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Resolving Structural Isomers of Monosaccharide Methyl Glycosides Using Drift Tube and Traveling Wave Ion Mobility Mass Spectrometry

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Abstract

Monosaccharide structural isomers including sixteen methyl-D-glycopyranosides and four methyl-N-acetylhexosamines were subjected to ion mobility measurements by electrospray ion mobility mass spectrometry. Two ion mobility-MS systems were employed: atmospheric pressure drift tube ion mobility time-of-flight mass spectrometry and a Synapt G2 HDMS system which incorporates a low pressure traveling wave ion mobility separator. All the compounds were investigated as [M + Na]⁺ ions in the positive mode. A majority of the monosaccharide structural isomers exhibited different mobility drift times in either system, depending on differences in their anomeric and stereochemical configurations. In general, drift time patterns (relative drift times of isomers) matched between the two instruments. Higher resolving power was observed using the atmospheric pressure drift tube. Collision cross section values of monosaccharide structural isomers were directly calculated from the atmospheric pressure ion mobility experiments and a collision cross section calibration curve was made for the traveling wave ion mobility instrument. Overall, it was demonstrated that ion mobility-mass spectrometry using either drift tube or traveling wave ion mobility is a valuable technique for resolving subtle variations in stereochemistry among the sodium adducts of monosaccharide methyl glycosides.

Introduction

Carbohydrates or glycans play important roles in a wide variety of biological processes^{1–5} and their structural elucidation is an essential prerequisite for understanding their many functions. They are highly variable in structure owing to differences in their monomer stereochemistries, inter-residue linkage positions, and general branching patterns.^{6–8} Moreover, their preparation from biological sources is frequently accompanied by complex mixtures, often isomeric mixtures, of molecules. NMR spectroscopy is useful for evaluation of isomeric heterogeneity, and for structural elucidation, but it is highly preferable to isolate single molecular species prior to determining the structures of unknowns using NMR.^{9–13} Typically, physical separation of the molecules is time consuming, usually involving more than one LC separation in more than one orthogonal LC mode.^{13–18} Mass spectrometric methods^{19–23} can analyze samples at far greater sensitivity, but have their own limitations.

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One critical issue is that mass spectrometry is not well suited for the evaluation of isomeric heterogeneity. With different variants of multi-stage mass spectrometry (MS^n) this applies not only to precursor ions having the same m/z but also to many product or multi-stage product ions. Mass spectra at any stage of isolation/dissociation might result from dissociation of more than one isomeric precursor or product ions. We sought to answer the question as to whether small carbohydrate ions, in this case methyl glycosides varying in their stereochemistries, could be physically resolved in the gas phase as a requisite for evaluating the isomeric heterogeneity of small product ions derived from larger oligosaccharide precursors. The means employed here was ion mobility spectrometry (IMS), which is capable of physically separating isomeric ions based on their different drift velocities through an electric field in a counter flow of neutral gas.^{24–29}

IMS separates ions based on an ion's collision cross section (Ω) to charge ratio. Ions of the same chemical formula but different sizes and configuration potentially can be resolved by IMS. When IMS is coupled to MS, it can provide valuable stereochemical information about analytes. Drift tube ion mobility mass spectrometry (DTIMMS) has been applied in the field of carbohydrate research and it has been reported that both isobaric carbohydrate standards and isomeric biological glycans could be unambiguously distinguished by IMS.^{30–35} Dwivedi et al.³⁰ initially demonstrated that monosaccharide methyl glycosides could be resolved by atmospheric pressure ion mobility time-of-flight mass spectrometry. However, a complete set of methyl glycoside isomers were not investigated and systematic structural information associated with the mobility of isomers was not provided. Moreover, IMMS has also been demonstrated as a valuable tool to analyze complex glycan samples. Measurements of glycan conformational and isomeric distributions by IMMS can give a more complete picture of the complexity of glycans accompanying disease states.^{34–35} The mass-mobility correlation band occupied by carbohydrates^{34, 36–37} can provide a general metric for assigning unknown signals to particular molecular classes. Recently, traveling wave ion mobility spectrometry (TWIMS) has been developed^{38–40} and applied to a wide range of applications^{41–46} including glycomics.^{47–50} The electric field, pressure and design of the system^{38–40} are different from traditional DTIMS, and so conducting identical studies on both systems would be beneficial to further characterize the TWIMS separation.

Here we compare the performance of an atmospheric pressure drift tube IMS, employing a novel resistive glass tube design and a traveling wave IMS in a Waters Synapt G2 instrument, at low pressure, in differentiating 20 monosaccharide methyl glycoside structural isomers. Many anomeric and epimeric pairs of methyl glycosides were resolved in analyses performed on the millisecond time scale on both systems. A detailed and direct comparison was made for the first time between the two different types of IMS systems based on the study of structural isomers of small, relatively rigid molecules.

Experimental Section

Electrospray Ionization Ambient Pressure Resistive Glass Drift Tube Ion Mobility Time of Flight Mass Spectrometer (ESI-AP-DTIM-TOFMS)

The instrument was described previously in detail⁵¹ by Kaplan et al. in 2010. This system will be simply referred to as the DTIMS in the following text. In this study, voltages applied to the ion gate and IMS exit were 9007 V and 773 V respectively, which resulted in a homogeneous drift field of 412 V/cm. The gate pulse width was 200 μ s. A counter flow of nitrogen (1.5 L/min) was introduced at the exit end of the drift tube to provide both the drift gas and also to aid efficient desolvation of ions prior to the ion gate. The IMS tube was placed in a stainless steel cylindrical tube of the same length that did not touch the resistive glass, with an air gap of ~ 5 mm in between. The stainless steel tube was surrounded by heating jackets and heated. The buffer gas temperature was measured to be 92 °C.

TofDaqViewer software, developed by TOFWERKS AG, was used to view and collect all the data from the instrument. The data from each sample could either be completely or selectively exported based on the user-specified time range in the form of a 2D text file. IDL virtual machine software (www.exelisvis.com) was then used to generate 2D IMMS correlation spectra based the data exported from TofDaqViewer. The ESI was constructed at WSU. Detailed information on its construction and operation are given in the supporting information. The voltage applied to the ESI needle was 14.5 KV, producing a 3KV difference between the ESI needle and the entrance of the ion mobility spectrometer. ESI solvent used in this study was a 50:50 (v/v) methanol: water mixture.

