Supporting Information

Discovery of a Heparan Sulfate 3-*O*-Sulfation Specific Peeling Reaction

Yu Huang[†, ‡], Yang Mao[†, ‡, *], Chengli Zong[§], Cheng Lin[†], Geert-Jan Boons[§], and Joseph Zaia[†*]

[†]Dept. of Biochemistry, Boston University Medical Campus, Boston, MA, 02118

[§]Complex Carbohydrate Research Center, University of Georgia, Athens, GA, 30602

[‡] These authors contributed equally to this work

[*] Co-corresponding authors:

Joseph Zaia

Center for Biomedical Mass Spectrometry

Boston University Medical Campus

670 Albany St., Rm. 509

Boston, MA 02118

(v) 617-638-6762

(f) 617-638-6761

(e) jzaia@bu.edu

Yang Mao

Center for Biomedical Mass Spectrometry

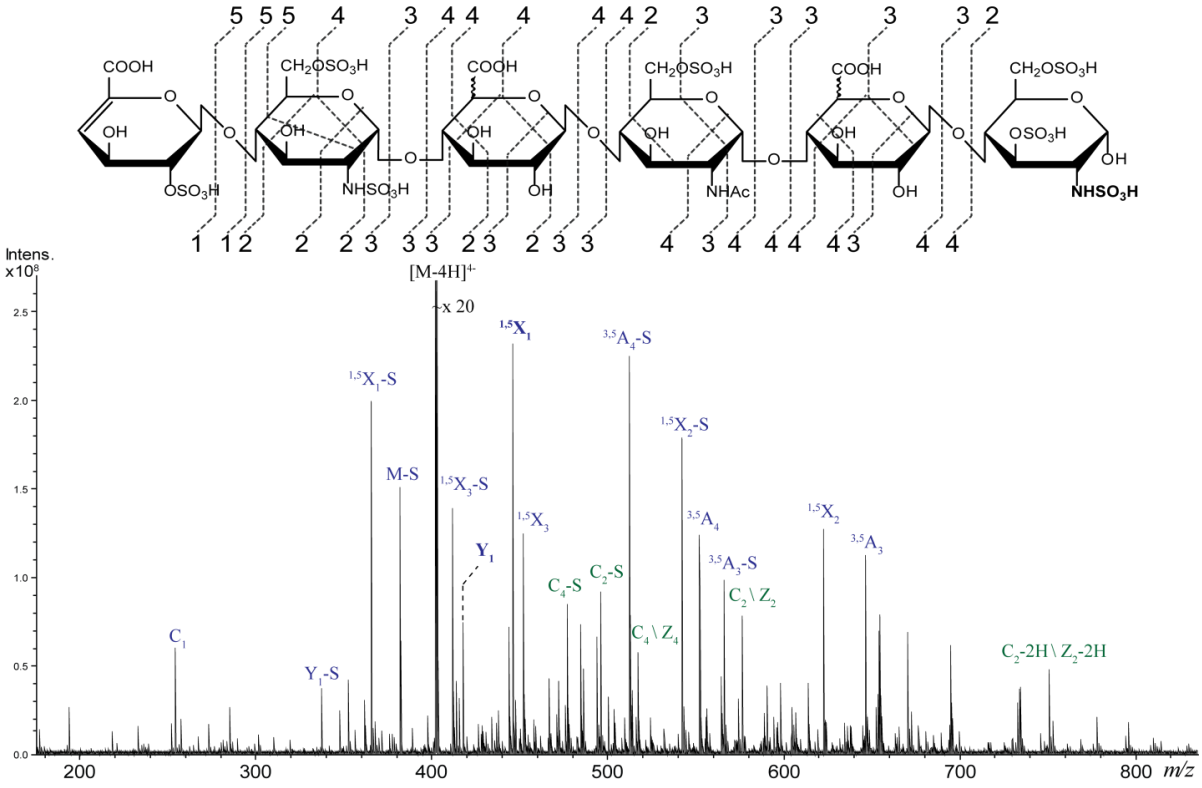
Boston University Medical Campus

670 Albany St., Rm. 504

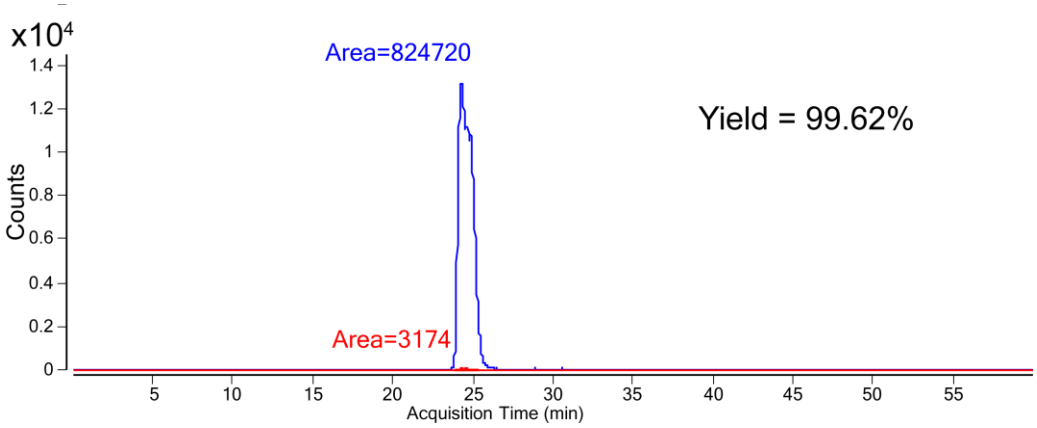
Boston, MA 02118

(e) yangmao@bu.edu

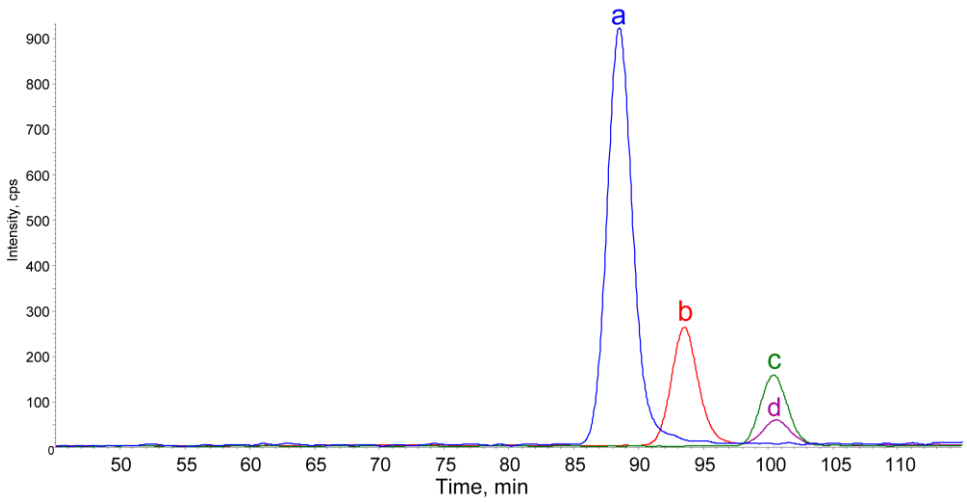
Supplemental Figure 1. Structural determination of the base-susceptible hexasaccharide [1,2,3,1,7] by EDD tandem mass spectrometry. The numbers on the cleavage map indicate the maximum number of sulfates found from the fragmentation on that cleavage position.



