ELECTRONIC SUPPORTING INFORMATION

Differentiation of Oligosaccharide Isomers by Direct Infusion Multidimensional Mass Spectrometry

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1. Experimental Method

Apparatus for direct infusion nano-electrospray ionization (nESI). Theta glass capillary was employed for non-contact nESI to deliver/ionize all sample solutions toward the mass spectrometer (**Figure 1a**). This setup consists of a silver (Ag) metal electrode and a theta borosilicate (disposable) glass capillary (O.D 1.5 mm) pulled to a sharp tip using a micropipette puller (Model P-97, Sutter Instrument Co., Novato. CA, USA). In the non-contact mode, the Ag electrode is not in physical contact with sample solution or reagent in the theta capillary, providing an air gap between the reagent solution and the Ag electrode. We placed 5 µL of oligosaccharide sample (in urine or in water) in one barrel of the pulled borosilicate theta capillary while 5 µL of aqueous solution of the ammonium salt (NH_4Cl or NH_4Br) was placed in the other barrel as illustrated in **Figure 1a**. To initiate electrospray, 1.5 kV direct current (DC) voltage was applied to the Ag electrode, which induces an electric field that charges the reagent solution electrostatically, facilitating charged microdroplets to be released from the tip of the pulled theta capillary. We found that the result is the same regardless of the barrel to which the DC voltage is applied. That is, the release of charged microdroplets is achieved from both barrels allowing in-situ mixing of reagent with the analyte solution followed by adduct formation between the oligosaccharide and halide in negative-ion mode and oligosaccharide and ammonium in positive-ion mode. The theta capillary was stationed 5 mm away front of the mass spectrometer inlet. This enabled direct transfer of the charged microdroplets containing the adducts to the mass spectrometer for subsequent characterization via MS/MS. All analyses were performed in the non-contact mode of the nESI platform.

Mass spectrometry. The data were collected using a Thermo Fisher Scientific Velos Pro ion trap mass spectrometer at a full MS range (San Jose, CA, USA). The following MS parameters were employed: 3 microscans, 100 ms ion injection time, and 250 °C inlet capillary temperature. Spectra collection was done for at least 30 s. A distance of 5 mm was kept constant between the nESI tip and the MS inlet. All data collection and processing were done using Thermo Fisher Scientific Xcalibur 2.2 SP1 software. Identification and characterization of oligosaccharide isomers were achieved using tandem MS with collision-induced dissociation (CID) at 30% manufacturer's unit and 1.5 Th (mass/charge units) for isolation window of normalized collision energy.

Chemicals and Reagents. Cellotriose, gentianose, 1-kestose, isomaltotetraose, maltotetraose, verbascose, cellopentaose, maltopentaose, and stachyose were purchased from Biosynth International Inc. Nigerotriose was obtained from Neogen corporation (Lansing, MI) Cellotetraose and Isomaltotriose were obtained from VWR International (Radnor, PA). Maltotriose and melezitose were purchased from Sigma-Aldrich (St. Louis, MO). Ammonium bromide and ammonium chloride were also purchased from Sigma-Aldrich (St. Louis, MO).

Preparation of urine sample. Pooled human urine sample was purchased from Innovative research, Novi MI. 5 mL of the sample was centrifuged using a Microsep™ Advance 3k MW cutoff Centrifugal Filter (Pall Corp., Ann Arbor, MI) at ~15000 rpm for 15 minutes. 20 uL of the filtered urine sample was dissolved in 80 mL of 100% water containing 50 µM each of the 14 oligosaccharides.

2. Full MS Analysis of Isomers via Chloride Adduction

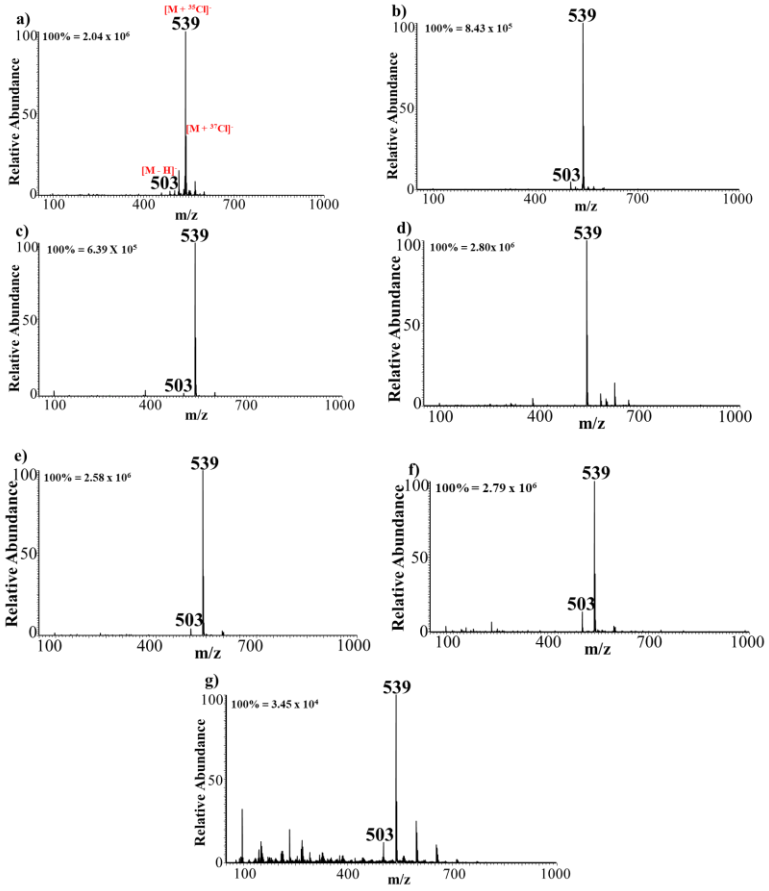


Figure S1. Negative-ion mode mass spectra showing formation of chloride adduct with 100 µM **a)** Isomaltotriose **b)** Melezitose **c)** Maltotriose **d)** Cellotriose **e)** 1-Kestose **f)** Nigerotriose **g)** 0.5 µM Gentianose.