Synapt G2 HDMS System-Traveling Wave Ion Mobility Spectrometry (TWIMS)

The Synapt G2 HDMS (high definition mass spectrometry) (Waters Corp., Manchester, UK) is a hybrid quadrupole/IMS/orthogonal TOFMS instrument and has been previously described.^{38–40, 52, 53} The moving/non-uniform electric field in Synapt G2 is called the traveling wave or T-wave. In the IM cell of the Synapt G2, the ability of the ions to pass through the neutral gas under the influence of a traveling wave electric field is dependent on its mobility. Ions with high ion mobility are more able to keep up with the traveling wave and are overtaken by the pulses less often than the ions with low mobility.^{38, 39} This system will be simply referred to as TWIMS hereafter. In order to enhance the IM resolution, second-generation IM technology has been incorporated into the Synapt G2 instrument.^{52, 53} The resolving power of the TWIM cell in the Synapt G2 was increased 3~4 times compared to the traditional Synapt.

ESI voltage was at 3.0 KV and nitrogen was used as the drift gas at 90 mL/min. The TWIMS cell in this study was operated at nominally 3 mbar N₂ with a 40V, 900 m/s T-Wave. A complete set of instrumental parameters are included in the supporting information. Masslynx 4.1 (Waters Corporation, Milford, MA, USA) was used to collect and process all the data.

Chemicals and Solvents

All sugars used were the D-enantiomers. The α - and β -methyl glycopyranosides of galactose, glucose, mannose and 2-acetamido-2-deoxy-glucose were purchased from Sigma. The α - and β -methyl glycopyranosides of 2-acetamido-2-deoxy-galactose were from Calbiochem. Those of allose, altrose, gulose, idose and talose were synthesized by Fischer-type glycosidation. The synthetic method, NMR spectra, chemical shifts and *J*-couplings are presented in the supporting information section.

For ion mobility studies, 20 μ L of a 500 μ M sample stock solution and 10 μ L of a 1 mM NaCl stock solution were added to 1mL ESI solvent, resulting in a sample concentration of 10 μ M and sugar/NaCl ratio of 1 : 1. Glycosides were examined to avoid any configurational interconversion or additional complexities observed for reducing sugars, which are typically present in aqueous solvent mixtures in multiple anomeric configurations and ring forms. HPLC grade solvents (methanol, water) were used and purchased from J. T. Baker (Phillipsburgh, NJ).

Reduced Mobility K_o , Collision Cross Section Ω , Separation Factor α and Resolving Power R_p

The mobility (K), reduced mobility (K_o) and collision cross section (Ω) of an ion and their calculations have been widely described.^{24, 27, 30–34} Values of Ω can be directly determined from experimental data obtained using DTIMS. Due to the non-uniform electric field in TWIMS, there is currently no method to calculate Ω values directly from experimental data. However, Ω values can be estimated based on the calibration methods detailed previously in

the literature^{45, 46, 54–56} for TWIMS. Briefly, ions with known Ω values are used as calibrants and their drift times on TWIMS are measured. In general, a calibration curve by plotting $(\Omega \mu^{0.5})/(ze)^{45, 46, 54–56}$ values versus TWIMS corrected drift times, t_d' , is made. The Ω values for unknown compounds in TWIMS are thus determined using the calibration curve and drift time information obtained under exactly the same instrumental conditions. In this study we plot directly the Ω values obtained from the DTIMS versus t_d' obtained using the TWIMS. Adjustment of Ω for reduced mass and charge state is not essential here since the reduced mass is almost the same for all species studied and all are singly charged. The t_d' values have been corrected for m/z dependent and independent offsets in the TWIMS as described elsewhere.^{45, 46} A power trend line of the form $\Omega=A(t_d')^B$ is used to fit the data, where A is an incorporated correction factor for the electric field and other parameters on the TWIMS system; B is dependent on many parameters such as T-Wave amplitude and velocity and accounts for the non-linear effects in TWIMS.

The separation factor (α) is by $\alpha = t_{d(2)}/t_{d(1)}$ where $t_{d(2)}$ is the drift time of the slower drifting ion and $t_{d(1)}$ is the drift time of the faster drifting ion. The experimental resolving power (R_p) is traditionally defined by the drift time t_d divided by the peak full width at half maximum (FWHM): $R_p = t_d/\Delta t_d$. The R_p of TWIMS was theoretically studied by Shvartsburg and Smith in 2008⁴⁰ where they showed that due to the non-linear relationship between mobility and drift time on the TWIMS, the mobility resolving power is approximately twice the temporal resolving power, R_p . Zhong et al.⁵³ have experimentally characterized the ion mobility resolution of the second generation TWIMS as a function of different experimental parameters. Both theory and experiment show that the resolution is dependent on wave height, wave velocity, IM cell length, pressure and other parameters.

Results and Discussion

Structures of 20 monosaccharide methyl glycoside isomers and MS spectra

Monosaccharides are the basic units of larger carbohydrate molecules. For the methyl-D-pyranosides of hexoses, where the chirality at C-5 by definition is invariant, four additional chiral carbons are present that may vary in stereochemistry (C-1 to C-4), giving rise to 16 isomeric forms with the same exact mass and chemical formula. In addition, 4 isomers of the methyl glycosides of two important *N*-acetylhexosamines: *N*-acetylgalactosamine and *N*-acetylglucosamine, were also included in this study. The structures and nomenclature for all 16 methyl-D-glycopyranosides and the 4 methyl-*N*-acetyl-D-hexosamines are shown in Fig. 1a. Representative mass spectra for the sodiated adduct of α -Me-glucopyranoside at m/z 217 and α -Me-*N*-acetylglucosamine at m/z 258 are shown in Fig. 1b and Fig. 1c. Other methyl-glycopyranosides and methyl-*N*-acetylhexosamines had identical mass spectra to those shown in Fig. 1b and Fig. 1c, respectively. Therefore using MS alone, it is impossible to differentiate those isomers that have identical m/z values. Carbohydrates have an especially large number of stereo-isomeric variants differing at anomeric and/or epimeric positions. Anomers are cyclic structures that only differ in the configuration at the acetal carbon for glycosides (carbon 1) which is called the anomeric carbon. For the 4C_1 chair conformations as drawn in Fig. 1a, α -glycosides have the $-\text{OCH}_3$ in the axial position, and β -glycosides have the $-\text{OCH}_3$ in the equatorial position. Epimers are diastereomers that differ at only one asymmetric carbon. For example, α -methyl-talopyranoside and α -methyl-galactopyranoside are epimers differing only at carbon two (Fig. 1a). Overall, all the structures are categorized into β and α configurations in Fig. 1a with their corresponding names shown in the right column. It is evident that all the isomers only vary in subtle structural differences.

