Available online at www.sciencedirect.com
ScienceDirect
Carbohydrate Research 342 (2007) 217–235
Carbohydrate RESEARCH

Differentiation of the anomeric configuration and ring form of glucosyl-glycolaldehyde anions in the gas phase by mass spectrometry: isomeric discrimination between *m/z* 221 anions derived from disaccharides and chemical synthesis of *m/z* 221 standards

Tammy T. Fang,[a] Joseph Zirrolli[b] and Brad Bendiak[a,*]

[a]*Department of Cellular and Developmental Biology and Biomolecular Structure Program, University of Colorado at Denver and Health Sciences Center, Aurora, CO 80045, USA*
[b]*School of Pharmacy, University of Colorado at Denver and Health Sciences Center, Aurora, CO 80045, USA*

Received 8 August 2006; received in revised form 14 November 2006; accepted 20 November 2006
Available online 23 November 2006

Abstract—Mass spectrometry of disaccharides in the negative-ion mode frequently generates product anions of *m/z* 221. With glucose-containing disaccharides, dissociation of isolated *m/z* 221 product ions in a Paul trap yielded mass spectra that easily differentiated between both anomeric configurations and ring forms of the ions. These ions were shown to be glucosyl-glycolaldehydes through chemical synthesis of their standards. By labeling the reducing carbonyl oxygen of disaccharides with ^{18}O to mass discriminate between monosaccharides, it was established that the *m/z* 221 ions are comprised solely of an intact nonreducing sugar with a two-carbon aglycon derived from the reducing sugar, regardless of the disaccharide linkage position. This enabled the anomeric configuration and ring form of the ion to be assigned and the location of the ion to the nonreducing side of a glycosidic linkage to be ascertained. Detailed studies of experimental factors necessary for reproducibility in a Paul trap demonstrated that the unique dissociation patterns that discriminate between the isomeric *m/z* 221 ions could be obtained from month-to-month in conjunction with an internal energy-input calibrant ion that ensures reproducible energy deposition into isolated *m/z* 221 ions. In addition, MS/MS fragmentation patterns of disaccharide *m/z* 341 anions in a Paul trap enabled linkage positions to be assigned, as has been previously reported with other types of mass spectrometers.

Keywords: Carbohydrate mass spectrometry; Discrimination of disaccharide product anions; Assignment of anomeric configuration; Differentiation of ring form; Synthetic product ion standards

1. Introduction

Mass spectrometry (MS) is a valuable tool for analysis of oligosaccharides. A number of different techniques have been advocated as they often contribute different information and provide overlapping coverage for structural determination.[1–3] The power of multiple isolation/dissociation steps, carried out in ion traps or Fourier transform ion cyclotron resonance (FTICR) instruments, now permits many oligosaccharides to be broken down stepwise in the gas phase to isolable substructures. We sought to address the question as to whether reducing disaccharides, the smallest substructures derived from oligosaccharides that still contain a glycosidic linkage between two monosaccharides, can be effectively differentiated and structurally elucidated as gas-phase species. Disaccharide fragmentation patterns have been the topic of some important fundamental investigations, both in the positive-[4–9] and negative-ion[10–16] modes. Alkyl and acyl derivatives have also proven valuable.[3,17–19]

* Corresponding author. Tel.: +1 303 724 3453; e-mail: Brad.Bendiak@uchsc.edu

0008-6215/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2006.11.021

In the negative-ion mode, previous studies of disaccharide fragmentation have been carried out with major emphasis on the assignment of linkage positions, with characteristic fragmentation patterns in the gas phase clearly enabling the substitution position on the reducing sugar to be identified.[10–15] Although these studies were carried out under a wide range of dissociation conditions, some consensus was reached concerning fragmentation products. The cross-ring cleavages of the precursor ion m/z 341 $[M-H]^-$ generated product ions having higher masses than a monosaccharide of m/z 323, 311, 281, 263, 251, and 221. These correspond to losses of water, one to four –CHOH-backbone units, and, in the case of the m/z 263 anion, two –CHOH-units and one water. The presence and abundance of product ions was dependent on linkage position and apparent energy deposition into the ions using different methods. Mechanisms have been proposed that the reducing monosaccharide may initially rearrange to an open-chain form, whereupon dissociation occurs highly preferentially on the reducing sugar.[11,12,16] Similar proposals were made in the positive-ion mode with metal-ion adduction.[4–6,8] These results were interesting at the time and warrant further detailed investigation now that MS^n has become widely available and controlled disassembly of larger oligosaccharides to disaccharide substructures is possible.

