

Supplementary Information

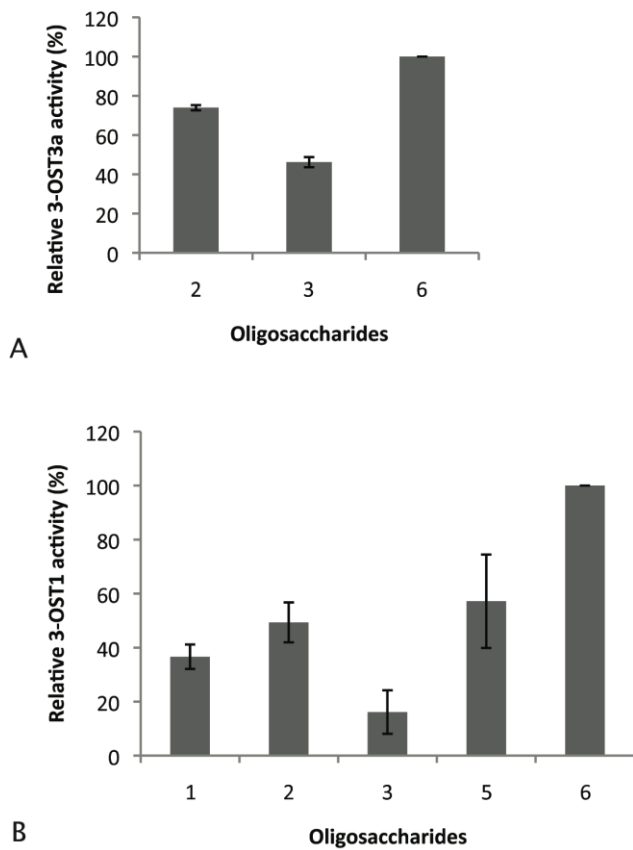


Figure S1. Relative activity of 3-OST1 (**A**) and 3-OST3a (**B**) on synthetic oligosaccharides. The values presented are calculated from the AUC of 3-*O*-sulfated oligosaccharide product peaks that are shown in Figures 1 and 2. Data has been normalized to the AUC for oligosaccharide **6**. The error bar indicates the standard error of the mean (SEM) from three independent experiments.