3. Analysis of Positional Isomers via Bromide Adduction

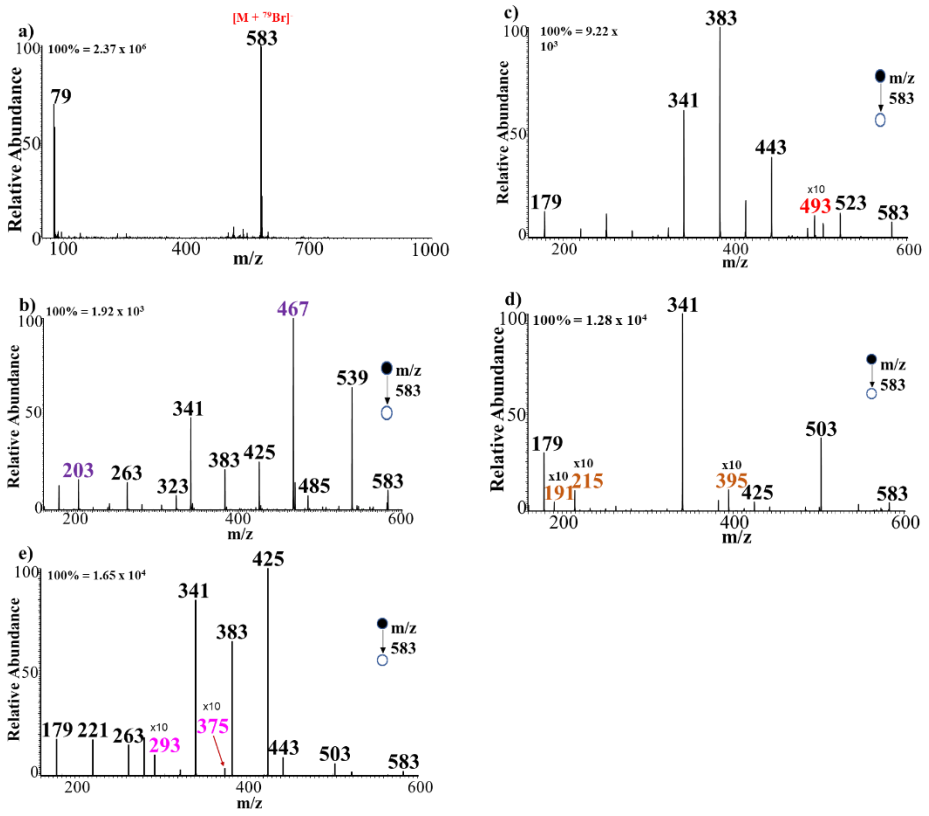


Figure S2. **a)** Representative negative-ion mode mass spectrum showing formation of bromide adduct with isomaltotriose. Product ion tandem MS recorded for **b)** Cellotriose **c)** Maltotriose **d)** Nigerotriose **e)** Isomaltotriose all at m/z 583.

4. Mixture of Trisaccharide Positional Isomers

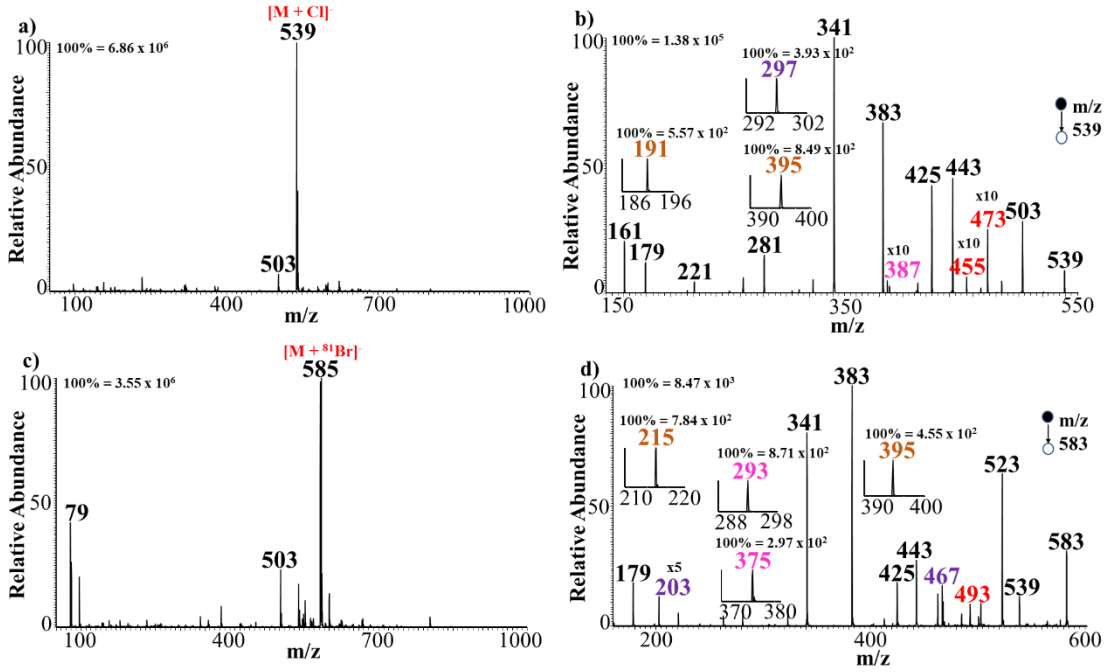


Figure S3. a) Negative-ion mode mass spectra showing chloride adduct formed from spraying mixture of cellotriose, isomaltotriose, maltotriose, nigerotriose and ammonium chloride from theta capillary **b**) Negative-ion mode tandem MS/MS analysis of the mixture **c)** Negative-ion mode mass spectrum showing bromide adduct formed from spraying mixture of cellotriose, isomaltotriose, maltotriose, nigerotriose and ammonium bromide from theta capillary **d**) Negative-ion mode tandem MS analysis of adduct, inserts showing diagnostic ions extracted from different regions.

5. Tandem MS Analysis of Other Trisaccharide Isomers via Chloride Adduction

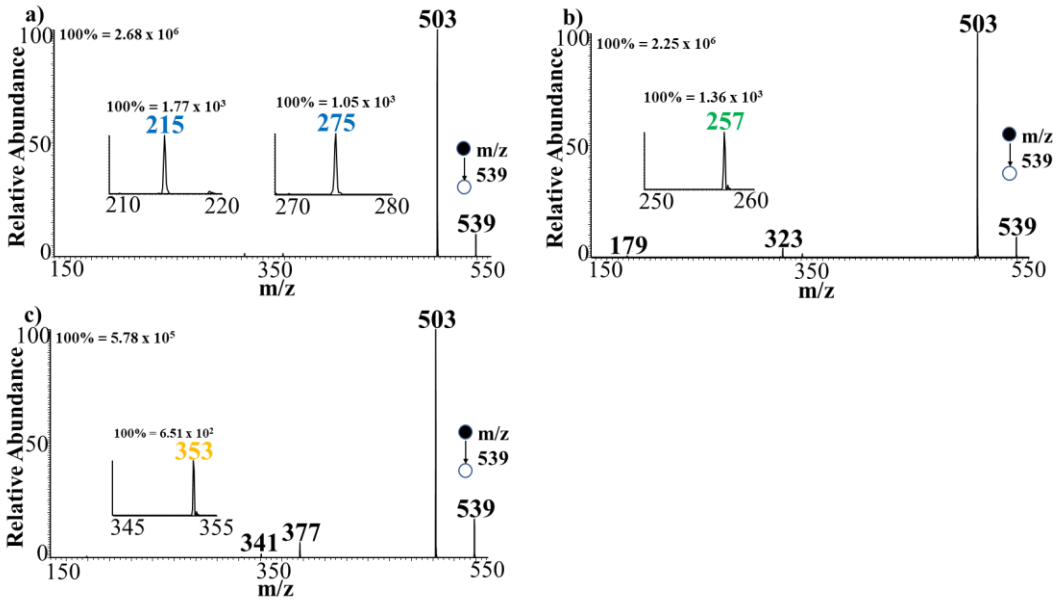


Figure S4. Product ion tandem MS/MS recorded for **a**) 1-Kestose **b**) Melezitose **c**) Gentianose all at m/z 539.