Fewer studies have been carried out with the intent to discriminate anomeric configuration and ring forms of monosaccharides within disaccharides where the disaccharides have first been isolated as gas-phase ions. As disaccharides comprise a very large set of isomers, establishing their structures by MS entirely in the gas phase is not a trivial matter, without hydrolysis and GC–MS of individual monomers,[20,21] or using permethylation followed by hydrolysis or reductive cleavage in solution.[22,23] Discrimination between the α and β anomeric configurations of the nonreducing sugar of reducing disaccharides in the gas phase has been possible through direct in-source dissociation using electrospray-ionization,[14,15] using mass-analyzed ion kinetic energy (MIKE) spectrometry and kinetic energy release studies,[24] upon collision-induced dissociation (CID) of isolated disaccharide ions in the negative-ion mode as alkoxy anions[12] and chloride adducts,[25] and recently using two-dimensional variable-wavelength infrared multiple photon dissociation/mass spectrometry.[9] Also, differentiation of anomeric configuration has been observed in some cases following derivatization, using alkyl derivatives of increasing length,[17] upon examining a synthetic set of galactose–fucose isomers having a benzyl group at the reducing end,[26] and more recently, using a synthetic set of methylated disaccharide standards having pyranosyl-1-ene sugars at the reducing end.[3] As yet, though, no general method is available to determine the configuration of a glycosidic linkage in the gas phase. We have found no related studies that discriminate between anomeric configuration and ring forms of sugars as isolated product ions, which may in part be due to a lack of commercially available disaccharides having nonreducing aldofuranosides. Nevertheless, furanosides in general and those of glucose are found in nature.[27–30]

Studies carried out with disaccharides in the negative-ion mode[10–16] prompted four questions concerning their dissociation and the potential of garnering additional information about their structures: (1) Do any of the product ions of disaccharide dissociation, upon isolation and further fragmentation, permit differentiation of anomeric configuration and/or ring form? Of the above studies in the negative-ion mode,[10–15] only one further dissociated the ions derived by cross-ring cleavage of the disaccharides.[12] Of the ions investigated, one ion, the m/z 221 anion, was reported to show some potential to differentiate anomers. However, there was enough reported variability in the spectral data for this ion from disaccharides having different linkages to question whether it was solely derived by dissociation events occurring on the reducing sugar.[12] (2) Do the dissociation spectra of product ions match those of synthetic compounds? Although product ions derived from disaccharides are often considered transitory intermediates, some, for example, have been hypothesized to be anions of a hexopyranoside linked to a pentose, tetrose, glyceraldehyde, or glycolaldehyde.[11,12,16] If they are indeed identical, their spectra should presumably match. Moreover, such synthetic compounds could provide useful reference spectra provided they are the same molecules as isolated gas-phase species. (3) Are the mass spectra of the precursor and product ions reproducible enough to enable their identification from month-to-month in the same instrument, and even between instruments of the same general type (such as Paul traps)? (4) Are the product ions that have a larger m/z than a monosaccharide (m/z 221 or greater) actually mixtures of isomers themselves? Hypothesized mechanisms have proposed that these 'cross-ring cleavages' occur on the open-chain form of the reducing sugar.[11,12,16] In the positive-ion mode, substitution of hexose-containing disaccharides at the carbonyl position with ^{18}O has been performed to selectively mass label ions derived from the reducing monosaccharide.[5,6,8] However, in the negative-ion mode, to our knowledge, just one report of lactose (β-D-Gal*p*-(1→4)-D-Glc) ^{13}C-labeled at the glucose C-1 position[13] has been performed to effectively mass discriminate between the two monosaccharides. Their results were inconclusive in answering the above hypothesis that cross-ring cleavages solely occur on the reducing sugar. For example, these authors observed m/z 281 and 282 ions in a ratio of 70:30. The m/z 282 anion could have either resulted from cleavage of the C-5 to C-6 portion of the reducing sugar or from a two-carbon

fragment loss from the nonreducing sugar. Nonetheless, such studies are, in general, very valuable as they enable the origins of many ions to be clearly identified.

