

ASSESSMENT OF DIMERIC METAL-GLYCAN ADDUCTS VIA ISOTOPIC LABELING AND ION MOBILITY-MASS SPECTROMETRY

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INTRODUCTION

- Adduction of divalent metal cations with tetrasaccharides has been shown to have noticeable impacts on isomer drift time separation and alternate fragmentation pathways.^{1,2}
- However, arrival time distributions also have revealed the formation of dimeric metal-glycan species appearing at the same nominal m/z as the corresponding monomers.
- This has the potential to be problematic for interpretation of tandem MS spectra if heterodimeric species are formed.
- Using cobalt cation adduction, these heterodimers have been observed under some circumstances, thereby highlighting the need for mobility separation before tandem MS of metal adducted glycan species.

AIMS

- Use a set of isomeric tetrasaccharides—one of which was isotopically labeled—and two other glycans to assess the speciation of metal-glycan dimers formed with Co^{2+} .
- Determine if homodimers, heterodimers, or both are formed and what the implications may be for tandem MS of glycan mixtures.

METHODS

- Drift time, single stage MS, and tandem MS spectra were obtained using a dual gate, atmospheric pressure drift tube IM-MS system (Figure 1).
- A frequency chirp of 5-10,005 Hz applied to both gates modulated ion current such that drift times were frequency encoded for ions of interest (Figure 3).
- Arrival time distributions obtained for fragment ions are representative of the drift time profiles of the corresponding precursor producing each fragment—not of the fragments themselves—thereby permitting the identification of a given fragment's source based upon drift time.

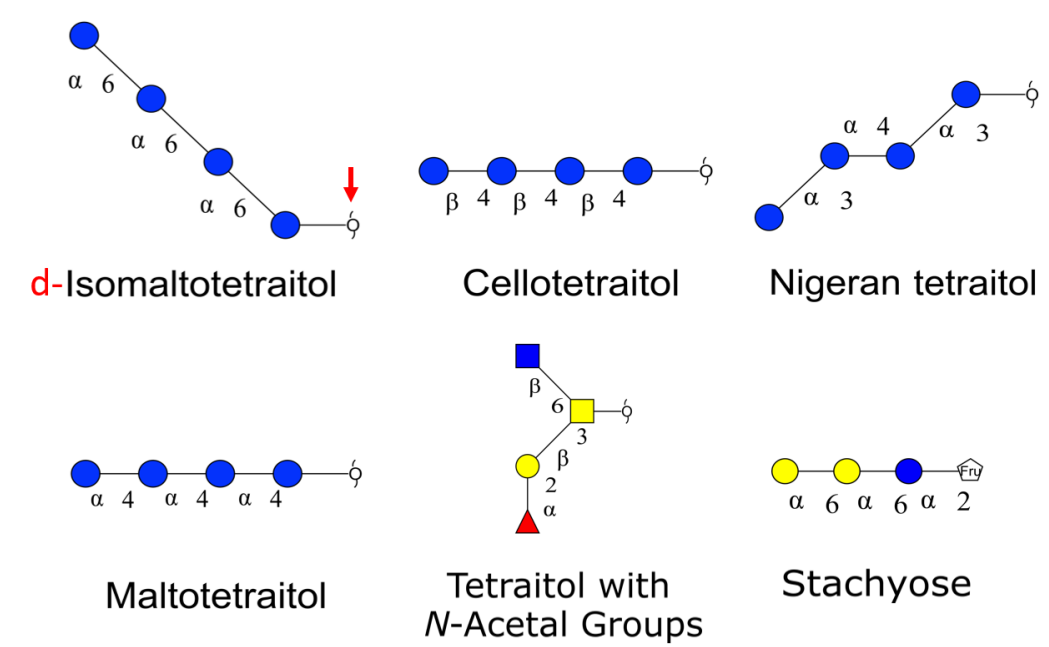


Figure 2. The six tetrasaccharides analyzed, with the first deuterated on the reduced reducing end hydroxyl.