6. Analysis of Other Trisaccharides via Bromide Adduction

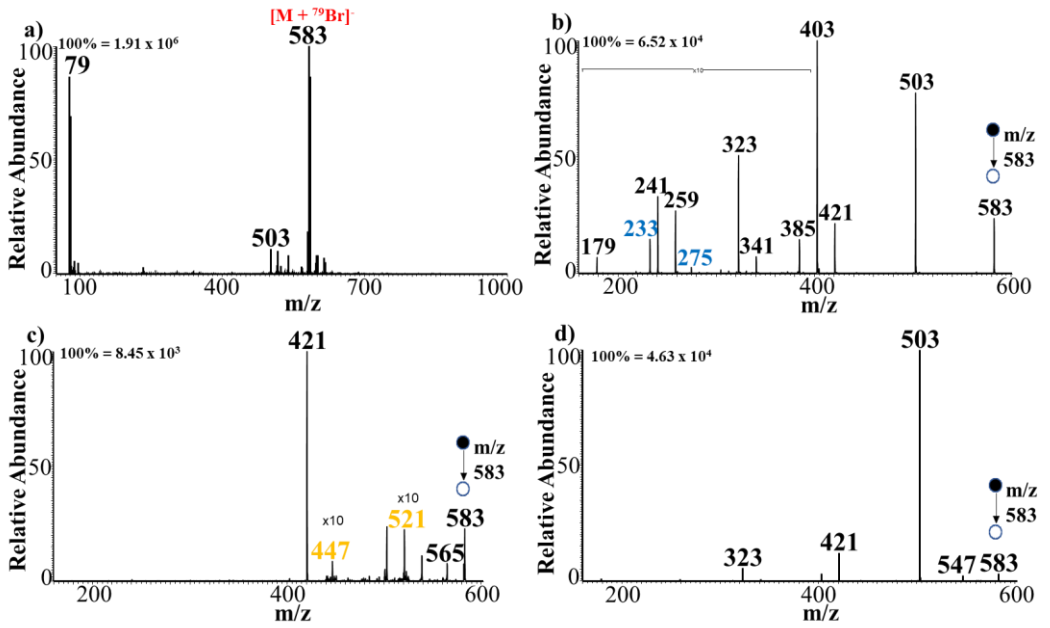


Figure S5. **a**) Representative negative-ion mode mass spectrum showing bromide adduct formed from spraying melezitose and ammonium bromide from theta capillary. Product ion tandem MS recorded for **b**) 1-Kestose **c**) Gentianose **d**) Melezitose, all at m/z 583.

7. Mixture of Seven Isomers via Chloride Adduction

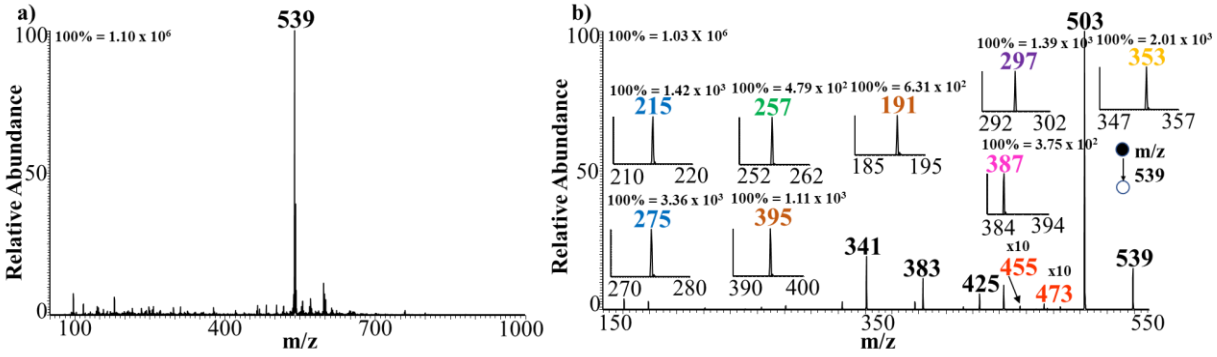


Figure S6: a) Negative-ion mode mass spectrum showing chloride adduct formed from spraying mixture of seven trisaccharide isomers and ammonium chloride from theta capillary. **b**) Product ion tandem MS recorded for the mixture at m/z 539, inserts showing diagnostic ions at different ranges.

8. Positive-Ion Mode Adduction of Oligosaccharide Isomers

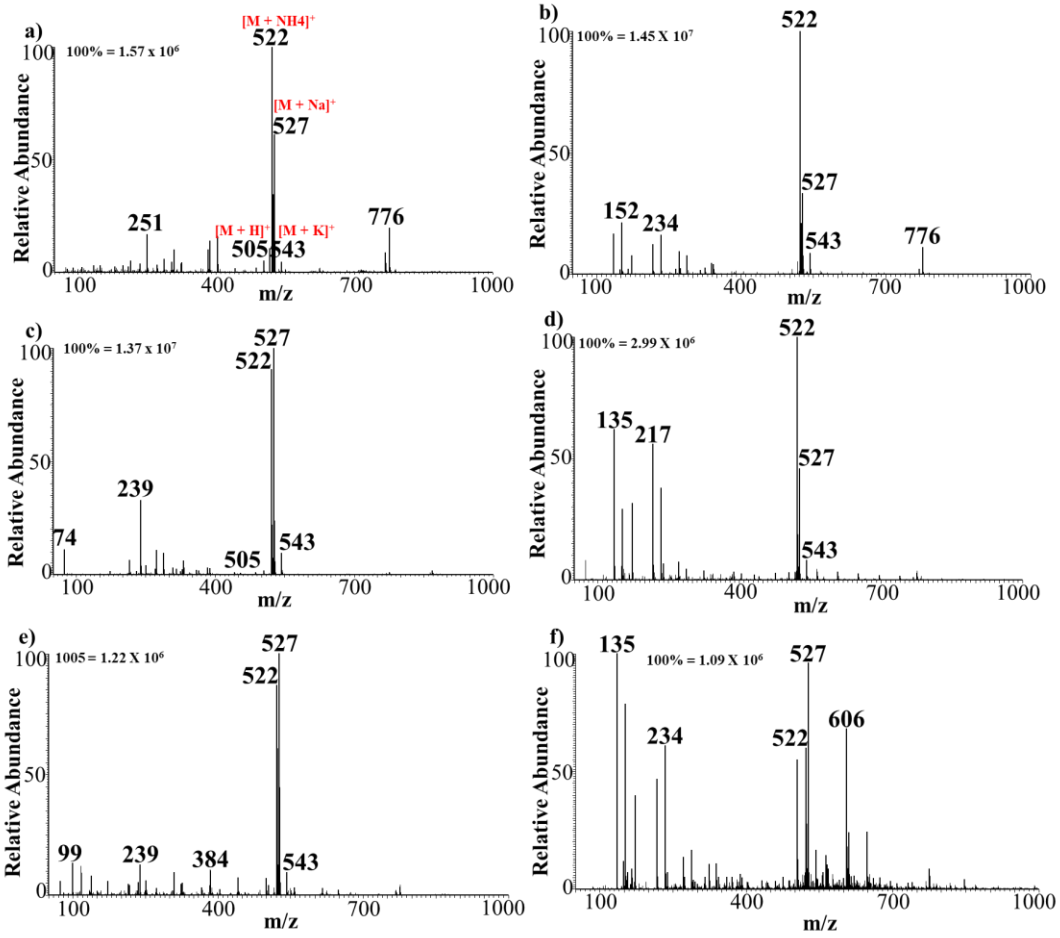


Figure S7. Positive-ion mode mass spectra showing adduction of **a)** Isomaltotriose **b)** 1-Kestose **c)** Maltotriose **d)** Gentianose **e)** Melezitose **f)** Cellotriose, with background subtraction**.**

