Supporting Information

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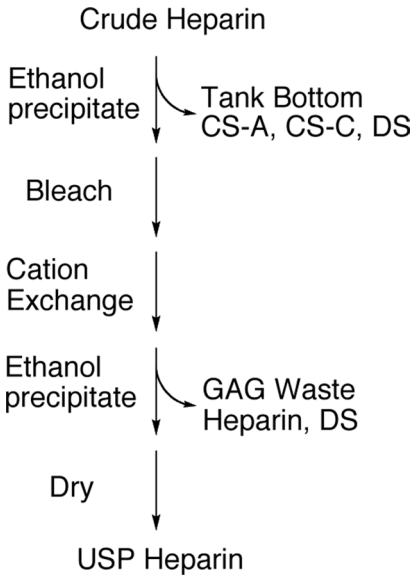

Fig. S1. Simplified Purification Schematic for heparin and identification of the origin of tank bottom and GAG waste material.

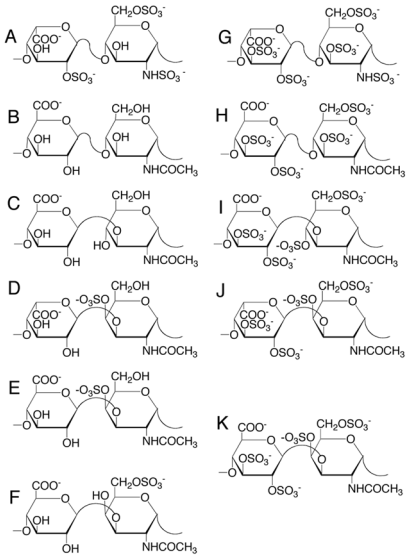

Fig. S2. Major disaccharide repeat units of various GAGs and persulfonated GAGs. (*A*) heparin. (*B*) HS. (*C*) HA. (*D*) DS. (*E*) CS A. (*F*) CS C. (*G*) OS heparin. (*H*) OSHS. (*I*) OSHA. (*J*) OSDS. (*K*) OSCS.