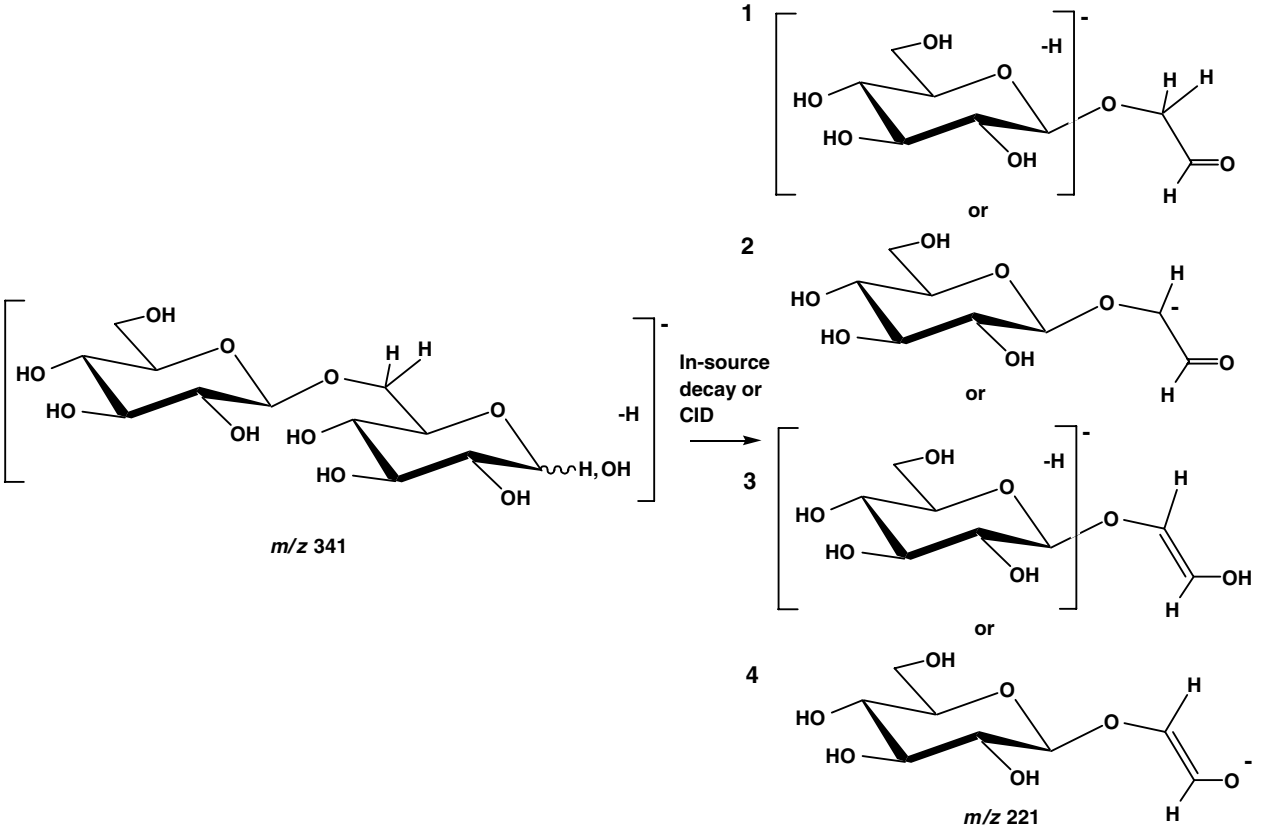
Bearing the above questions in mind, experiments were initiated, in a limited fashion with certain linkages and monosaccharides, to address them. It is demonstrated, for simple glucose-containing disaccharides having 2-, 4-, and 6-linkages, that glucosyl-glycolaldehyde product ions ($[M-H]^-$ 221) are derived solely from the nonreducing sugar of these disaccharides, bearing an additional two-carbon fragment derived from the reducing sugar. Synthesis of these (MW 222) structures demonstrates that as $[M-H]^-$ ions they dissociate identically to the *m*/*z* 221 ions derived from disaccharides and fragment in a way that enables their anomeric configuration and ring form to be easily and reproducibly assigned.