9. Tandem MS Analysis of Oligosaccharide Isomers in Positive-Ion Mode via NH^+ Adduction

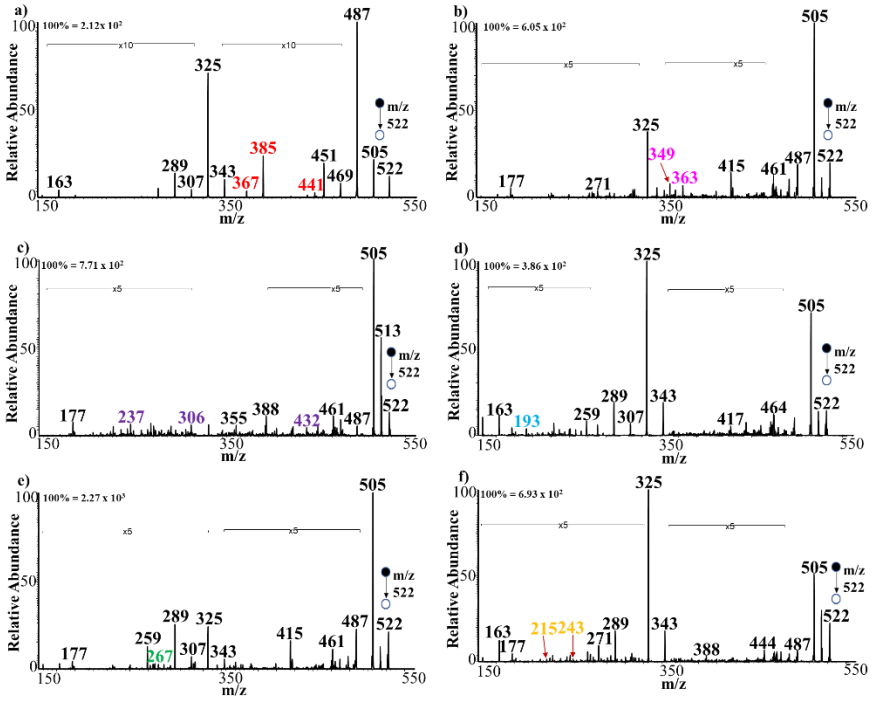


Figure S8. Product-ion tandem MS spectra recorded for **a)** Maltotriose **b)** Isomaltotriose **c)** Cellotriose **d)** 1-Kestose **e)** Melezitose **f)** Gentianose, all at m/z 522.

10. Tandem MS Analysis of Oligosaccharide Isomers in Positive-Ion Mode via Na^+ Adduction

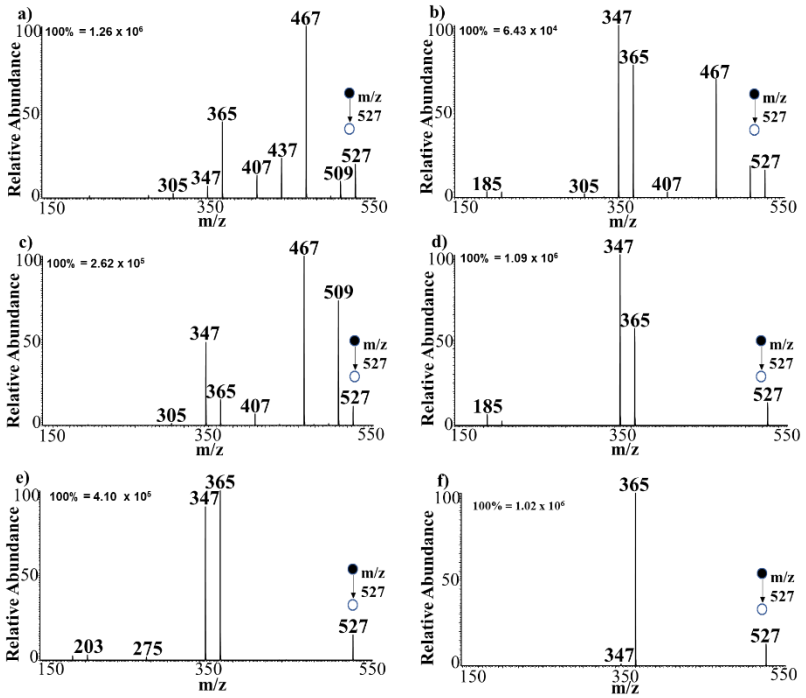


Figure S9. Product-ion tandem MS spectra recorded for **a)** Maltotriose **b)** Isomaltotriose **c)** Cellotriose **d)** 1-Kestose **e)** Melezitose **f)** Gentianose, all at m/z 527.

11. PCA Results from Hetero-linked Trisaccharide Isomers

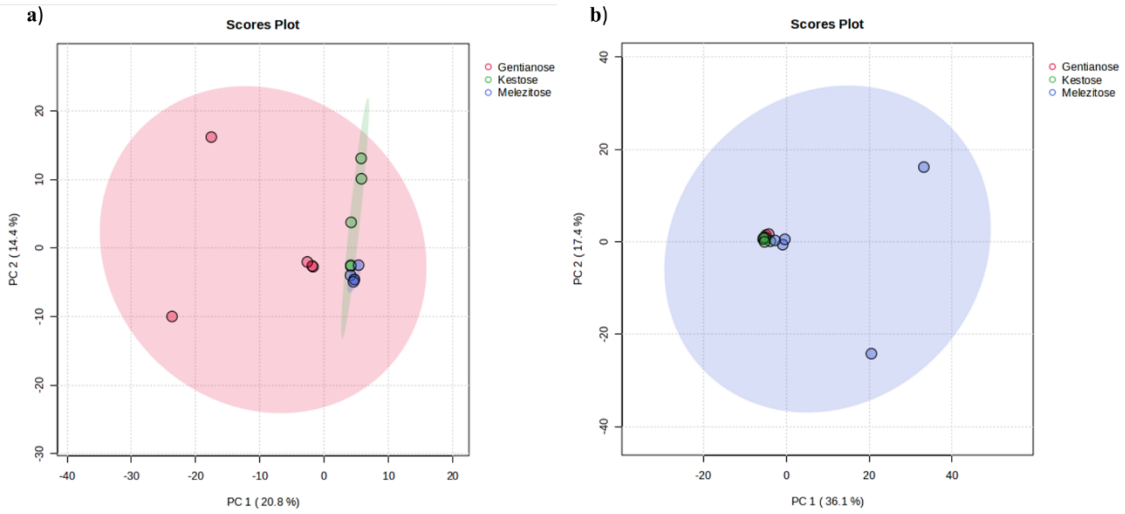


Figure 10. PCA scores plot obtained for **a)** Negative - ion mode tandem MS **b)** Positive - ion mode tandem MS, of hetero-linked trisaccharide isomers.

12. Heatmap of Hetero-linked Trisaccharide Isomers

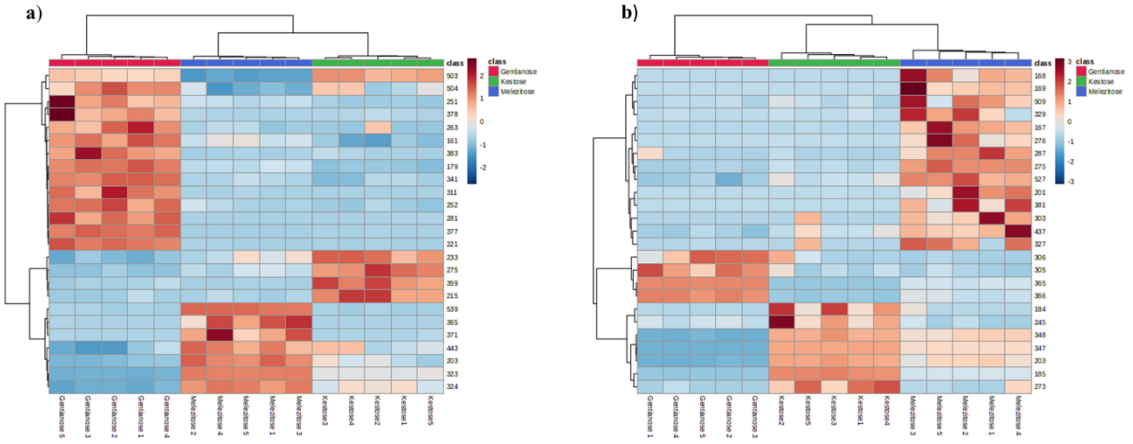


Figure 11. Heatmap results obtained for **a)** Negative - ion mode tandem MS **b)** Positive - ion mode tandem MS, of trisaccharide isomers.