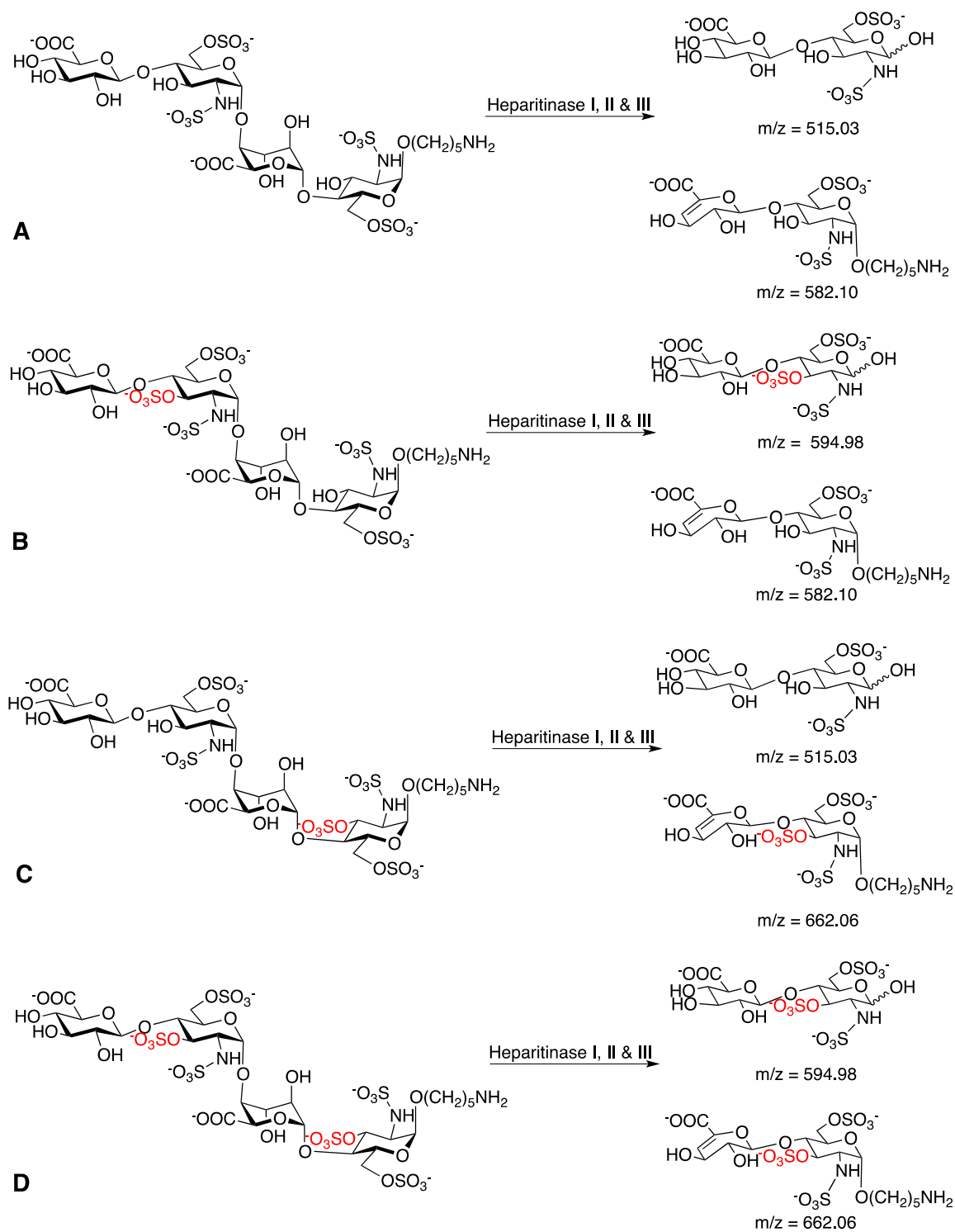


Figure S2. Theoretical 3-OST3a modification sites on oligosaccharide **2** and their respective disaccharide products upon digestion with heparitinase I, II & III. **A.** No 3-*O*-sulfate groups are added to the oligosaccharide. **B.** One 3-*O*-sulfate group is added to the first GlcNS6S residue. **C.** One 3-*O*-sulfate group is added to the second GlcNS6S residue. **D.** 3-*O*-sulfate groups are added to both GlcNS6S residues.