Overall mobility separation patterns of monosaccharide structural isomers by DTIMS and TWIMS

The overall ion mobility spectra of the 16 structural isomers of the methyl-D-glycosides obtained on the DTIMS and TWIMS instruments are displayed in Fig. 2a and Fig. 2b, respectively. Corresponding abbreviations are listed on the right. Each DTIMS mobility spectrum was acquired for 5 minutes with 3 $\mu\text{L}/\text{min}$ ESI sample flow rate. Each dataset shown in Fig. 2b from TWIMS was acquired for two minutes with a 5 $\mu\text{L}/\text{min}$ infusion of the sample. The results showed that the majority of monosaccharide structural isomers exhibited unique mobility drift times, even though not all of them were fully resolved. Many drift time orders matched between these two sets of data, but not all. Based on the traditional definition, R_p for DTIMS was ~75 with FWHM of 0.3 ms; for TWIMS the temporal R_p was ~15–20 with FWHM of around 0.16 ms, which translates to a mobility resolution of 30–40 (see above), which is in keeping with results presented previously for singly charged species.⁵² It should be noted that the experimental conditions for DTIMS in this study were for high sensitivity (operating conditions for the DTIMS were set to approximate the sensitivity of the TWIMS), not optimum resolving power; the measured resolving power for the DTIMS was ~83 % of its optimum resolving power under the same operating conditions.⁵⁷

In all of the spectra, the predominant carbohydrate ions produced by ESI were found to be the Na^+ adduct of the saccharide. The formation of metal ion-saccharide adducts are common in electrospray ionization. $[\text{M}+\text{H}]^+$ ions were also detected at low abundance for certain monosaccharide structural isomers, however, the mobility separation between isomers was less than for the sodiated ions (see supporting information). Cerda and Wesdemiotis⁵⁸ using *ab initio* calculations demonstrated that Na^+ interacts with sugars through multidentate coordination with oxygen lone electron pairs. The favored [monosaccharide + Na^+] structures contained pyranose rings in the chair or boat conformation that permits tri or tetradeinate coordination of Na^+ . Thus the resolution observed among structural isomers of monosaccharide methyl glycosides is attributed not only to their different stereochemistries but also to the conformational changes induced by the Na^+ metal ion. Clearly, different methyl glycosides vary in the compactness of their Na^+ adducts which is reflected in their overall cross-sectional areas and drift times. With both instruments, the α -Tal stereochemical arrangement showed the shortest drift time and α -Glc had the longest drift time. The major differences observed between mobility profiles in comparing the two instruments were between the α -, β -Ido, and α -, β -Gul configurations. With DTIMS, very similar drift times were observed, and reduced mobility differences (Table 1) were within the expected experimental variation and could be considered not separated. While TWIMS exhibited better separation, the α -Gul (2.89 ms) and β -Gul (2.81 ms) anomers had longer drift times than the α - and β -Ido anomers (2.70 ms). The TWIMS and DTIMS techniques, while similar, are different in pressure, electric field homogeneity and temperature, thus some differences in resolution and relative order of separations empirically might have been expected.

The mobility spectra of four methyl-*N*-acetylhexosamines that are commonly found in mammalian oligosaccharides are shown in Fig. 3. While separation between sodium adducts of the epimers was observed, no separation was seen between anomers. Interestingly, the α - and β -Me-GalNAc anomers drifted faster (higher mobility) than the α - and β -Me-GlcNAc anomers (lower mobility). This suggests, at least with sodium adducts, that the methyl-GalNAc species adopt more compact overall structures than those of the methyl-GlcNAc anomers. In addition, the drift time profile of these four isomers matched exactly between the two IMS systems. Overall, monosaccharides having different stereochemistries coordinate the sodium ion differently, depending on the electron donor groups available and their relative 3-dimensional spatial orientations. This clearly results in different overall

shapes and compactness for the coordination complexes of different methyl glycosides, thereby resulting in different ion mobilities.

Separation between anomers and epimers

All the monosaccharide structural isomers in this study can be categorized as 10 pairs of anomers based on the orientation of the $-OCH_3$ group in either the axial (α) or equatorial positions (β) as shown in Fig. 1a. Six pairs of anomers including the α and β -Tal, Man, Glc, Gal, Alt and All were baseline or fully separated in DTIMS. Separation for the same six pairs of anomers was also observed on TWIMS, however, with lower resolution. However, as mentioned previously, the anomer pair of α and β -Gul was partially separated in TWIMS, while no separation was observed in DTIMS. Examples of the separation profiles of 4 pairs of anomers on the two different systems are shown in Fig. 4. Two-dimensional IMMS plots overlaid from individual IMS spectra obtained from DTIMS are shown with m/z along the x axis and mobility drift time along the y axis having units in μs . For each anomeric pair, the equivalent 1D overlaid TWIMS spectra are also displayed.

It is worthy of note that in some cases (Fig. 4, panels a and d) the α anomer showed a shorter drift time than the β , but in other cases (Fig. 4, panels b and c), the reverse was true. It is important to point out that *ab initio* calculations⁵⁸ have indicated that some sugars coordinate with preferred conformations that can be either 4C_1 or 1C_4 chair forms, or boat forms, and more generally for all stereoisomeric variants, other forms such as the skew, half-chair or sofa forms would need to be seriously considered in theoretical calculations. Depending on the stereochemistry and relative orientation of hydroxyl groups, different methyl glycosides could participate in different multidentate coordination complexes having different shapes and compactness and with different positioning of the central Na^+ either above or below a plane drawn between C-1, C-3 and C-5, for example. While the detailed coordination complexes are beyond the scope and intent of this paper, it is evident that solely modifying the stereochemistry at C-1 can dramatically affect the ion mobility of sodium complexes, and their overall cross-sectional areas are experimentally significantly different as evaluated by ion mobility spectrometry.