13. Full MS Analysis of Tetrasaccharide Isomers via Chloride Adduction

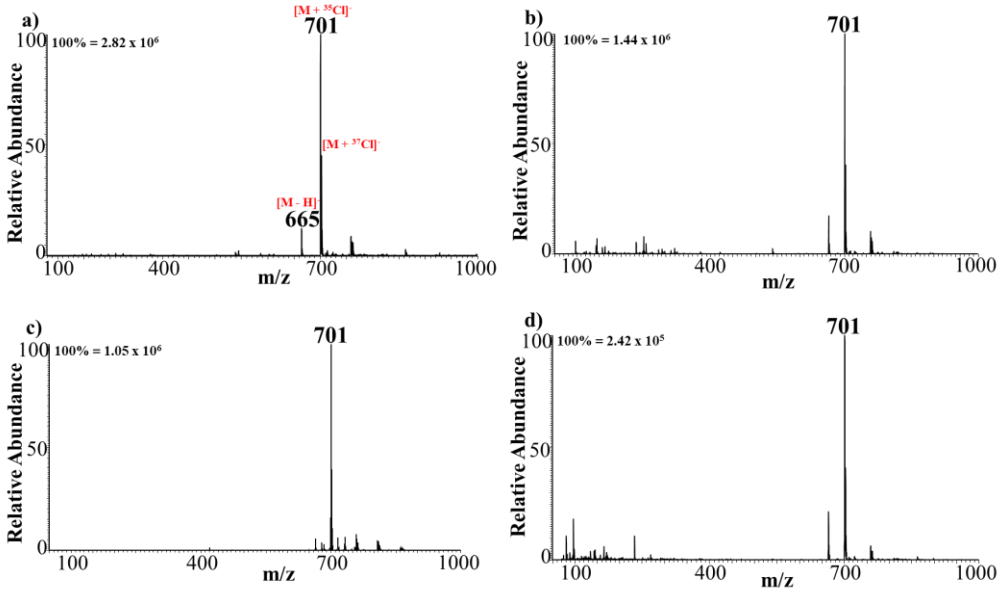


Figure S12. Negative-ion mode mass spectra showing formation of chloride adduct with **a)** Maltotetraose **b)** Stachyose **c)** Cellotetraose **d)** Isomaltotetraose.

14. Analysis of Tetrasaccharide Isomers via Chloride Adducts

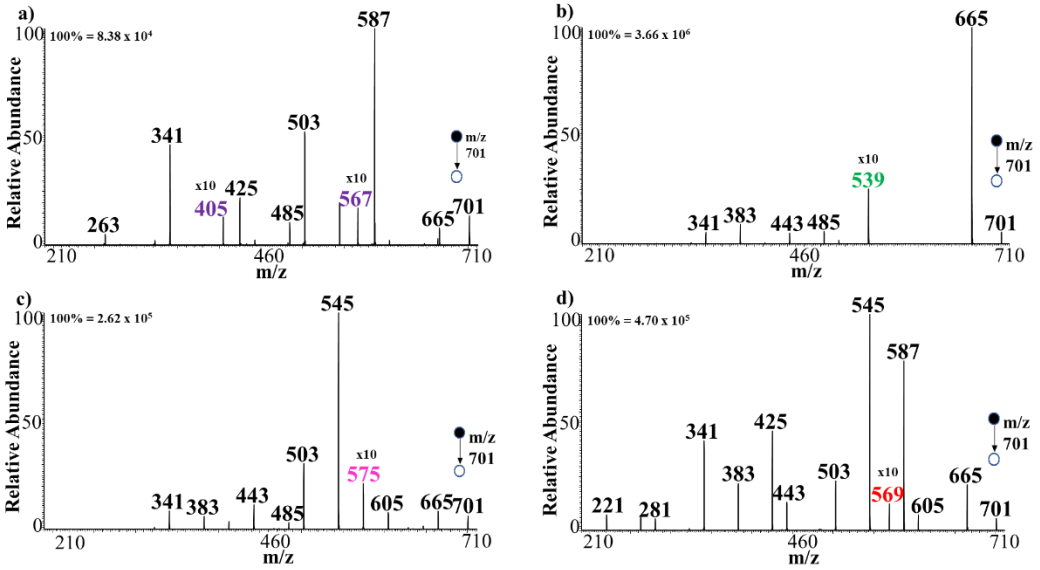


Figure S13. Product ion tandem MS recorded for **a**) Cellotetraose **b**) Stachyose **c**) Isomaltotetraose **d**) Maltotetraose, all at m/z 701.

15. Analysis of Tetrasaccharide Isomers via Bromide Adducts

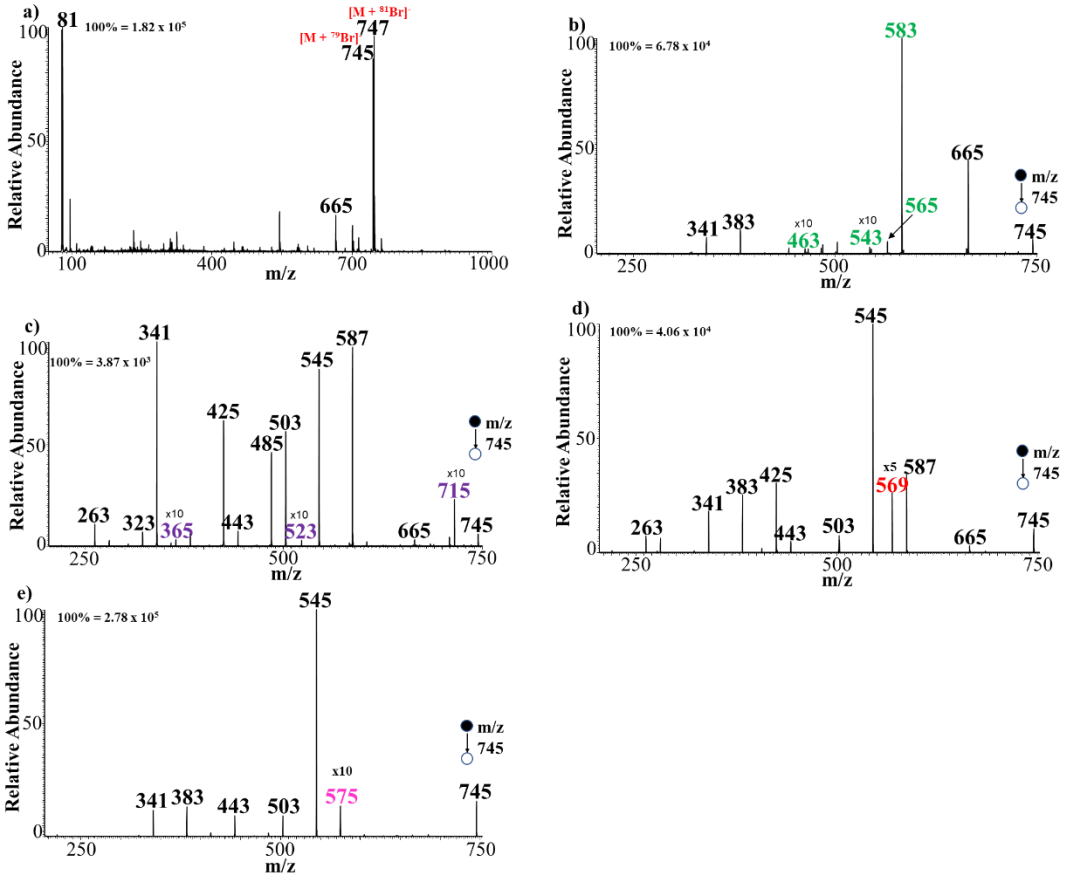


Figure S14. a) Representative negative-ion mode mass spectrum showing bromide adduct formed from spraying isomaltotetraose and ammonium bromide from theta capillary. Product ion tandem MS recorded for **b**) Stachyose **c**) Cellotetraose **d**) Maltotetraose **e**) Isomaltotetraose, all at m/z 745.