2. Results and discussion

2.1. Fragmentation patterns of *m*/*z* 221 anions isolated from disaccharide precursors in the negative-ion mode are identical to synthetic glucopyranosyl-glycolaldehydes

Dissociation of disaccharides in the negative-ion mode has been reported in the literature,[10–15] and four different structures that have been proposed for the *m*/*z* 221 product ions are shown in Scheme 1. In one report[12] it was proposed that 6-linked glucose-containing disaccharides may give rise to the aldehydo structure **1**, whereas 2-linked disaccharides may give rise to the enol structure **3**. As all the different tautomers/positional charge variants shown in Scheme 1 have the same *m*/*z*, it does not appear straightforward to be able to discriminate among them by mass spectrometry, and such ionic variants might rapidly equilibrate in the gas phase through proton transfer. Experiments were performed to determine whether *m*/*z* 221 anions, derived in the gas phase from related disaccharides having different linkage positions, were in fact the same ion or potentially different isomers, where the two-carbon fragment might originate from either monosaccharide of the disaccharides.

Two disaccharides varying solely in their anomeric configuration (isomaltose, α-D-Glc*p*-(1→6)-D-Glc, and gentiobiose, β-D-Glc*p*-(1→6)-D-Glc, precursor $[M-H]^-$ *m*/*z* 341) were dissociated in a Paul trap in the negative-ion mode, and the resultant *m*/*z* 221 product ions were isolated and further dissociated by MS^3. The spectra are shown in Figure 1, panels A and B. They differed strikingly in the abundance of *m*/*z* 161, 113, and 101 ions, under identical conditions in the Paul trap. Similarly, dissociation of the *m*/*z* 221 ions derived from the 2-linked disaccharides kojibiose, α-D-Glc*p*-(1→2)-D-Glc and sophorose, β-D-Glc*p*-(1→2)-D-Glc is shown in Figure 1,



Scheme 1.

