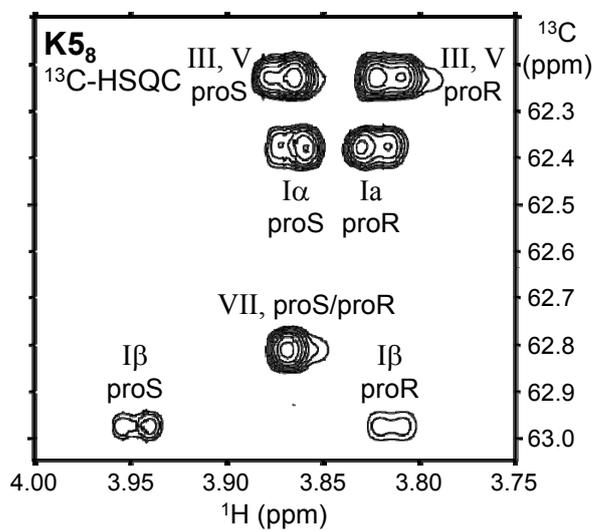
Supplementary Table 1. One-bond coupling constants in K5₆

¹ J _{H,X} (± 0.3 – 0.6 Hz)	GlcNAc			
	Iβ	Iα	III	V
H-1,C-1	162.9	171.0	175.3	175.5
H-2,C-2	141.5	140.8	141.3	137.7
H-3,C-3	146.2	147.6	148.0	146.7
H-4,C-4	151.0	145.8	145.5	147.6
H-5,C-5	146.3	145.8	145.3	147.6
H-6 <i>proR</i> ,C-6	142.2	145.2	145.4	144.0
H-6 <i>proS</i> ,C-6	144.0	143.1	142.6	144.0
H-Me,C-Me	128.7	128.7	128.7	128.7
H-N,N	-91.9	-93.0	-92.9	-93.1
		GlcA		Δ4,5-GlcA
	IIβ	IIα	IV	VI
H-1,C-1	162.9	162.9	163.1	168.6
H-2,C-2	145.3	145.8	145.3	148.2
H-3,C-3	144.4	144.4	144.8	146.9
H-4,C-4	148.7	148.7	148.4	167.6
H-5,C-5	148.7	148.7	148.7	n/a

References

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Supplementary Figure



Supplementary Figure. Partial [¹H,¹³C]-HSQC spectrum (900 MHz) of K5₈, showing the C-6 hydroxymethyl region for comparison with that of K5₆ in Figure 3A. Resonances from rings III and V are not resolved in K5₈, indicating that these central rings are approaching the polymer environment.