In the same way, all the 20 monosaccharide methyl glycosides can be classified as 26 pairs of epimers depending on the asymmetry at carbons 2, 3 and 4. For each α and β anomeric configuration, C-2 epimers include the pairs of Tal and Gal, Man and Glc, Ido and Gul and Alt and All; C-3 epimers include the pairs of Tal and Ido, Man and Alt, Gul and Gal and Glc and All; C-4 epimers include pairs of Tal and Man, Ido and Alt, Glc and Gal, Gul and All and GalNAc and GlcNAc. Six out of eight pairs of C-2 epimers, five out of eight pairs of C-3 epimers and eight out of ten pairs of C-4 epimers were baseline/fully separated using DTIMS. For TWIMS, five pairs of C-2 epimers demonstrated good separation and eight pairs of C-4 epimers were baseline or partially differentiated, however, only α -Glc and α -All showed significant separation for C-3 epimers. Fig. 5 shows the overlaid 2D IMMS plots and overlaid 1D IMS plots for 4 representative epimeric pairs including (a) C-2 epimers of β -Glc and β -Man; (b) C-3 epimers of α -Gal and α -Gul; (c) C-4-epimers of β -All and β -Gul; (d) C-4 epimers of α -GlcNAc and α -GalNAc. Specific epimeric carbons are highlighted by blue dots. These four epimeric pairs having the same m/z were all fully separated on the mobility scale in the DTIMS system. In the TWIMS system, separation between epimeric pairs of β -Glc and β -Man, β -All and β -Gul, and α -GlcNAc and α -GalNAc was observed, although peaks were partially overlapping. There was a little separation between α -Gal and α -Gul in TWIMS. The higher separation degree and resolution for these 4 epimeric pairs in the DTIMS compared to the TWIMS system is apparent. Clear separation was achieved when glycosides varied solely in the stereochemistry at one carbon as shown in Fig. 5, for many epimeric pairs. It can therefore be concluded that metal ion coordination of carbohydrates as characterized by ion mobility measurements in the gas phase is exquisitely

sensitive to changes in the saccharide stereochemistry. The stereochemistry at positions C-1, C-2, C-3 and C-4 all influence complexation with the metal ion as measured by ion mobility and evidently contribute to the shapes and overall conformations of the sugar complexes.

Separation of these compounds as mixtures was also considered an important point to demonstrate. Selected mixtures of different isomers having equal concentrations of 10 μM were examined and it was demonstrated that the same mobilities were observed in the mixture as seen with the compounds run individually (see supporting information).

K_o, Ω and α calculations

The parameters of drift time (t_d and t_d'), reduced mobility (K_o), collision cross section (Ω) and separation factor (α) for the anomeric pairs of all 20 monosaccharides are displayed in Table 1. Reproducibility was checked with selected individual samples (see supporting information), indicating variations in K_o of ± 0.01 ($\text{cm}^2 \cdot \text{V}^{-1} \cdot \text{S}^{-1}$) on the DTIMS system; Drift time variation is ± 0.03 ms on the TWIMS system. The reported K_o and Ω values were determined directly from the experimental data obtained from the DTIMS. Ambient pressure in Pullman, Washington ranged from 690 Torr to 700 Torr during this study. As listed, K_o values ranged from 1.53 to 1.28 $\text{cm}^2 \cdot \text{V}^{-1} \cdot \text{S}^{-1}$ with corresponding Ω values ranging from 127 to 152 \AA^2 for all 20 methyl glycoside structural isomers. Values of $\alpha^{(a)}$ were calculated by using the ratio of $K_{o(2)}/K_{o(1)}$ (where 2 is the ion having the higher K_o and 1 is the ion having the lower K_o). This ratio accounts for any pressure and temperature influence on the variation of drift time values. Values of $\alpha^{(b)}$ were obtained from the ratio of t_d' values obtained using the TWIMS system. Even though, as shown in Fig. 4, some isomeric mobility peaks were better separated in the DTIMS system as compared to TWIMS, the separation factors from the two systems for the same anomeric pairs generally matched with little variation. TWIMS had similar α values in most of the cases or even higher α values in some cases (such as between α - and β -Gul and α - and β -Glc) compared to DTIMS. In the same way, α between any pairs of monosaccharide structural isomers could also be calculated and compared. However, the data reported in Table 1 were determined using one set of experimental conditions on each system and the variation of α values between the two instruments could be observed under a range of different experimental conditions.

A plot of Ω vs. t_d' is shown in Fig. 6 by using the data in Table 1. Two points overlapped, therefore only 18 points are observable in the figure. Fitting the data using the power law expression given earlier, a curve with the relationship $\Omega = 73.06(t_d')^{0.59}$ and $R^2 = 0.91$ is found. Good correlations have been observed previously for TWIMS calibration curves of this type in a wide range of applications.^{45, 46, 54–56} However, in reality the isomeric monosaccharide methyl glycosides do not serve as particularly good calibrants since they all share *m/z* values that were too close and as shown in Fig. 1(b) and 1(c), very similar drift times hence only cover an extremely small drift time region (mainly distributed between 2.6–3.0 ms), which could contribute to the relatively low R^2 value. In addition, it was observed that some isomers were distributed in vertical or horizontal patterns in Fig. 6, which means that some specific isomers were separable in one mobility system (different Ω values or t_d' values), while not in the other (same t_d' values or Ω values). The TWIMS and DTIMS techniques, while similar, have fundamental differences in the way they separate ions such as electric field homogeneity, temperature and pressure, thus some differences in resolution is not unexpected.

Conclusions

This study demonstrated the separation of 20 structural isomers of monosaccharide methyl glycosides using two different ion mobility instruments: ESI-AP-DTIM-TOFMS (DTIMS) and the Synapt G2 (TWIMS). It was shown that stereoisomers of methyl glycopyranosides

exhibited different mobilities and although some of them were well resolved, some isomeric pairs showed overlapping peaks. Using only one separation gas with each instrument and only one alkaline earth metal, sodium, it was possible to baseline separate a number of isomeric compounds having only subtle structural differences. Coordination strength, -OH and -OCH₃ group configurations and coordination geometry induced by Na⁺ adduction all influenced ion mobility drift times of the different sugar stereoisomers. Different drift gases and metal ions^{30, 59} may be needed to resolve other pairs of isomers in future studies. As expected, the DTIMS system provided higher resolving powers than the TWIMS system but the separation factors for anomeric pairs between the two instruments were similar. Even though there were many similarities between the separations using DTIMS and TWIMS, some differences were observed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