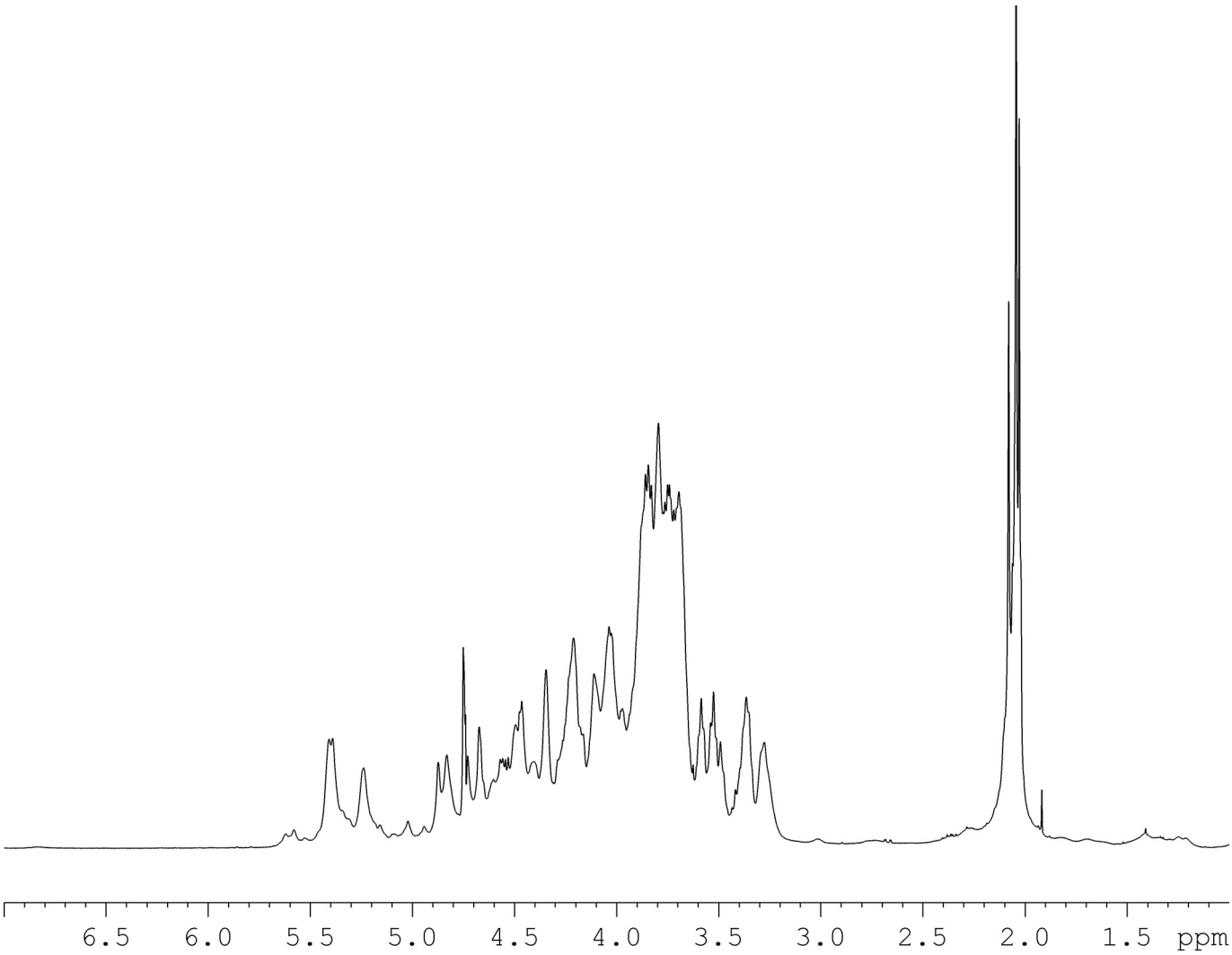

Fig. S3. 600 MHz Proton NMR profile of crude heparin.

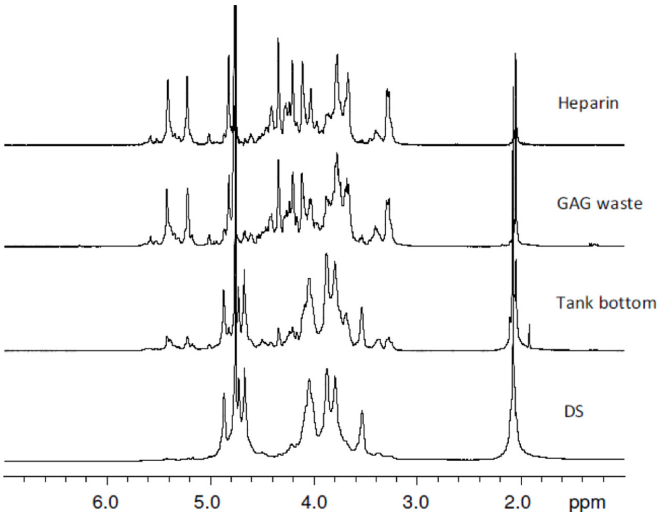

Fig. S4. Proton-NMR spectra of pure heparin, GAG waste, tank bottom, and pure DS. Based on spectral matching, tank bottom material largely consists of DS. Conversely, GAG waste material contains DS and heparin/HS.