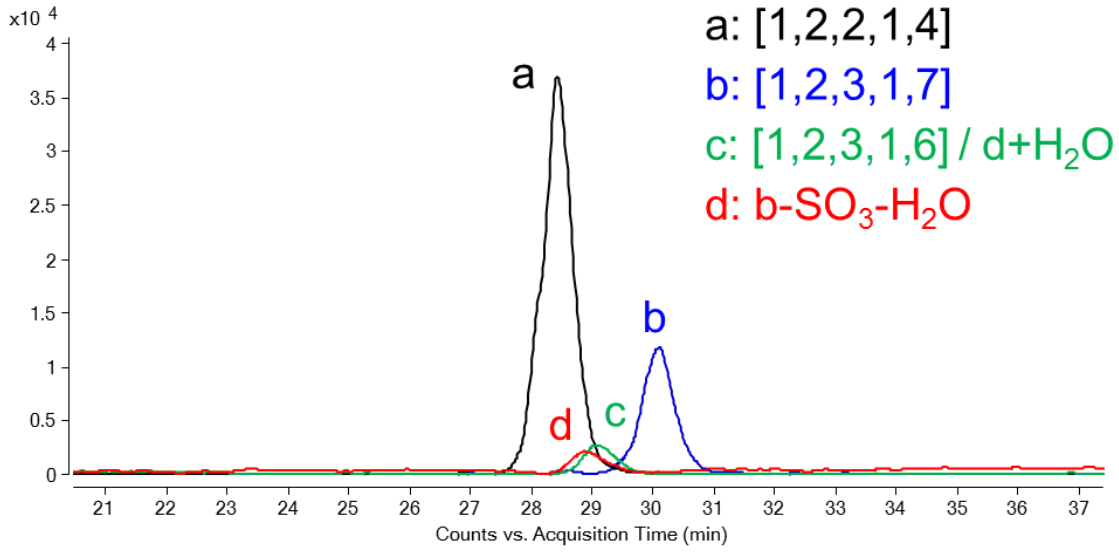
Supplemental Figure 2. The efficiency of Hs3st-1 modification on the tetrasaccharides. The desalted products were analyzed on HILIC-MS. Blue profile shows the charge state merged EICs of the Hs3st-1 modified synthetic tetrasaccharide. Red profile shows the charge state merged EICs of the intact tetrasaccharide.



Supplemental Figure 3. EICs of the SEC-MS analysis of the digestion and degradation products from the Hs3st-1 modified synthetic tetrasaccharide at pH 8.0. Peak a shows the charge state merged EICs of the digestion product ΔHex2S-GlcNS6S-R. Peak b shows the charge state merged EICs of the digestion product GlcA-GlcNS3S. Peak c shows the EICs of the degradation product with *m/z* 416.05. Peak d shows the EICs of the degradation product with *m/z* 434.06.



Supplemental Figure 4. EICs of the HILIC-MS analysis of the degradation products from the base-susceptible hexasaccharide [1,2,3,1,7] treated with 0.1 M $NH_3 \cdot H_2O$. Peak **b** shows the charge state merged EICs of [1,2,3,1,7]. Peak **a** shows the charge state merged EICs of the degradation product [1,2,2,1,4]. Peak **d** shows the EICs of the intermediate product with *m/z* matching to the composition of [1,2,3,1,7] after a loss of one water molecule (H_2O) and one sulfo group ($-SO_3$) . Peak **c** shows the EICs of the side product with *m/z* matching to the composition of [1,2,3,1,6] or the composition of the intermediate product **d** with an addition of one water molecule (H_2O).



Supplemental Figure 5. Elevated temperature speeds up the peeling reaction. The base-susceptible hexasaccharide [1,2,3,1,7] treated with 50 m M $NH_3 \cdot H_2O$ at either room temperature (RT) or 37 °C overnight. The products were analyzed on HILIC-MS with or without vacuum drying process. The control sample was incubated with ddH_2O at RT and analyzed without vacuum drying. Peak b shows the charge state merged EICs of [1,2,3,1,7]. Peak a shows the charge state merged EICs of the degradation product [1,2,2,1,4]. The result demonstrates that elevated temperature speeds up the peeling reaction on the base-susceptible hexasaccharide [1,2,3,1,7].