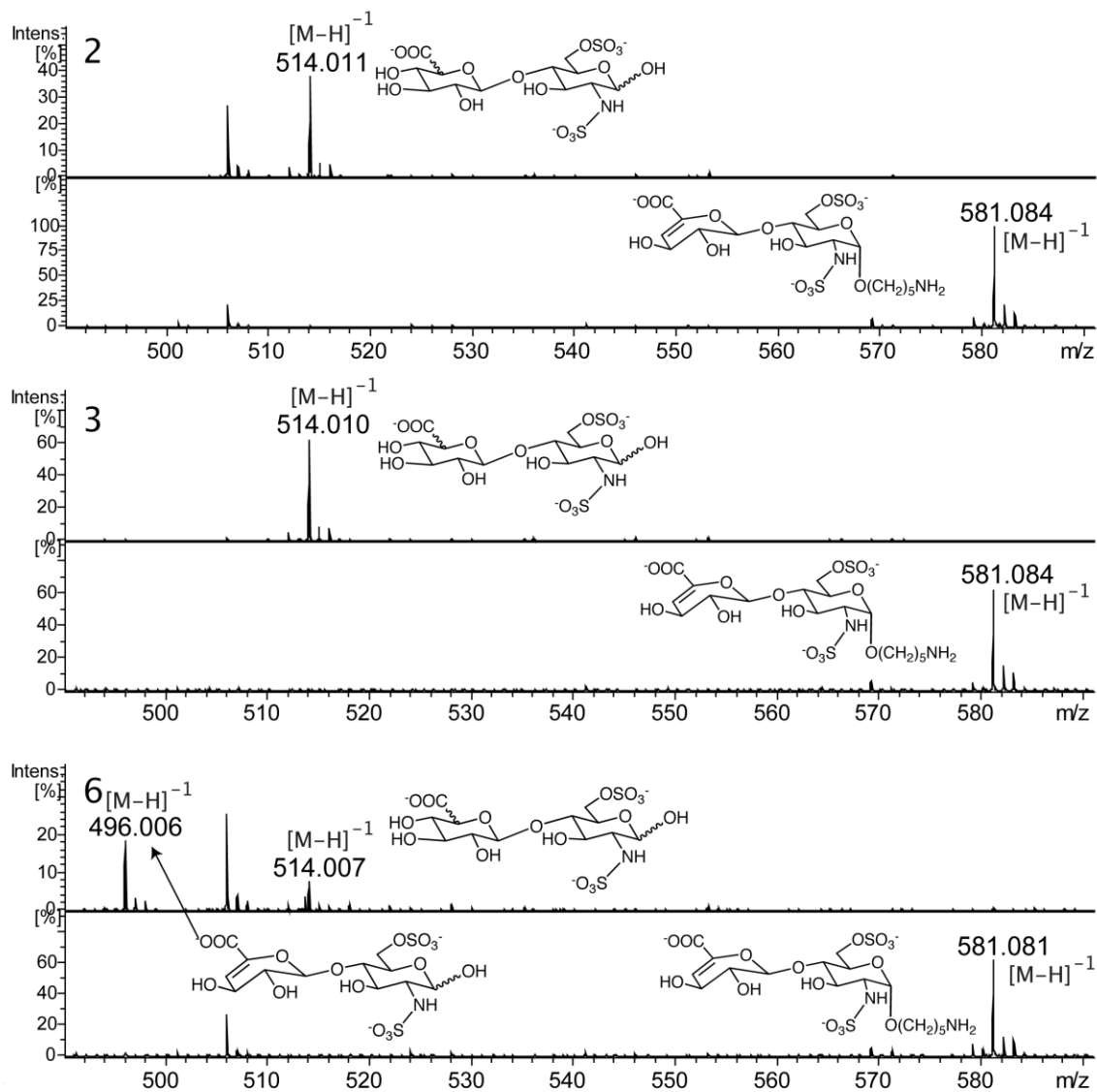


Figure S3. MS spectra of non-3-*O*-sulfated disaccharide products of oligosaccharides **2**, **3**, and **6**.

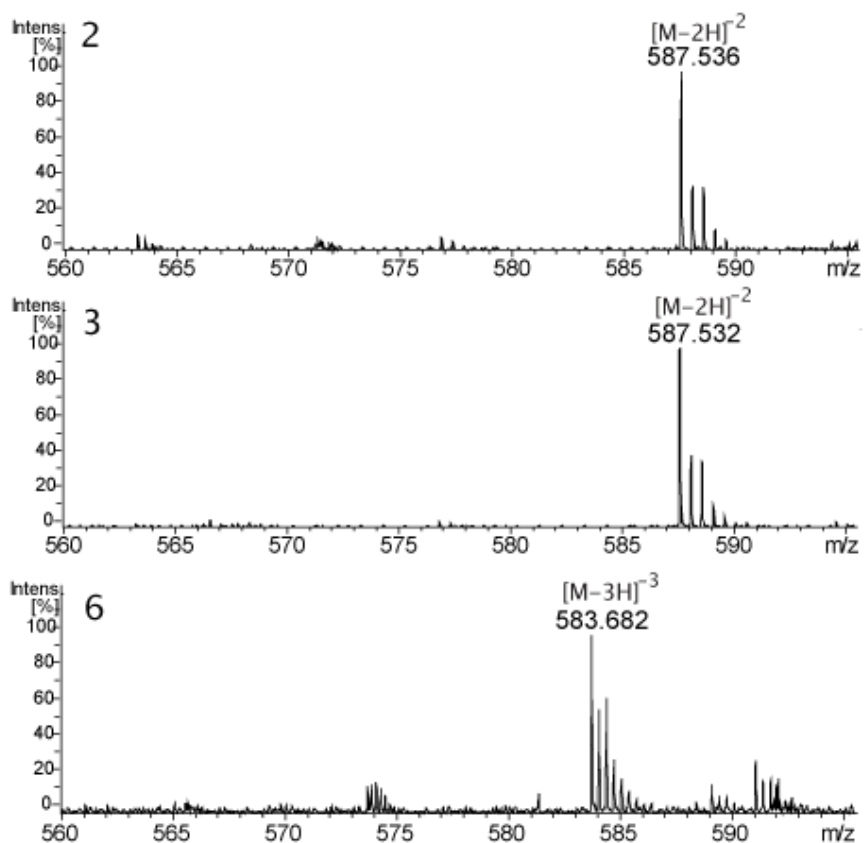


Figure S4. MS spectra of intact 3-*O*-sulfated oligosaccharide products derived from oligosaccharide substrates **2**, **3** and **6**.