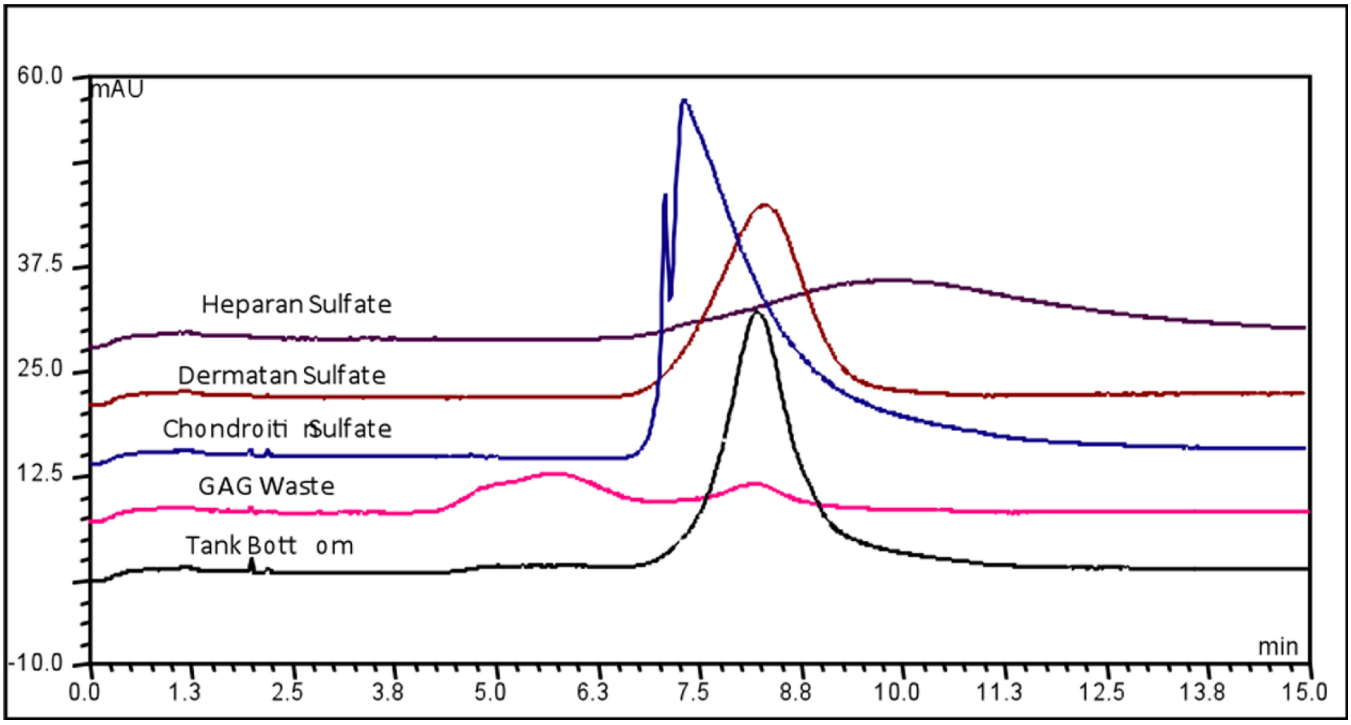

Fig. S5. Analysis of various GAGs by CE at 5 mg/mL. This information is tabulated in Table 1. Of note is the fact that purified heparin has a migration time of ≈5.0 min by this method.