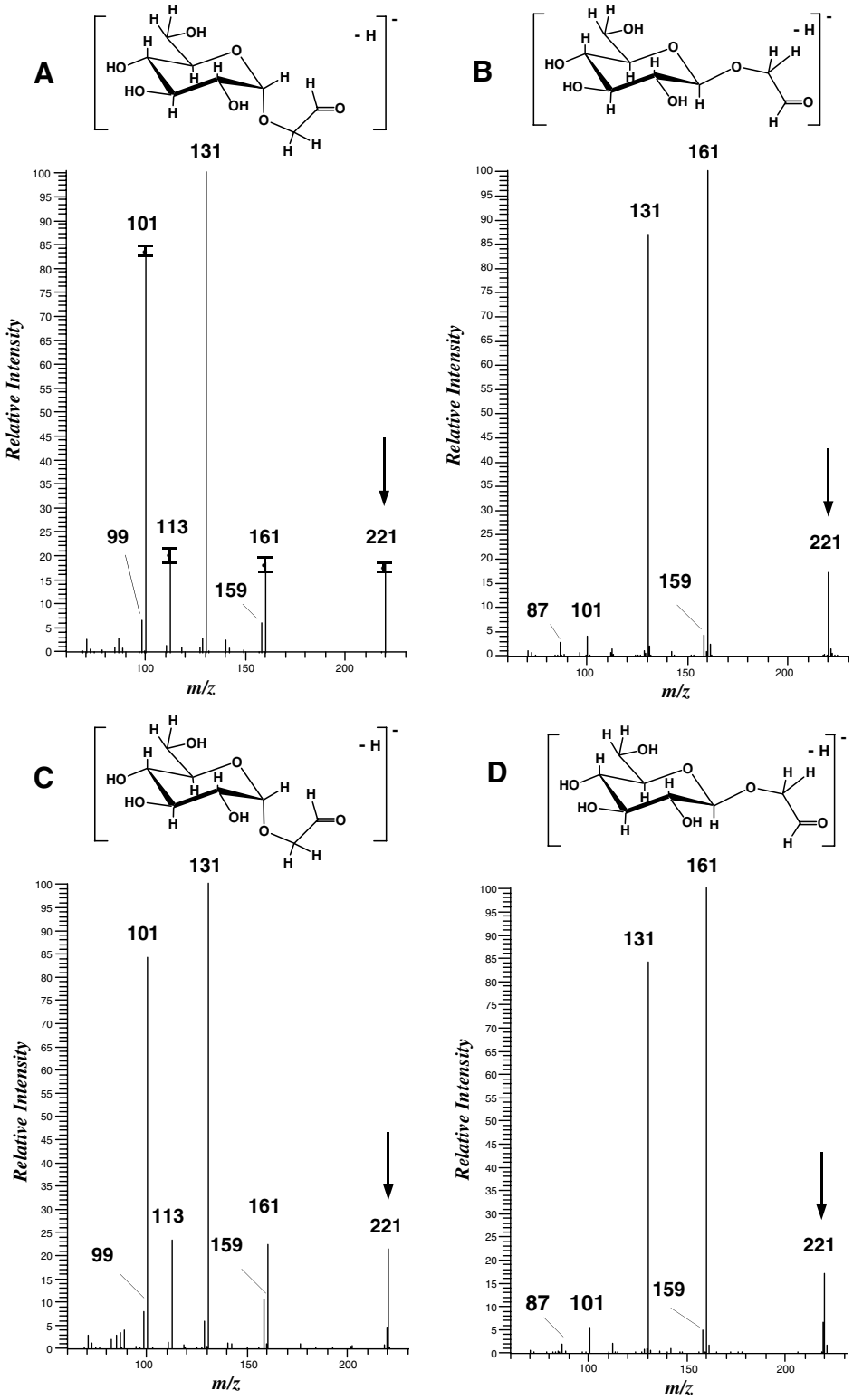
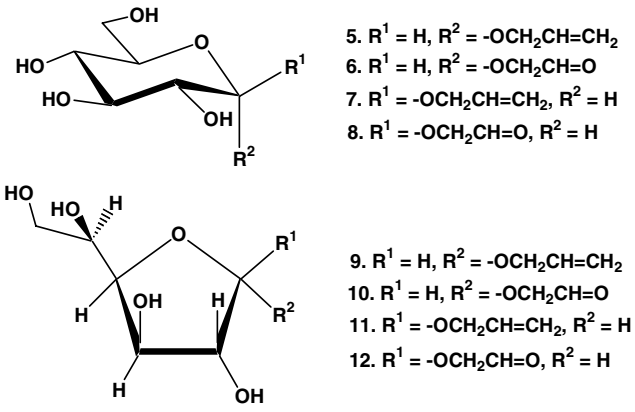


Figure 1. Dissociation of *m*/*z* 221 anions selected for MS^3 after disaccharide fragmentation in an LCQ Paul trap in the negative-ion mode. Disaccharide precursor ions $[M-H]^-$, *m*/*z* 341, were isolated and following dissociation, yielded *m*/*z* 221 anions that were isolated and subjected to a further round of dissociation at controlled collision energies. Mass spectra were recorded from the *m*/*z* 221 ion isolated from: (A) isomaltose, α-D-Glc*p*-(1→6)-D-Glc; (B) gentiobiose, β-D-Glc*p*-(1→6)-D-Glc; (C) kojibiose, α-D-Glc*p*-(1→2)-D-Glc; (D) sophorose, β-D-Glc*p*-(1→2)-D-Glc. Proposed structures of the anions are shown above each spectrum. They are based on similarity of mass spectra to the synthetic glucosyl-glycolaldehydes (Fig. 2) and data from ^{18}O labeling of disaccharides at the reducing carbonyl oxygen (Figs. 3–6). Error bars in panel A represent the standard deviation of the indicated ions calculated using the intensity of *m*/*z* 131 as exactly 100%, for 20 spectra accumulated over a 2-month period.