16. Tandem MS Analysis of Mixture of Tetrasaccharide Isomers

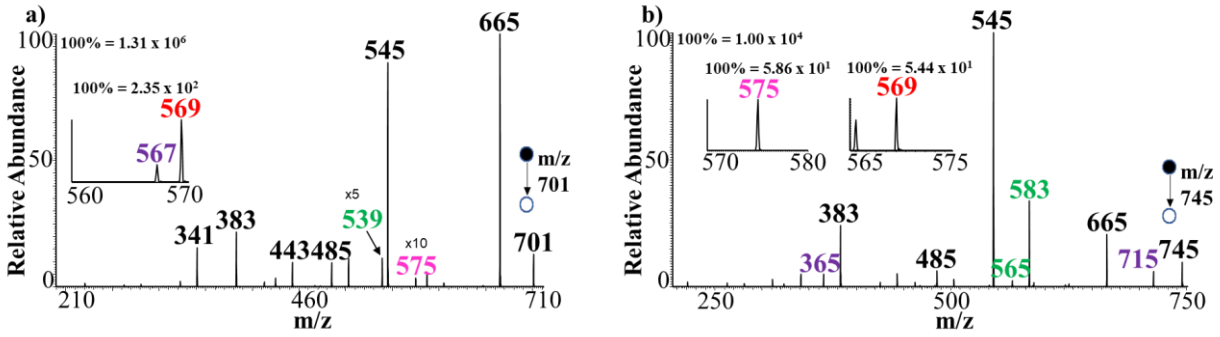


Figure S15. a) Negative-ion mode tandem MS analysis of chloride adducted mixture at m/z 701, inserts showing diagnostic ions for cellotetraose and maltotetraose. **b**) Negative-ion mode tandem MS analysis of bromide adducted mixture at m/z 745, inserts showing diagnostic ions for isomaltotetraose and maltotetraose.

17. Analysis of Pentasaccharide Isomers via Chloride Adduction

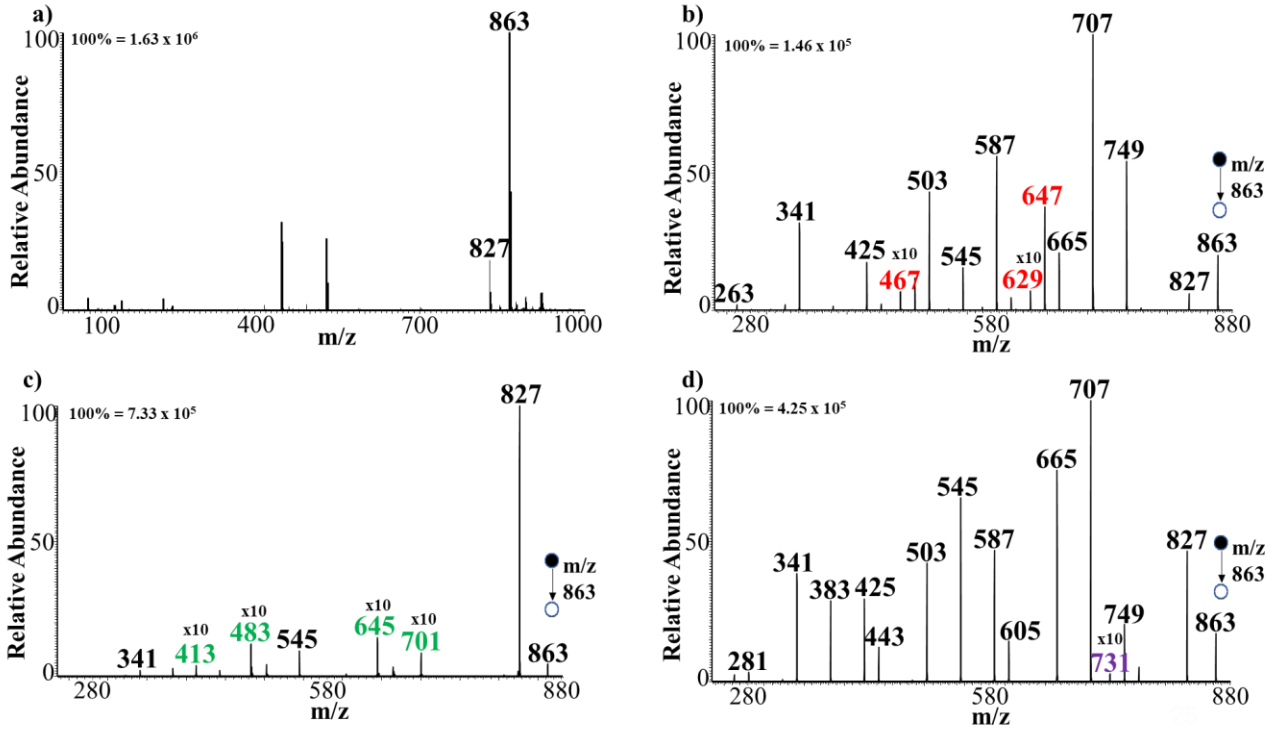


Figure S16. a) Representative negative-ion mode mass spectrum showing chloride adduct formed from spraying verbascose and ammonium chloride from theta capillary. Product ion tandem MS recorded for **b**) Maltopentaose **c**) Stachyose **d**) Cellopentaose, all at m/z 863.

18. Tandem MS Analysis of Mixture of Pentasaccharide Isomers

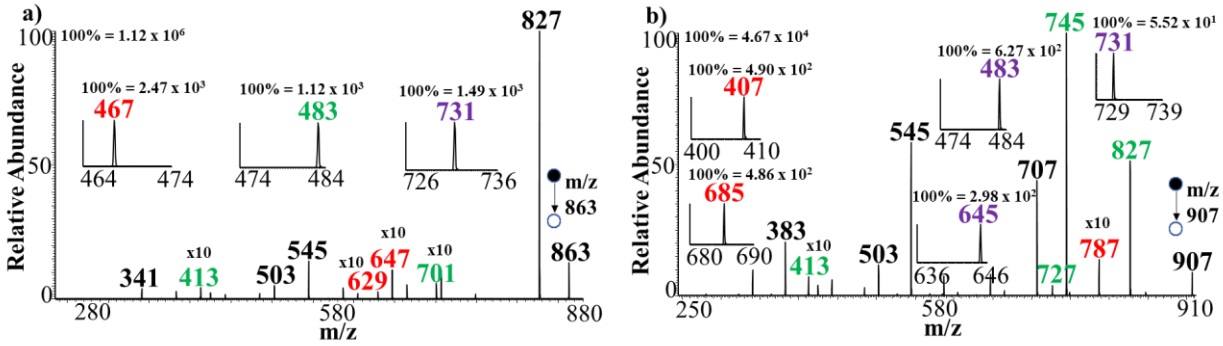


Figure S17. a) Negative-ion mode tandem MS analysis of chloride adducted mixture at m/z 863, inserts showing diagnostic ions. **b**) Negative-ion mode tandem MS analysis of bromide adducted mixture at m/z 907, inserts showing diagnostic ions for maltopentaose and cellopentaose.