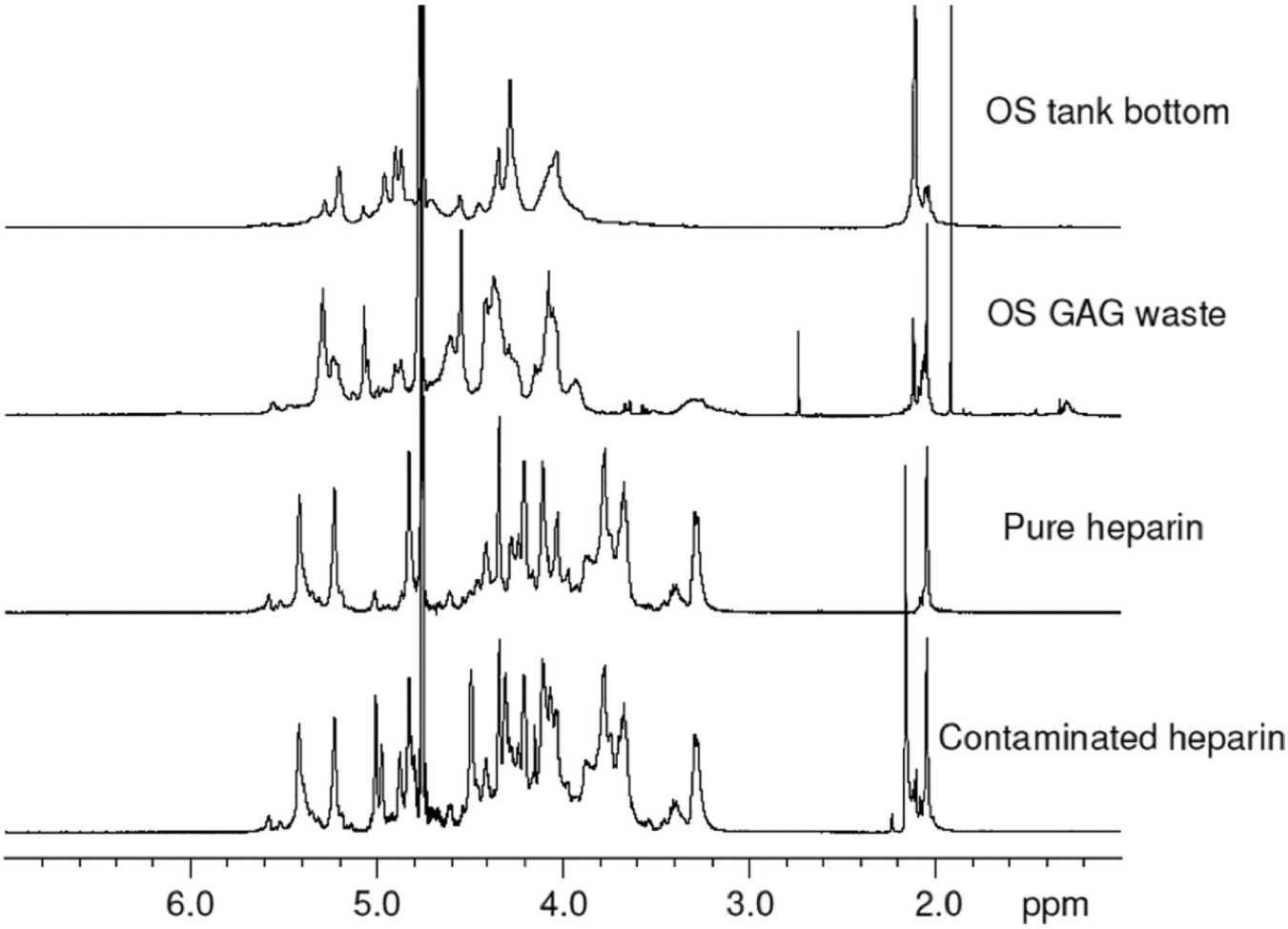

Fig. S6. Overlay of NMR spectra for heparin, contaminated heparin, oversulfated tank bottom material, and oversulfated GAG waste material.

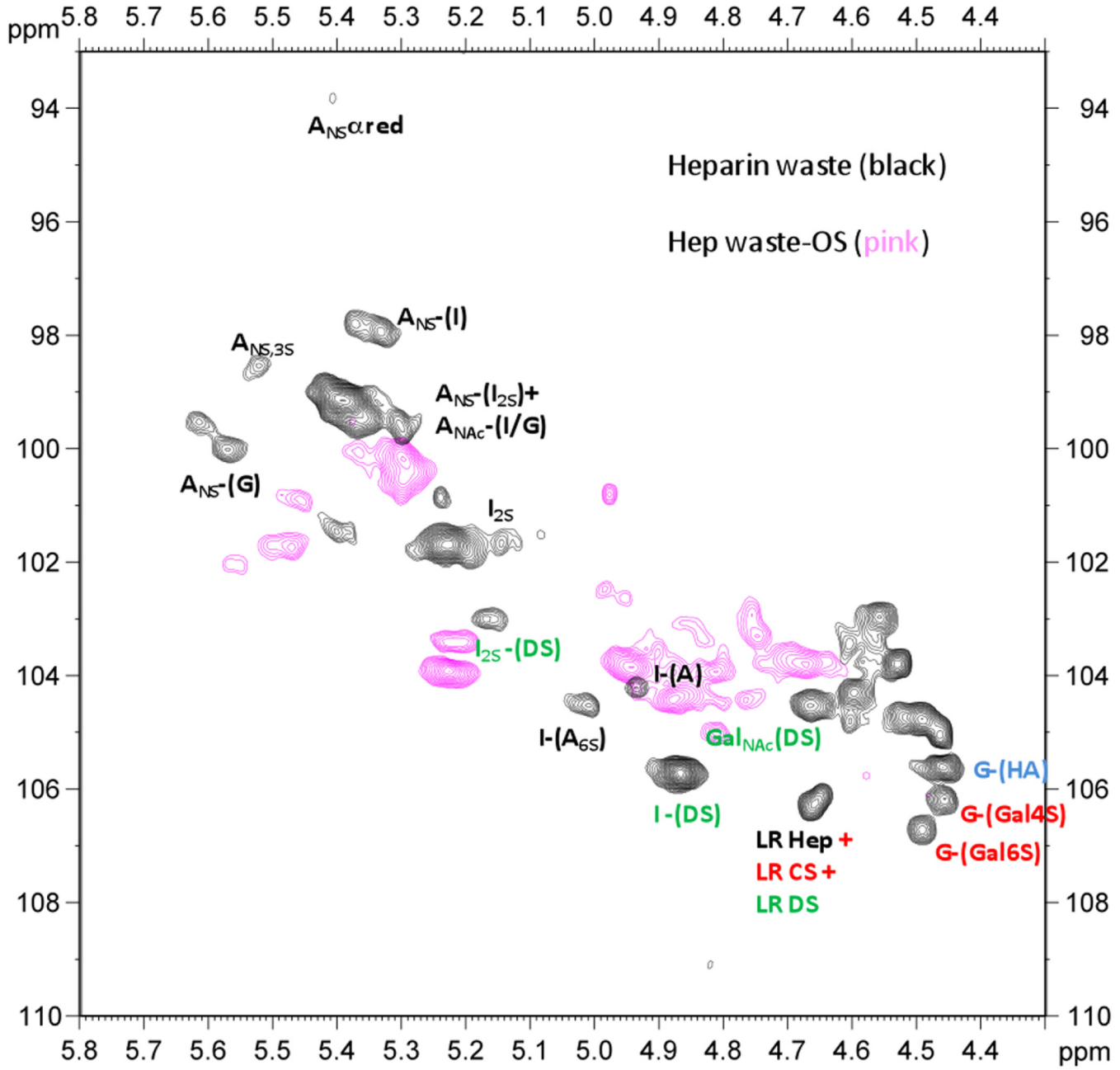

Fig. S7. HSQC Analysis of the anomeric region of crude heparin (black) compared with oversulfated crude heparin (pink). A_{NS}, *N*-sulfoglucosamine; A_{NAc}, *N*-acetylglucosamine; I, iduronic acid; G, glucuronic acid; Gal, galactosamine; LR, linkage region.