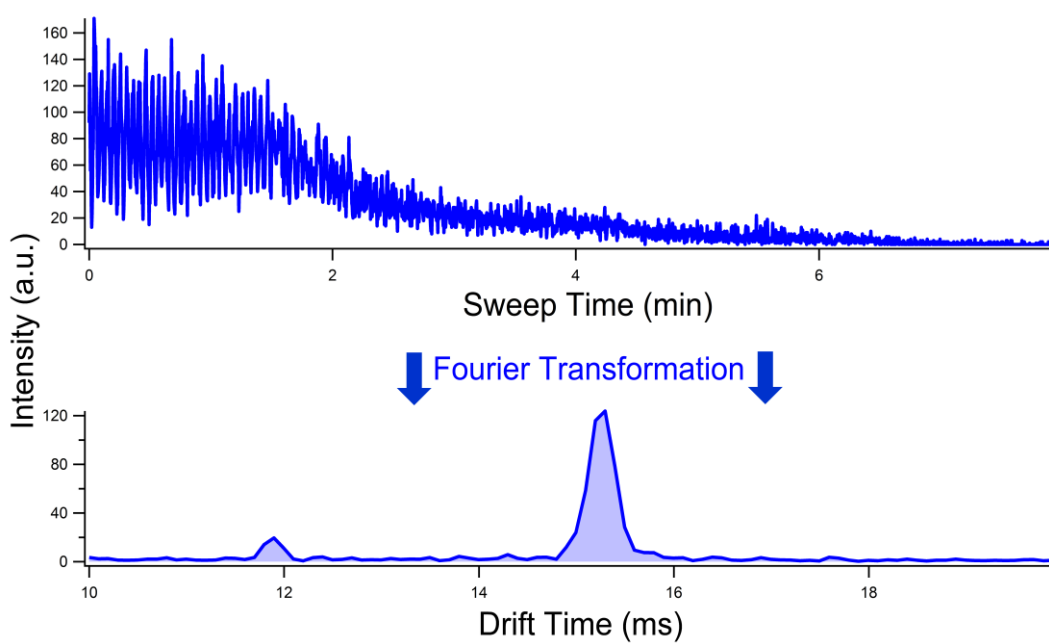


Figure 3. Example ion current decay and resulting arrival time distribution; d-isomaltotetraitol Co adduct is shown.

RESULTS

- Adduction of Co^{2+} with each tetrasaccharide resulted in each forming homodimers in individual solutions (Figure 4).
- Heterodimeric species have been observed to form under some circumstances, including with non-isomeric tetrasaccharide mixtures (Figure 5).
- The characteristic pattern of fragments for the cobalt-glycan dimer of each isomeric glycan species (Figure 6) can be used to monitor the components of dimers produced in isomer mixtures (Figure 7).
- Co dimers from mixtures of d-isomaltotetraitol with maltotetraitol and with nigeran tetraitol each have a single peak in the arrival time distributions, indicating that there may be a single dimer species.