19. Effect of Chain Length on Oligosaccharide Adduction

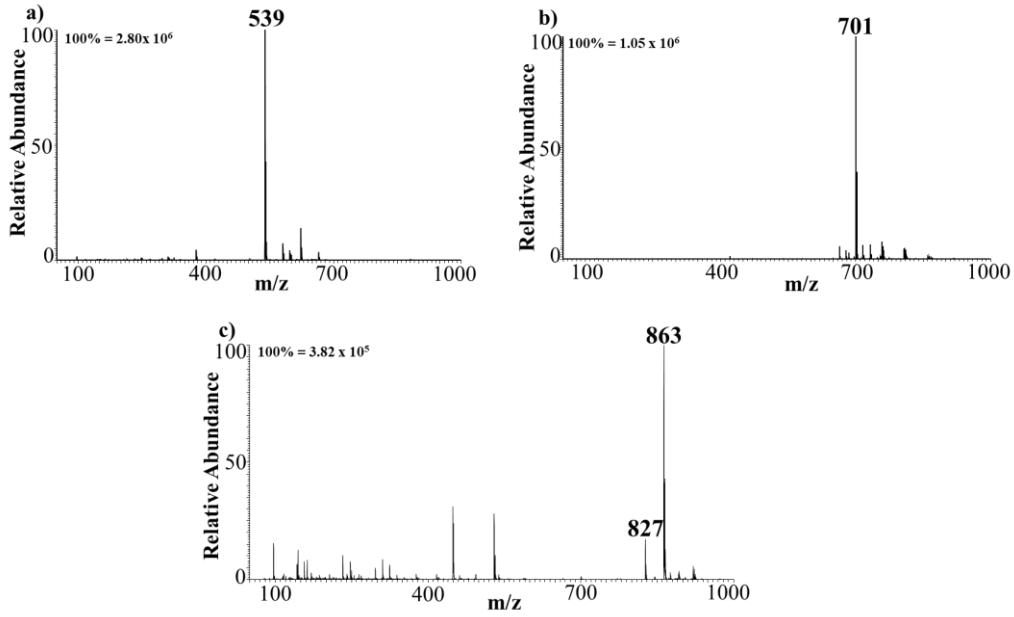


Figure S18. Representative negative-ion mode mass spectrum showing chloride adduct formed from spraying **a**)cellotriose **b**) cellotetraose **c**) cellopentaose and ammonium chloride from theta capillary.

20. Effect of Concentration on Adduct Formation

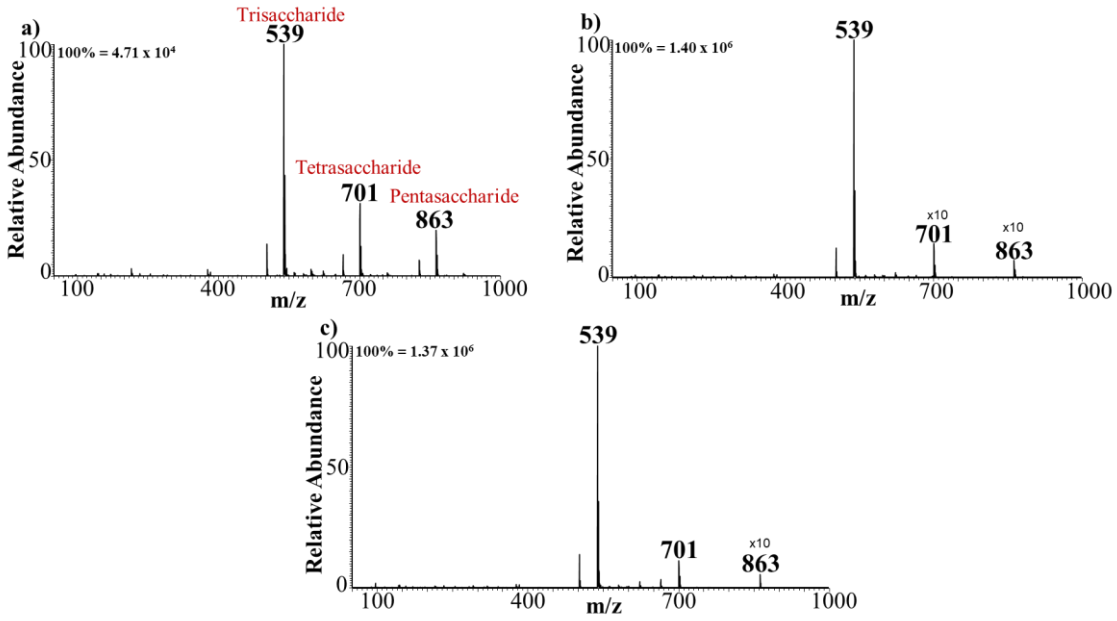
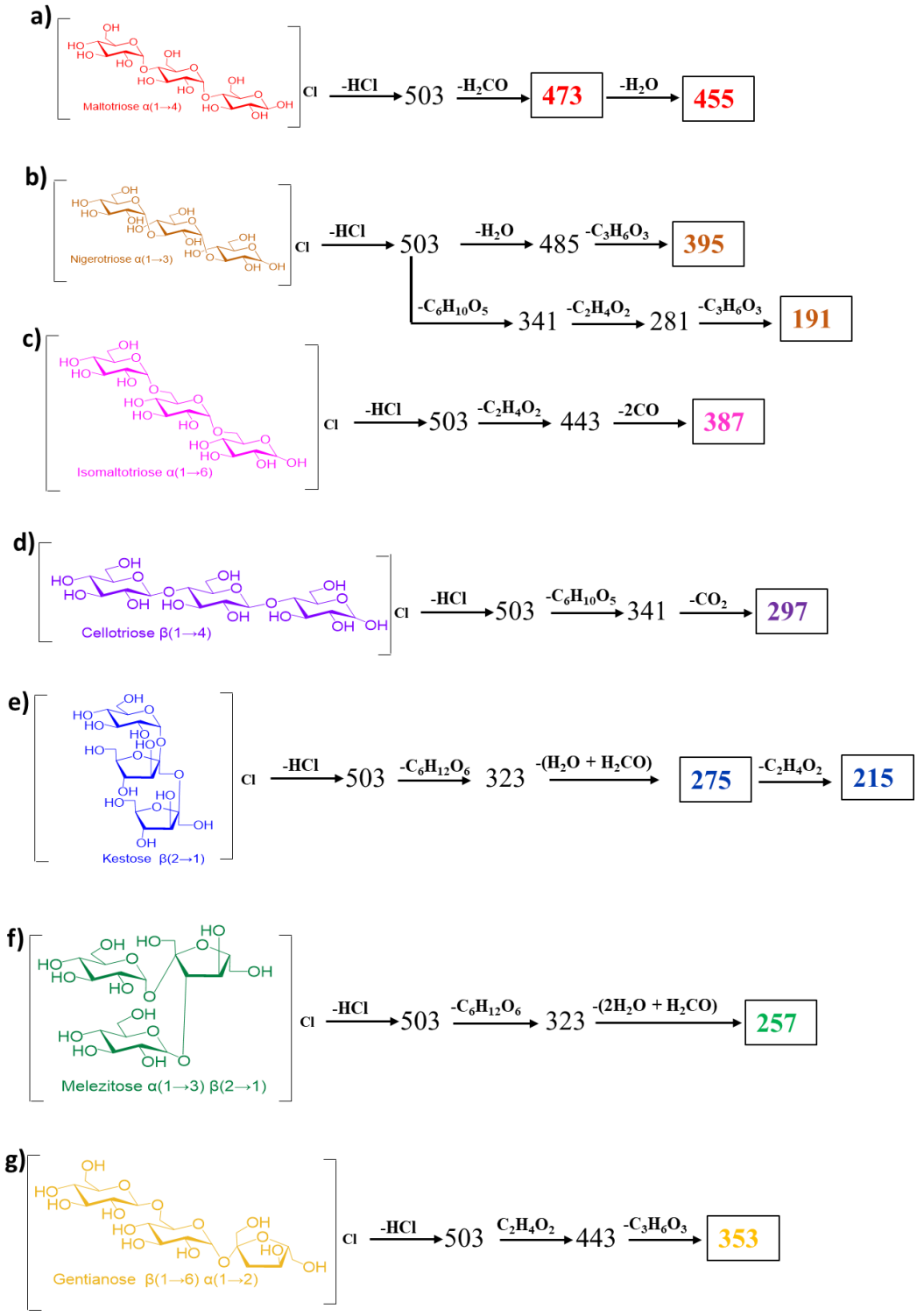