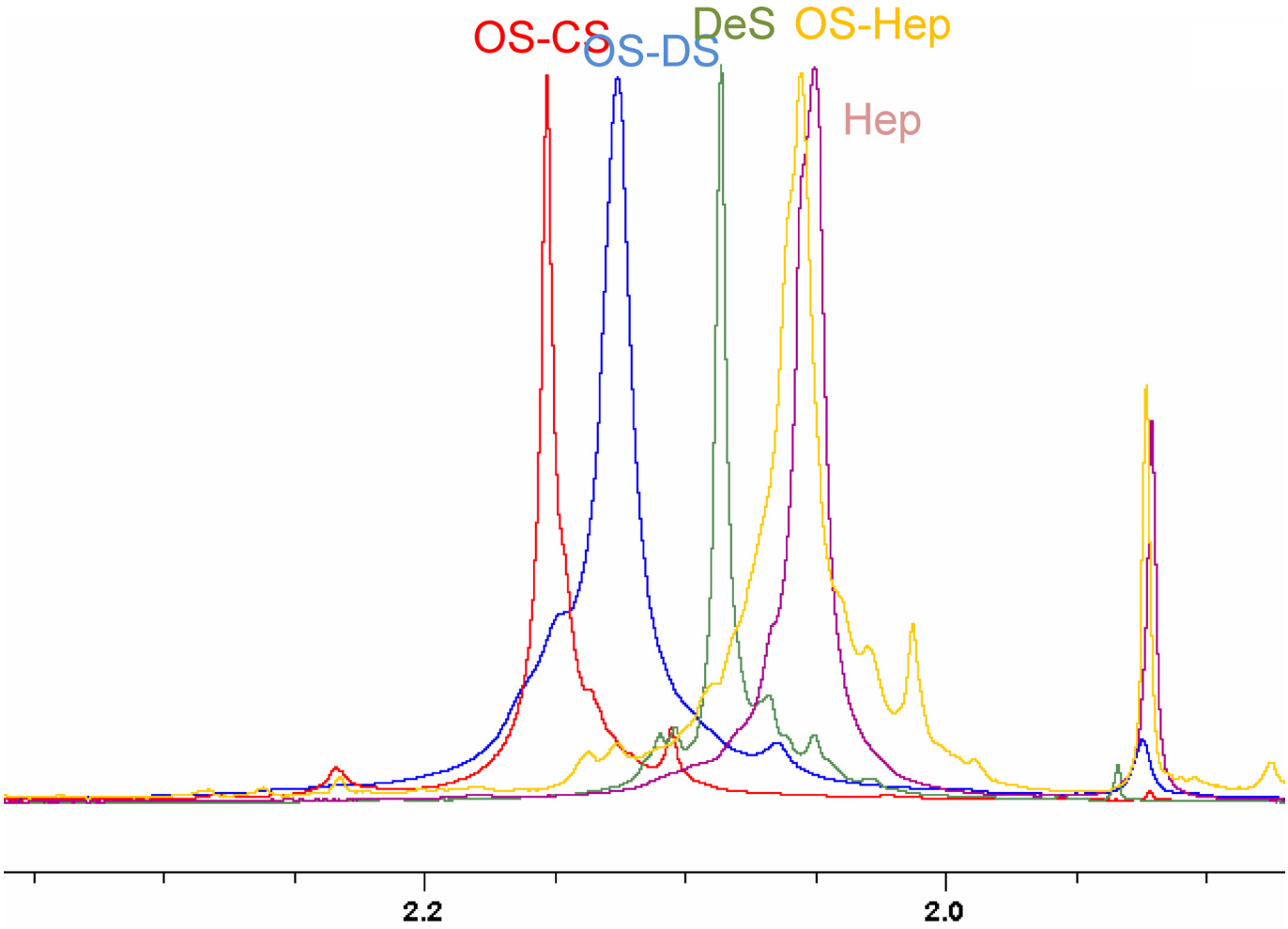

Fig. S8. Proton NMR analysis of oversulfated chondroitin sulfate (OSCS), oversulfated DS (OSDS), and oversulfated heparin (OS-Hep) compared with dermatan sulfate (DS) and heparin (Hep) in the region of 1.9–2.35 ppm. OSDS and OSCS are distinguishable from the *N*-acetyl signal for heparin, as is DS. Conversely, the chemical shift of the *N*-acetyl signal for OS-Hep is largely the same as that for heparin.