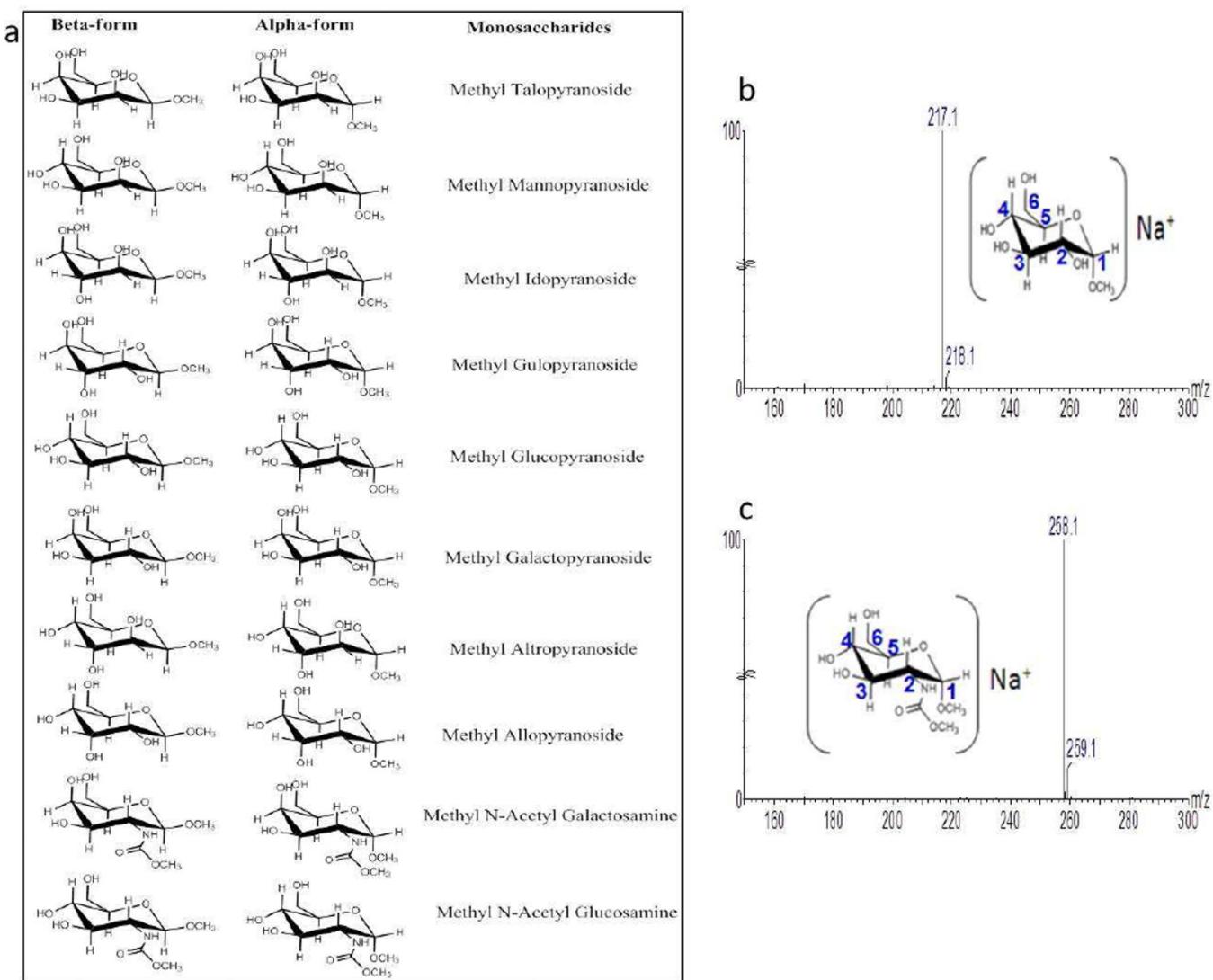
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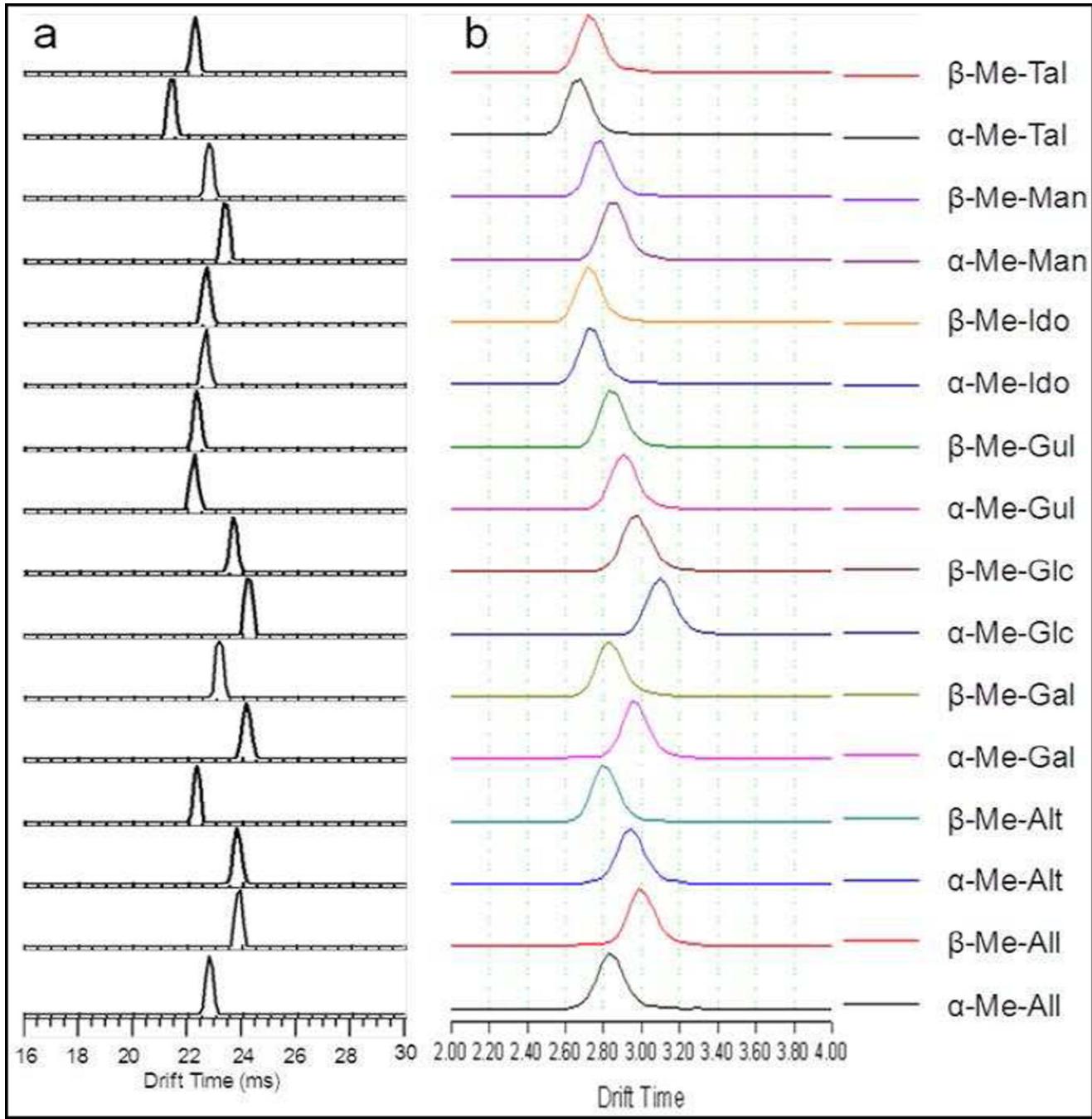
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**Figure 1.**