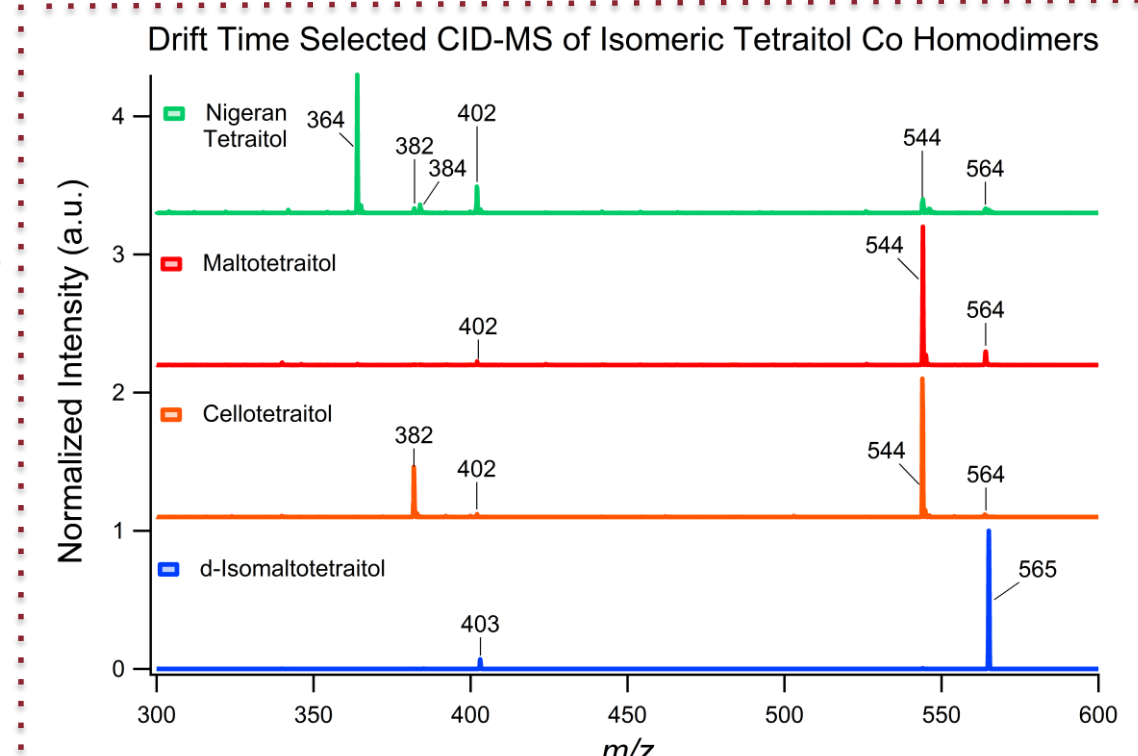


Figure 6. CID-MS spectra selected according to the individual, homodimeric Co-glycan adducts' drift times. Of note is that the fragment ions produced by deuterated isomaltotetraitol appear at 1 mass unit higher than comparable fragments from the other glycan species. Fragments at m/z 402/403 and 564/565 contain the reducing end, which is the location of the isomaltotetraitol deuterated hydroxyl.