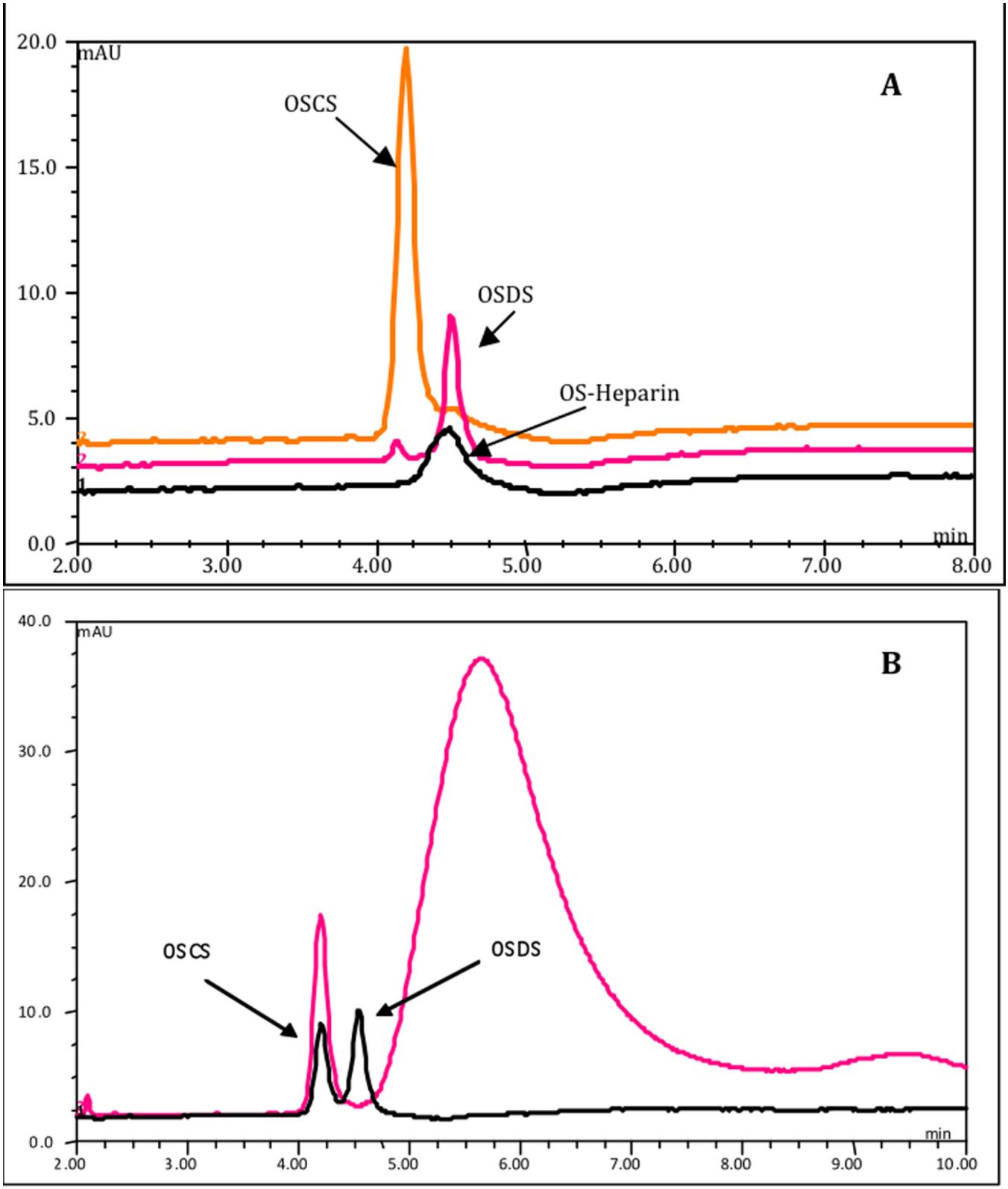

Fig. S9. CE analysis of persulfonated components of crude and eide-stream heparin. (*A*) CE analysis of OSCS, OSDS, and OS-heparin. The migration time for OSCS is distinct from that of OSDS and OS-heparin. Because of the presence of *N*-acetylgalactosamine, OSCS and OSDS have higher absorptivity at 195 nm than does OS-heparin. (*B*) Comparison of a mix of OSCS and OSDS with the USP reference standard (containing heparin + OSCS) indicates OSDS and, by extension, OS-heparin can be distinguished from heparin and OSCS. (*C*) Analysis of OS heparin alone. (*D*) Analysis of OSHS alone.