(a) Structures and nomenclature for the 16 methyl-D-glycopyranosides and 4 methyl-*N*-acetylhexosamines used in this study. (b) The mass spectrum of α -Me-glucopyranoside as a $[M+Na]^+$ adduct at m/z 217. The other 15 methyl-D-glycopyranosides showed essentially the same mass spectrum. (c) The mass spectrum of α -Me-*N*-acetyl glucosamine as a $[M+Na]^+$ adduct at m/z 258. The other 3 methyl-*N*-acetylhexosamines showed essentially the same mass spectrum. Sugar carbons are numbered as illustrated.

**Figure 2.**

(a) Overall mobility spectra of 16 structural isomers of methyl-D-glycopyranosides obtained on the AP-DTIM-TOFMS instrument. (b) Overall mobility spectra of the same 16 structural isomers collected using the Synapt G2 TWIMS instrument. All mobility spectra were extracted for sodiated ions $[M+Na]^+$ having m/z 217.

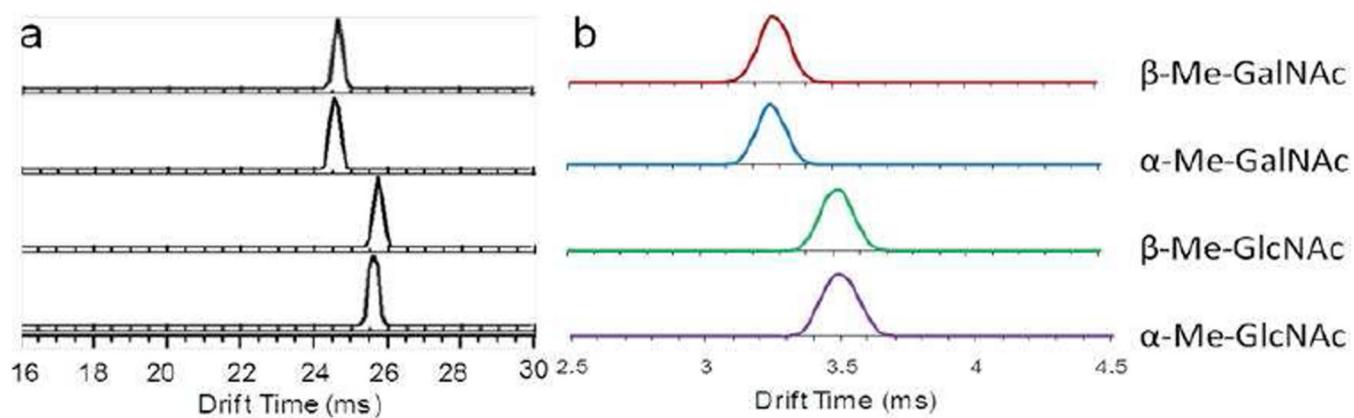


Figure 3.

(a) Overall mobility spectra of four structural isomers: α - and β -methyl-*N*-acetyl glucosamine and α - and β -methyl-*N*-acetylgalactosamine obtained on the AP-DTIM-TOFMS. (b) Overall mobility spectra of the same four structural isomers collected using the WATERS Synapt G2 instrument. All mobility spectra were extracted for sodiated ions $[M + Na]^+$ having m/z 258