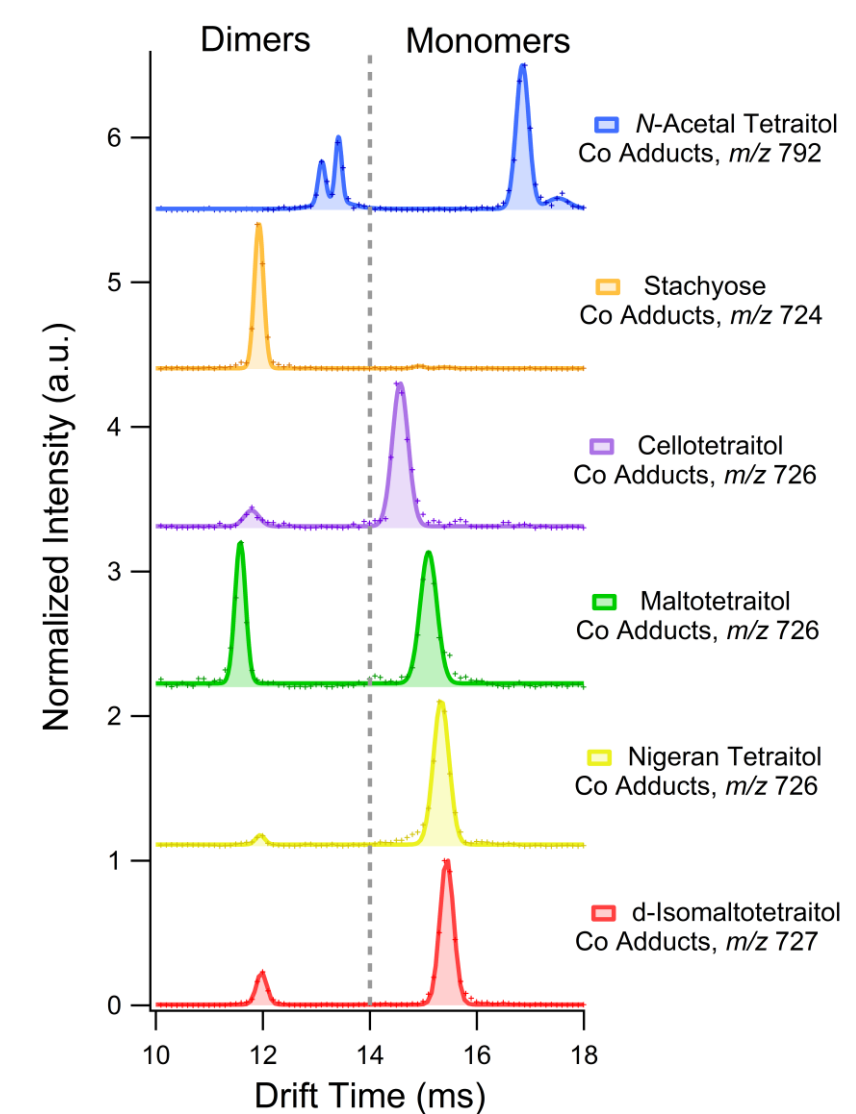


Figure 4. Arrival time distributions of cobalt-glycan adducts showing both monomeric and dimeric species.