panels C and D. Note that in both cases where the nonreducing sugars were linked in α configuration, isolated *m/z* 221 product ions dissociated to give essentially identical mass spectra (Fig. 1, panels A and C). Likewise, when sugars were linked in β configuration, the *m/z* 221 product ions fragmented identically (panels B and D), but very differently from their α-linked counterparts. Proposed structures of the *m/z* 221 ions derived from the disaccharides are shown with each mass spectrum. It is important to point out that solely based on the information given in Figure 1A–D, these proposed structures could have alternatives, such as the reducing sugar attached to a two-carbon piece from the nonreducing sugar. However, the structures proposed are based on additional evidence provided by the synthetic glucosyl-glycolaldehydes and studies carried out with ^{18}O-labeled disaccharides, described later in this report.

The *m/z* 221 ions were also observed in low abundance in fragmentation of the 4-linked disaccharides maltose, α-D-Glc*p*-(1→4)-D-Glc, and cellobiose, β-D-Glc*p*-(1→4)-D-Glc, in the Paul trap. With the 4-linked disaccharides, these anions were furnished in greater abundance directly from in-source fragmentation (as reported in Ref. 15). The spectrum of the isolated *m/z* 221 product ion (not shown) from maltose was identical to the α-linked ion (Fig. 1A and C), and the spectrum of the *m/z* 221 product ion from cellobiose (not shown) was identical to the β-linked ion (Fig. 1B and D). Consequently, the *m/z* 221 anion appears to be identical whether it originates from a 2-, 4-, or 6-linked disaccharide, and it dissociates markedly differently when derived from an α-linked disaccharide as compared to one that is β-linked. It is worth noting that the *m/z* 221 product ion has been generated in much higher abundance for 4-linked disaccharides under high energy CID conditions in a sector instrument[12] as compared to CID conditions available in the LCQ Paul trap. We also observed more abundant *m/z* 221 product ions using collision conditions available with a triple quadrupole instrument, described later.

The *m/z* 221 anion was observed in dissociation of 3-linked disaccharides in the Paul trap, but only in trace quantities (<1% of the base product ion peak) in spectra obtained with an adequate number of scans to obtain very high signal-to-noise ratios. It was therefore impractical to isolate and perform MS^3 studies on the ion derived from 3-linked disaccharides in the Paul trap. However, as much as a 12% abundance as compared to the base product peak has been noted using in-source fragmentation in a triple quadrupole instrument,[15] and a small abundance has been seen in higher-energy sector instruments using CID.[12] We have also noticed small quantities of the *m/z* 221 ion from nigerose (α-D-Glc*p*-(1→3)-D-Glc) and laminaribiose (β-D-Glc*p*-(1→3)-D-Glc) using MS/MS in Q2 of a triple quadrupole instrument (1–5% of the base product ion peak). It appears that isolation of appreciable *m/z* 221 from 3-linked disaccharides will require higher energy excitation conditions and perhaps finer control of energy input into their *m/z* 341 anions than is available in a Paul trap.



Scheme 2.

Mass spectra of the synthetic glucopyranosyl-glycolaldehydes in a Paul trap (compounds **6** and **8**, Scheme 2) showed *m/z* 221 anions $(M-H)^-$ and small amounts (<20%) of *m/z* 239 anions (the hydrates). In Figure 2, panels A and B are shown the MS/MS spectra of the synthetic α- and β-D-glucopyranosyl-glycolaldehydes (*m/z* 221 anions), respectively. Dissociation patterns of the synthetic compounds having defined anomeric configurations and ring forms were essentially identical to those of the respective *m/z* 221 product ions derived from disaccharides having the same anomeric configurations and ring forms. They were easily discriminated by their fragmentation patterns. The data strongly indicate that the synthetic glucopyranosyl-glycolaldehydes are the same as the *m/z* 221 anions isolated by fragmentation of the 2-, 4-, and 6-linked disaccharides, and that they readily discriminate anomeric configuration. A potential product ion having an intact reducing sugar with a two-carbon fragment derived from the nonreducing sugar does not appear to be a dissociation pathway of significant abundance based on this data, which was supported by ^{18}O-labeling studies of disaccharides described later.