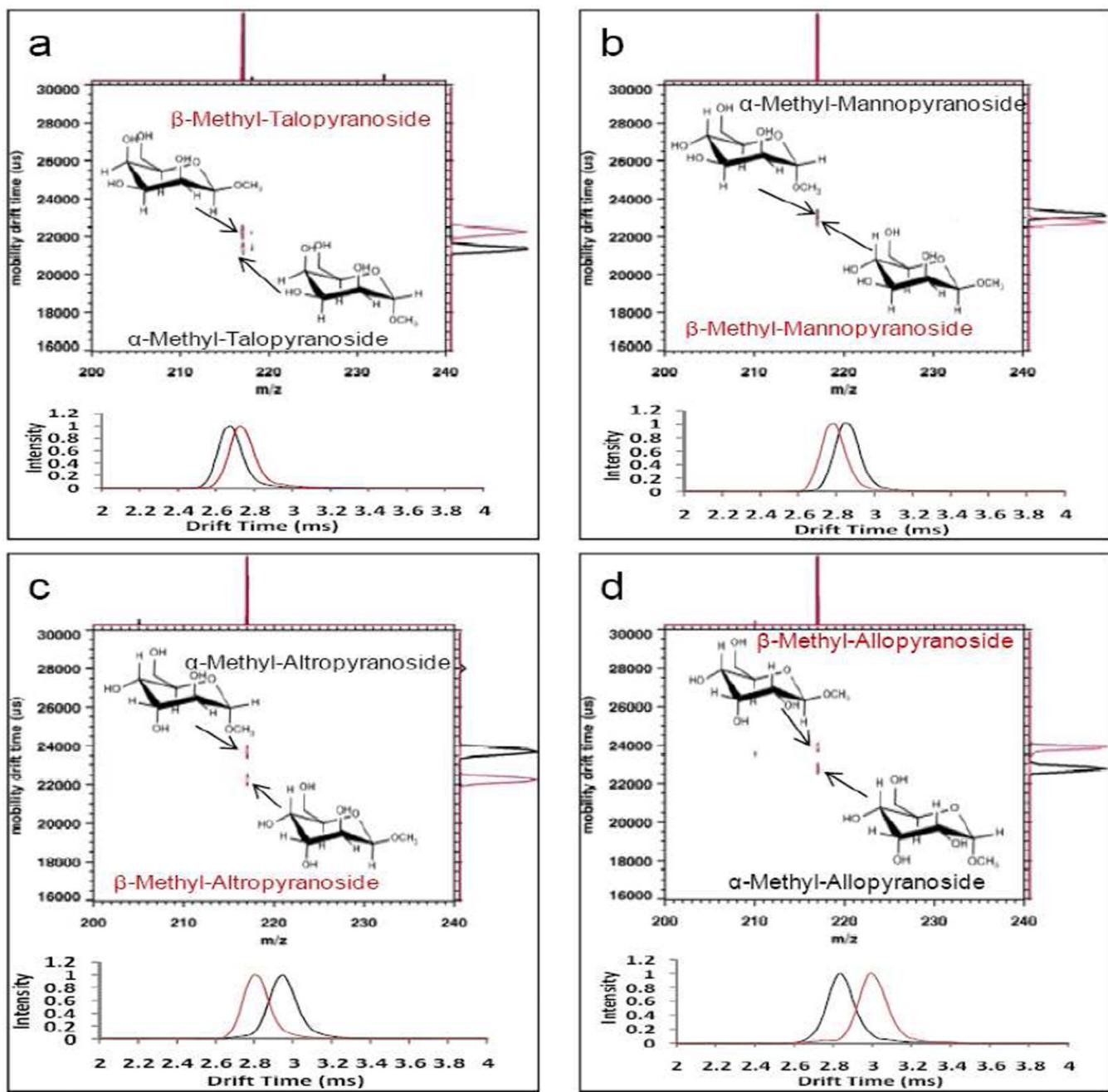
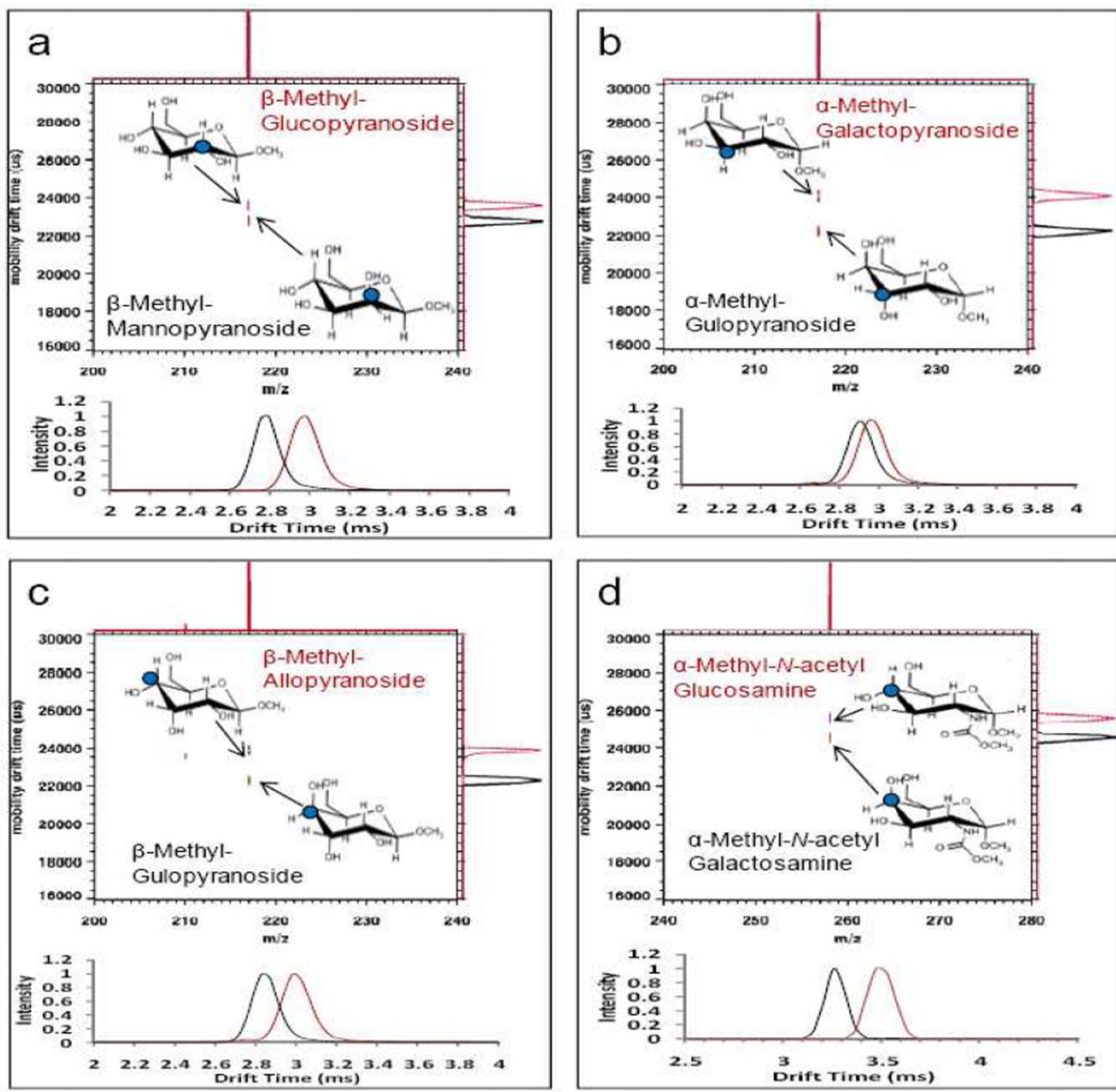


Figure 4.
Overlaid two-dimensional IMMS plots (top of each panel, from DTIMS) and IMS plots (1-D data from TWIMS, bottom of each panel) of selected pairs of anomeric methyl glycopyranosides: (a) Overlaid α - and β -methyl-talopyranosides; (b) Overlaid α - and β -methyl-mannopyranosides; (c) Overlaid α - and β -methyl-altrypyranosides; (d) Overlaid α - and β -methyl-allopyranosides. All the β configurations are in red color.

**Figure 5.**

Overlaid two-dimensional IMMS plots (top of each panel, from DTIMS) and IMS plot (1-D data from TWIMS, bottom of each panel) of selected pairs of epimeric methyl glycopyranosides: (a) Overlaid C-2 epimers: β -methyl-glucopyranoside and β -methyl-mannopyranoside; (b) Overlaid C-3 epimers: α -methyl-galactopyranoside and α -methyl-gulopyranoside; (c) Overlaid C-4 epimers: β -methyl-allopyranoside and β -methyl-gulopyranoside; (d) Overlaid 4-epimers of methyl-N-acetylhexosamines: α -methyl-N-acetylglucosamine and α -methyl-N-acetylgalactosamine. All the slower drifting (longer drift time) epimeric ions are shown in red.