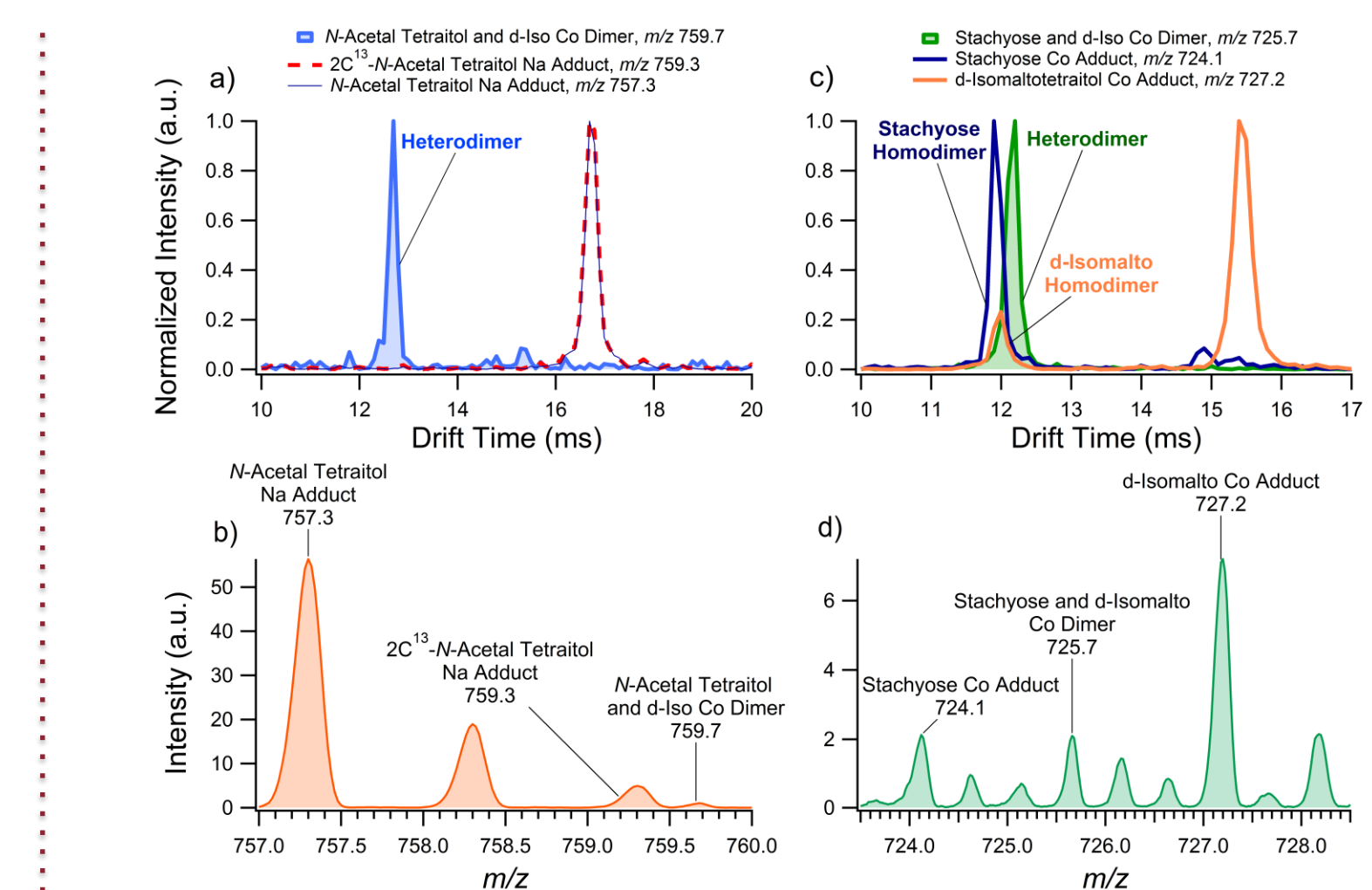


Figure 5. a) Drift time spectra for the Co heterodimer containing N-acetyl and isomalto tetraitol, appearing at a drift time distinct from the N-acetyl tetraitol Na adduct, b) despite being close in m/z . c) The individual Co homodimers for stachyose and for d-isomaltotetraitol have noticeably shorter drift times compared to the d-isomaltotetraitol-stachyose Co heterodimer, d) m/z 725.7.