2.2. Differentiation among the four isomeric D-glucosyl-glycolaldehydes (α- and β-pyranosides and furanosides) in the negative-ion mode

To further substantiate the nature of the *m/z* 221 product ions derived from disaccharides, the α- and β-D-glucofuranosyl-glycolaldehydes (**10** and **12**, Scheme 2) were prepared. Their dissociation spectra, under identical conditions described for their pyranosyl counterparts, are shown in Figure 2, panels C and D. Clearly, these molecules dissociate markedly differently than

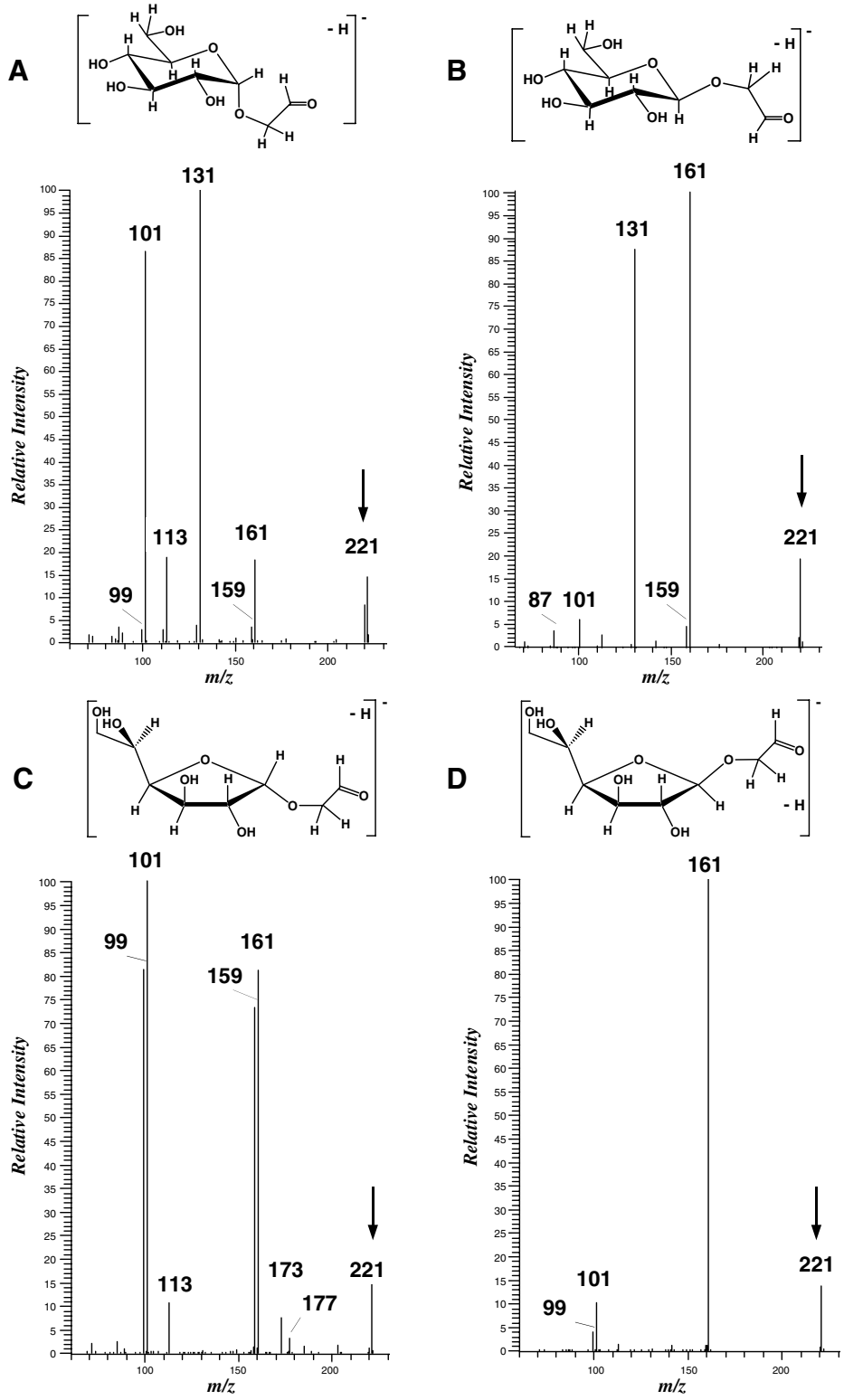


Figure 2. Differentiation of synthetic glucosyl-glycolaldehyde isomers having different anomeric configurations and ring forms by MS/MS in the negative-ion mode. The $[M-H]^-$, m/z 221, precursor anions were isolated and MS/MS spectra recorded following dissociation in an LCQ Paul trap. The spectra were recorded from: (A) α-D-glucopyranosyl-2-glycolaldehyde; (B) β-D-glucopyranosyl-2-glycolaldehyde; (C) α-D-glucofuranosyl-2-glycolaldehyde; (D) β-D-glucofuranosyl-2-glycolaldehyde. Structures of the synthetic compounds are shown above each spectrum.

the glucopyranosyl-glycolaldehydes. Each dissociation spectrum is unique and enables, for all four isomeric variants, both the anomeric configuration and ring form of the ions to be assigned.

2.3. The monosaccharide origin of the *m/z* 221 anion derived from 2-, 4-, and 6-linked disaccharides based on ^{18}O-labeling the carbonyl oxygen of the reducing glucose

The purpose of these labeling studies was to experimentally determine, in the negative-ion mode, whether *m/z* 221 anions originated by dissociation of either the reducing or nonreducing sugar of disaccharide precursor ions or whether the two-carbon fragment arose only from one of them.

Previously, disaccharides exchanged at the carbonyl oxygen of the reducing sugar with ^{18}O[5,8] have been investigated as single Li^+ adducts in the positive-ion mode. In those cases the equivalent ion to the *m/z* 221 anion, in the positive-ion mode, was an *m/z* 229 ion where the intact sugar residue was solely derived from the nonreducing monosaccharide in the case of 6- or 4-linked disaccharides. We followed the same labeling protocol that worked well and effectively exchanged the reducing sugar carbonyl oxygen for the two 6-linked disaccharides isomaltose and gentiobiose, the 4-linked disaccharides maltose and cellobiose, and the 2-linked disaccharides sophorose and kojibiose. It should be noted that labeling the reducing carbonyl oxygen with ^{18}O to mass-discriminate ions derived from disaccharides in the negative-ion mode has not previously been reported.