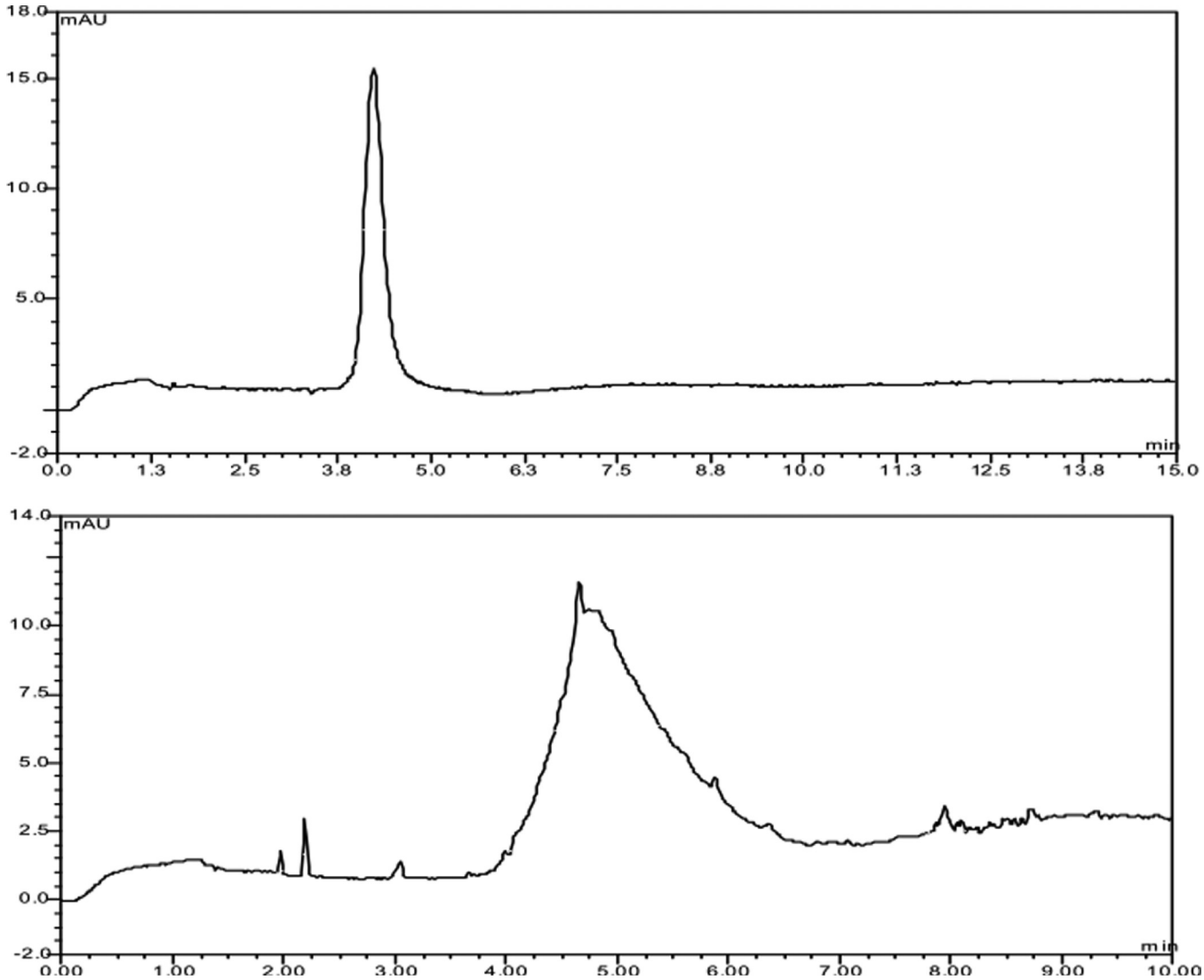

Fig. S9 (continued).