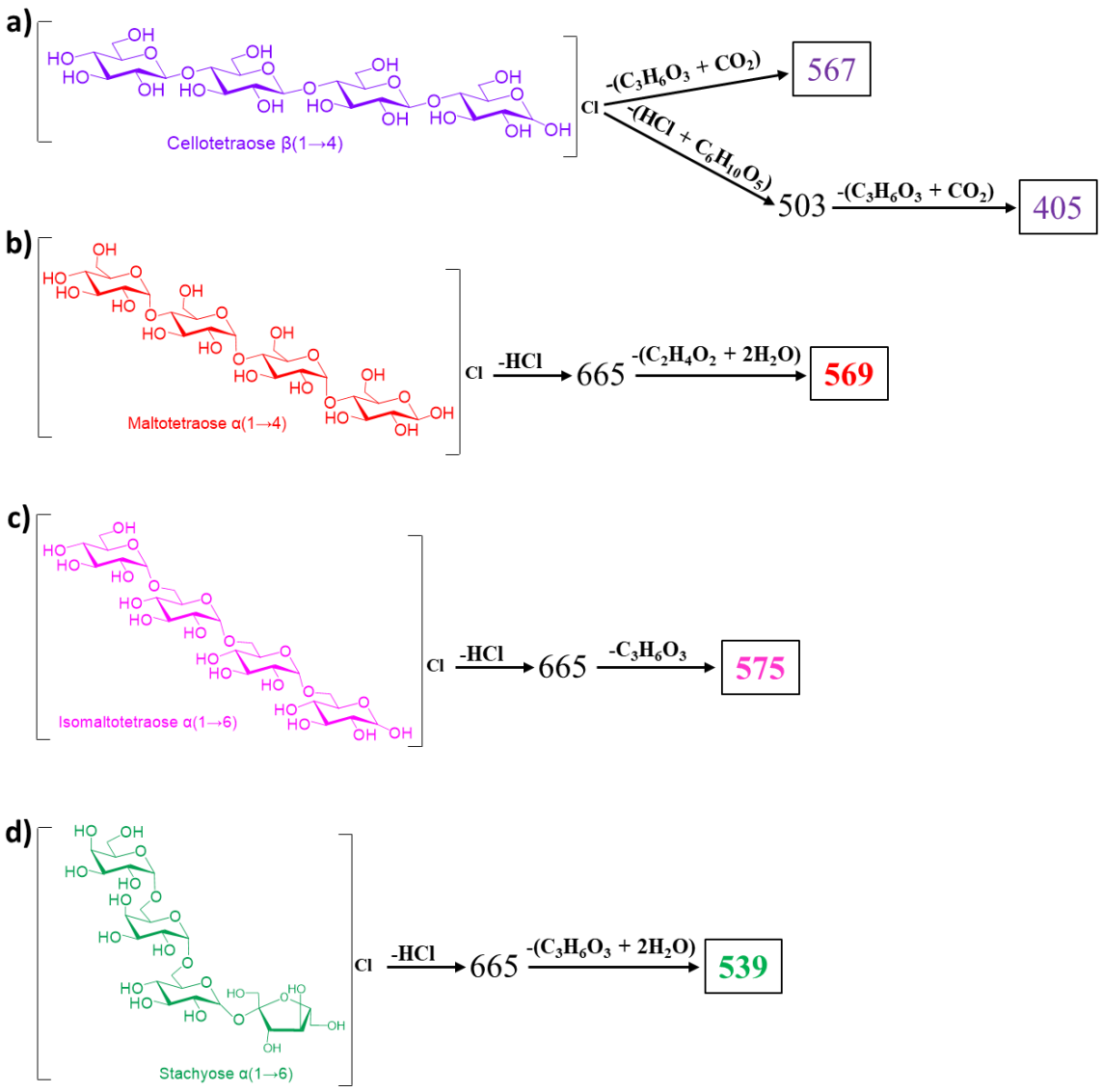
Figure S19. Negative-ion mode mass spectrum showing chloride adduct formed from spraying **a**) 5 µM Trisaccharides, 25 µM Tetrasaccharides, and 50 µM Pentasaccharides **b**) 50 µM Trisaccharides, 5 µM Tetrasaccharides, and 25 µM Pentasaccharides **c**) 25 µM Trisaccharides, 50 µM Tetrasaccharides, and 5 µM Pentasaccharides and ammonium chloride from theta capillary.

21. Fragmentation Patterns of Trisaccharides Isomers



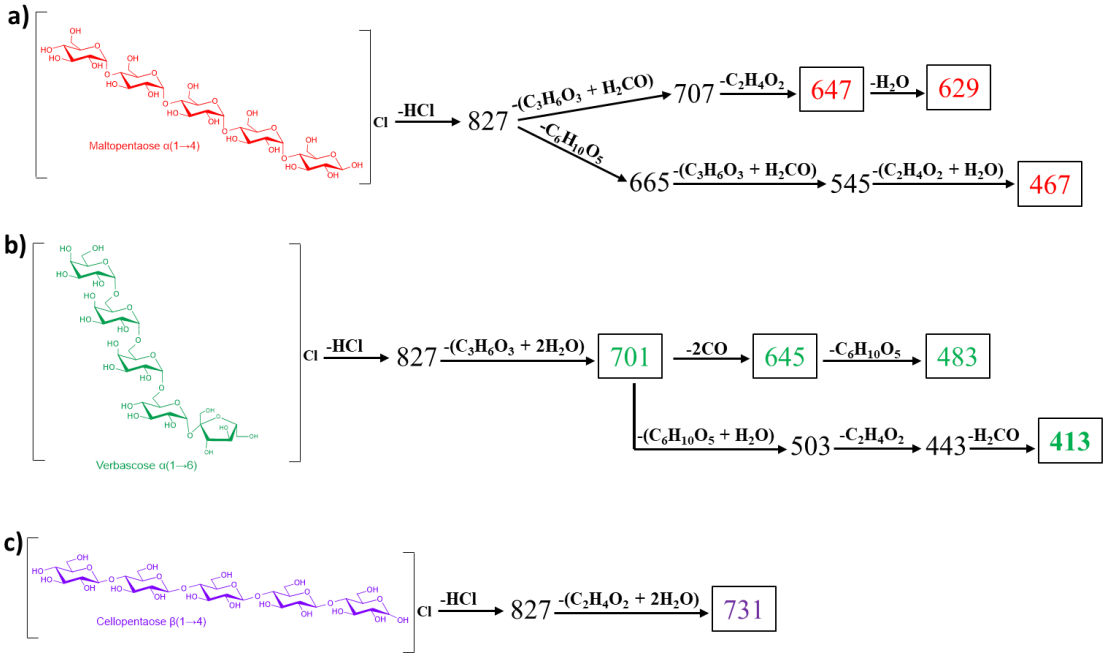
Scheme S1. Illustration of fragmentations observed for a) Maltotriose b) Nigerotriose c) Isomaltotriose d) Cellotriose e) Kestose f) Melezitose g) Gentianose

22. Fragmentation Patterns of Tetrasaccharide Isomers



Scheme S2. Illustration of fragmentations observed for a) Cellotriose b) Maltotetraose c) Isomaltotetraose d) Stachyose

23. Fragmentation Patterns of Pentasaccharide Isomers



Scheme S3. Illustration of fragmentations observed for a) Maltopentaose b) Verbascose c) Cellopentaose