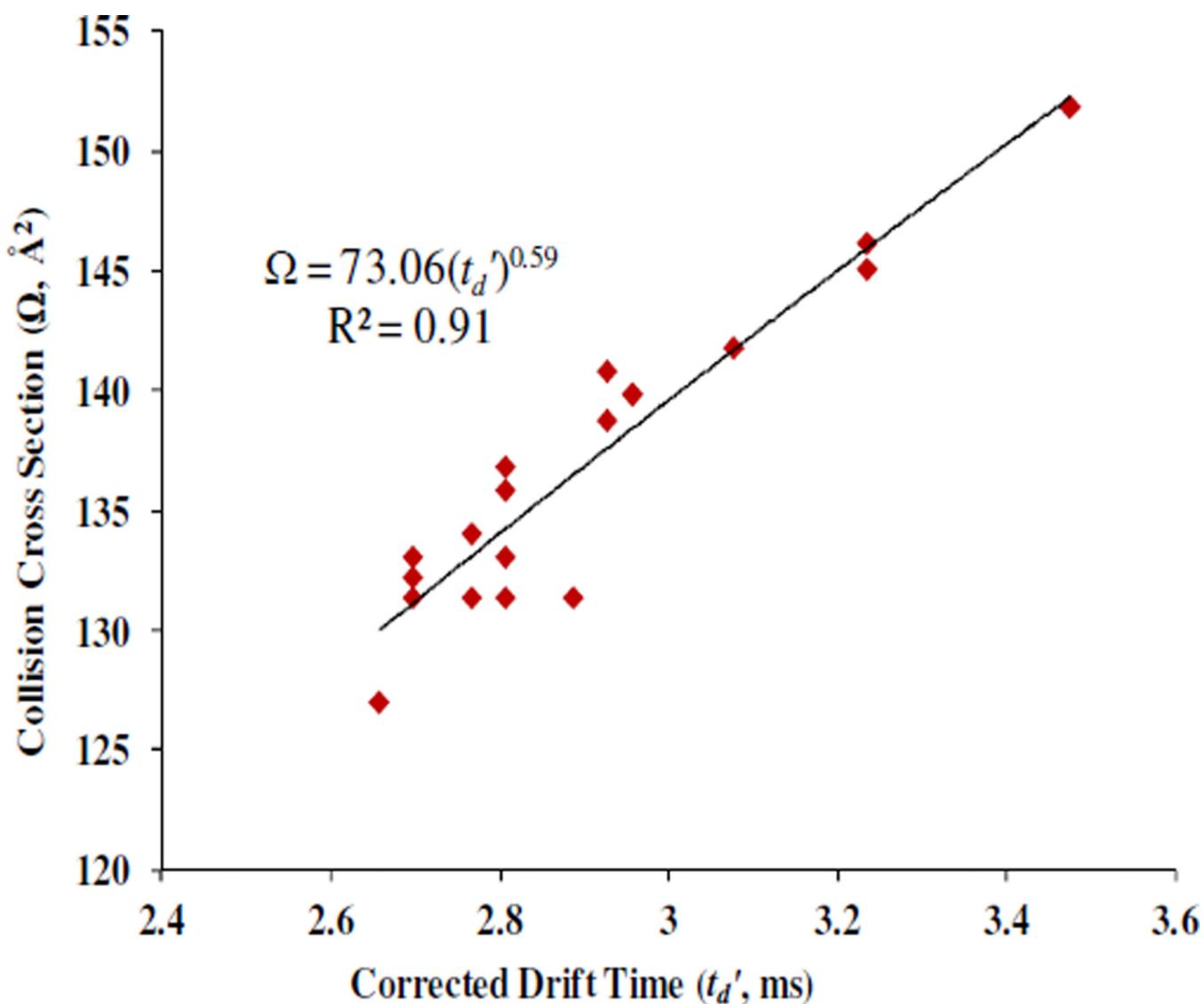


Figure 6.

Plot of Ω vs. t_d' for TWIMS using Ω values of 20 monosaccharide methyl glycosides structural isomers calculated from DTIMS (y axis) and their corrected drift time values derived from TWIMS (x axis).

Table 1

Drift times (t_d and t_d'), reduced mobilities (K_d), collision cross sections (Ω) and separation factors (α) for the sodiated adducts of all 16 structural isomers of Me-D-glycopyranosides and 4 structural isomers of methyl-*N*-acetylhexosamines employing ion mobility mass spectrometry

Compound	t_d ^a (ms)	t_d' ^b (ms)	K_d ^a (ms)	K_d ^a	α (^a)	α (^b)	Ω (^a) (Å ²)
β-Tal	22.24	2.70	1.48				131.34
α-Tal	21.40	2.66	1.53	1.03	1.02		126.98
β-Man	22.81	2.77	1.45				134.01
α-Man	23.34	2.81	1.42	1.02	1.01		136.80
β-Ido	22.65	2.70	1.47				132.19
α-Ido	22.63	2.70	1.46	1.01	1.00		133.04
β-Gul	22.33	2.81	1.48				131.34
α-Gul	22.30	2.89	1.48	1.00	1.03		131.34
β-Glc	23.65	2.96	1.40				139.83
α-Glc	24.22	3.08	1.37	1.02	1.04		141.77
β-Gal	23.11	2.81	1.43				135.83
α-Gal	24.14	2.93	1.38	1.04	1.04		140.80
β-Alt	22.31	2.77	1.48				131.34
α-Alt	23.78	2.93	1.40	1.06	1.06		138.74
β-All	23.87	2.96	1.39				139.83
α-All	22.76	2.81	1.46	1.05	1.05		133.04
β-GalNAc	24.65	3.23	1.33				146.14
α-GalNAc	24.56	3.23	1.34	1.01	1.00		145.05
β-GlcNAc	25.70	3.47	1.28				151.84
α-GlcNAc	25.61	3.47	1.28	1.00	1.00		151.84

Note:

^a data obtained or derived from ESI-DTIM-TOFMS (DTIMS).

^b data obtained or derived from ESI-TWIM-TOFMS (TWIMS).