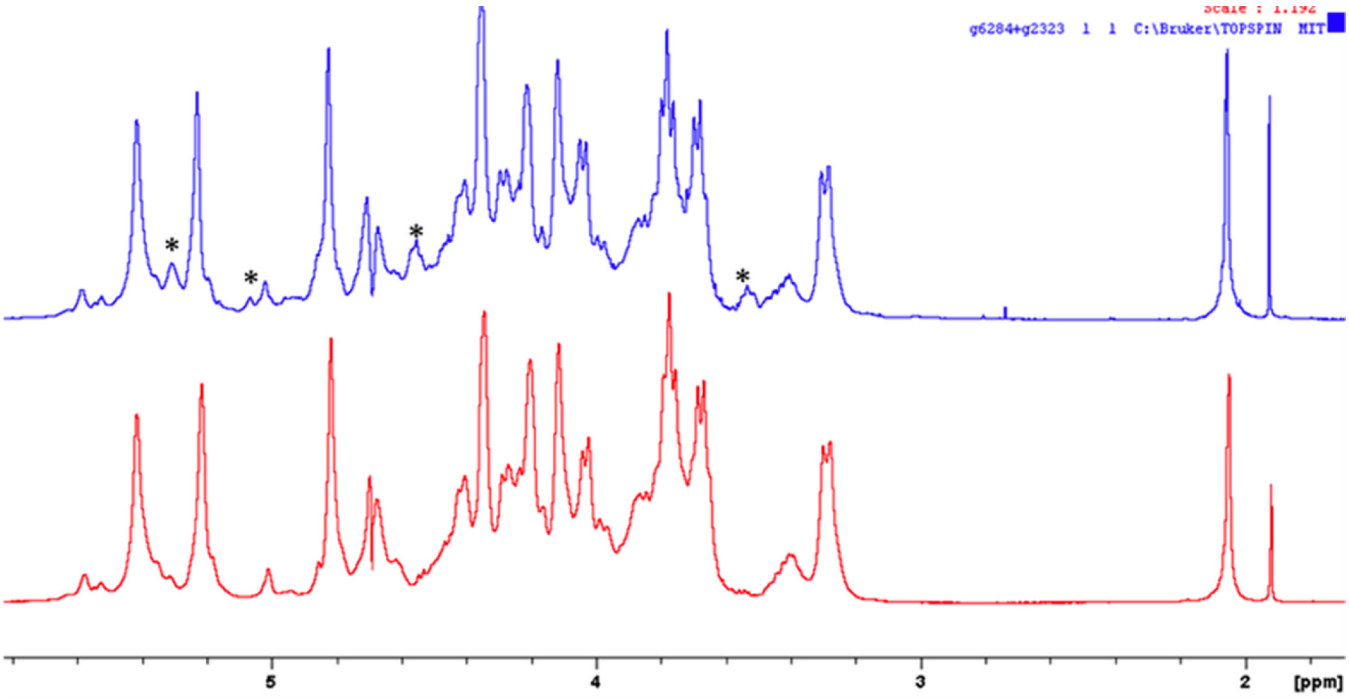

Fig. S10. ^{1}H NMR analysis of purified heparin (red) compared with heparin spiked with OS-heparin (blue). Features of the spiked sample which diverge from that of purified sample are marked (*).

Table S1. NMR chemical shifts of possible monosaccharides in oversulfated HS/Heparin

	GlcNS,3S,6S (1)	GlcNS,3S,6S (2)	GlcNS,3S,6S (3)	GlcNAc,3S,6S (4)
H1/C1	5.48	5.32/99.6	5.44/100.2	5.19/100.0
H2/C2	3.83	3.50/59.3	3.48/58.4	4.22/54.7
H3/C3	4.80	4.48/82.9	4.62/77.9	4.61/79.7
H4/C4	4.12	4.04/76.8	3.95/77.0	3.98/77.7
H5/C5	4.12	4.05/72.1	4.08/71.4	4.08/72.7
H6,6′/C6	4.38	4.27–4.41/68.7	4.31–4.52/67.6	4.29–4.58/68.4
	I2,3S (1)	I2,3S (2)	G2,3S (3)	G2,3S (4)
H1/C1	5.36	5.32/100.8	4.91/102.4	4.96/103.2
H2/C2	4.90	4.55/73.6	4.39/79.3	4.41/80.4
H3/C3	4.62	4.72/72.9	4.65/80.8	4.72/80.7
H4/C4	4.50	4.39/73.3	4.17/79.7	4.27/78.5
H5/C5	5.20	5.05/69.8	3.88/79.5	3.83/79.2

1. Toida T, et al. (1999) Preparation and anticoagulant activity of fully O-sulphonated glycosaminoglycans. *Int J Biol Macromol* 26, 233–241.
2. Yates EA, et al. (2000) Effect of substitution pattern on 1H, 13C NMR chemical shifts and 1J(CH) coupling constants in heparin derivatives. *Carbohydr Res* 329, 239–247.
3. Casu B, et al. (1994) Heparin-like compounds prepared by chemical modification of capsular polysaccharide from E. coli K5. *Carbohydr Res* 263, 271–284.
4. Guerrini M, Naggi A, Guglieri S, Santarsiero R, Torri G (2005) Complex glycosaminoglycans: profiling substitution patterns by two-dimensional nuclear magnetic resonance spectroscopy. *Anal Biochem* 337, 35–47.