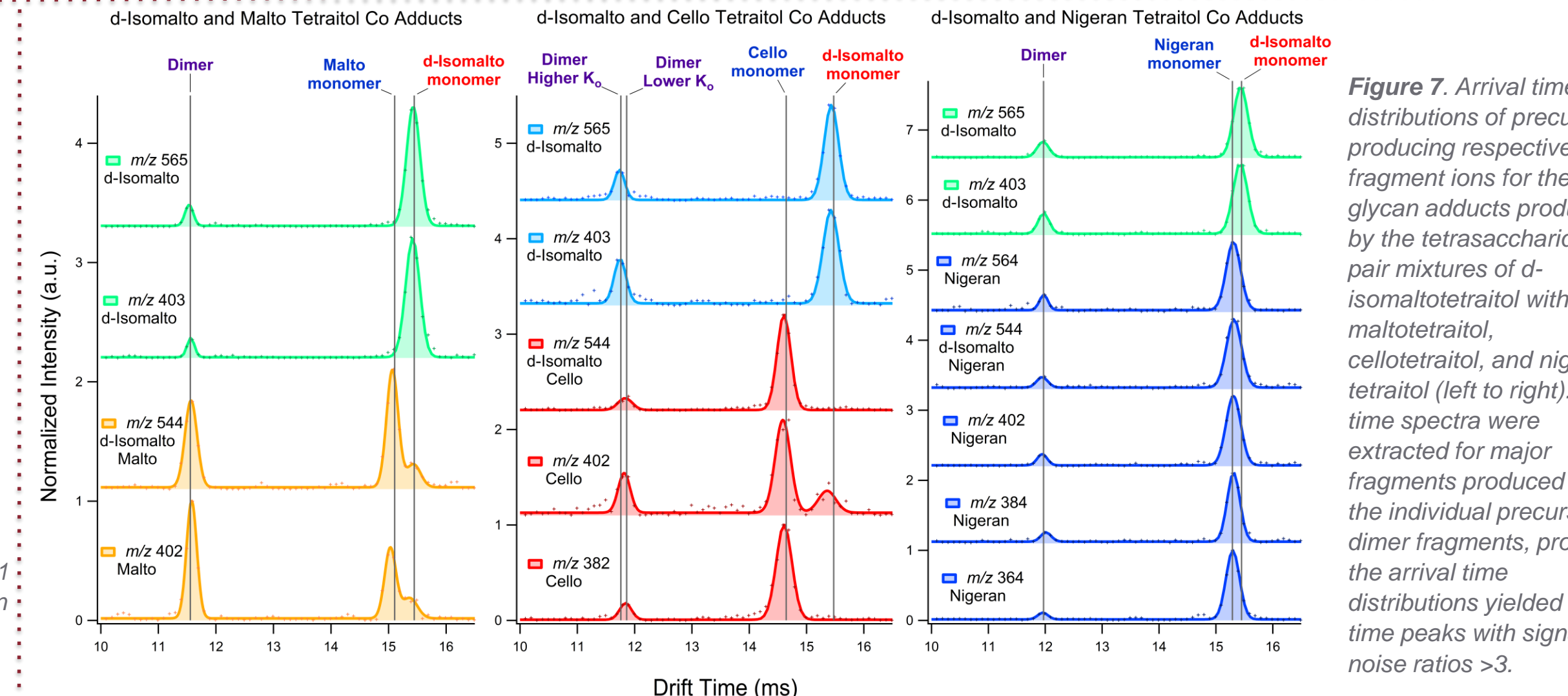


Figure 7. Arrival time distributions of precursors producing respective fragment ions for the Co-glycan adducts produced by the tetrasaccharide pair mixtures of d-isomaltotetraitol with maltotetraitol, cellotetraitol, and nigeran tetraitol (left to right). Drift time spectra were extracted for major fragments produced by the individual precursor dimer fragments, provided the arrival time distributions yielded drift time peaks with signal-to-noise ratios >3.

CONCLUSIONS

- Dimeric tetrasaccharide and Co^{2+} adduct formation with a deuterium labeled glycan indicated that metal-glycan heterodimers have the potential to form, even involving two non-isomeric glycans.
- Metal adduction to carbohydrates can provide benefits such as altered fragmentation pathways,^{2,3} but the possibility of heterodimers forming may confound attempts at glycan structure determination if no mobility separation is performed.
- Combining tandem MS techniques with a second dimension of separation in the drift time domain for analysis of glycan species will largely function to counteract complications from interfering heterodimers.

FUTURE DIRECTIONS

- Assess heterodimer formation involving polysaccharides with differing number of monosaccharide residues.
- Characterize influence of cobalt cationization on drift times of larger glycans.

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ACKNOWLEDGEMENTS

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ATMOSPHERIC PRESSURE, DUAL-GATE ION MOBILITY LINEAR ION TRAP MASS SPECTROMETER

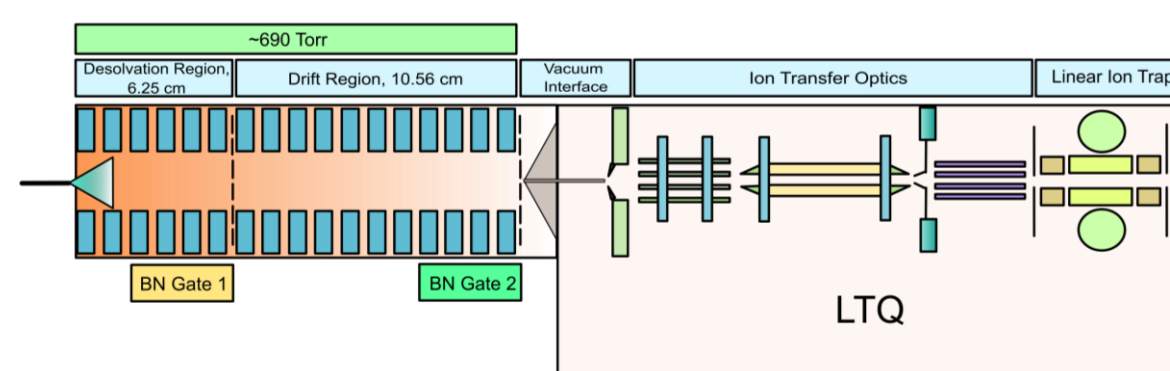


Figure 1. ExcellIMS MA3100 atmospheric pressure, dual-gate drift tube ion mobility spectrometer coupled to a Thermo LTQ linear ion trap mass spectrometer. The drift tube mounts to the LTQ mass spectrometer without any modifications to either system.