The MS/MS spectra of the unlabeled 6-linked disaccharides in the negative-ion mode (isolated precursor ions of *m/z* 341) are shown in Figure 3, panels A and B, and spectra of their ^{18}O-labeled isotopomers (precursor ions of *m/z* 343) are shown in panels C and D. Notably, *m/z* 221 product ions were found in both cases, with no observable *m/z* 223 product ions derived from the ^{18}O-labeled molecules. This indicates that for 6-linked disaccharides, the *m/z* 221 anions essentially solely contain an intact nonreducing sugar with an additional two-carbon aglycon derived from positions 5 and 6 of the reducing sugar. This is consistent with the results of fragmentation of the *m/z* 221 anions isolated from these disaccharides in MS^3 studies, where dissociation patterns matched those of synthetic glucosyl-glycolaldehyde ions. It is also worthy of note that ions of *m/z* 281 and 251, previously hypothesized to arise by two- and three-carbon losses from the reducing end of the reducing sugar,[10–15] were unchanged in *m/z* for the ^{18}O-labeled molecules, supporting previous proposals.

The MS/MS spectra of the disaccharides maltose and cellobiose, having linkages at the 4-position, are shown in Figure 4, panels A and B, and spectra of their isotopomers ^{18}O-labeled at the carbonyl position of the reducing sugar are shown in Figure 4, panels C and D. These experiments were carried out under higher energy collision conditions using a triple-quadrupole instrument with unit mass resolution for selection of the respective *m/z* 341 or 343 precursor ions. Disaccharides having 4-linkages yielded relatively low quantities of the *m/z* 221 ions in the Paul trap, although they were clearly observable (1–5% of the base product ion peak); more *m/z* 221 product ion was generated with in-source fragmentation using the trap. As with the 6-linked disaccharides, the characteristic product ion of *m/z* 221 was observed for the ^{18}O-labeled 4-linked disaccharides, with no observable *m/z* 223 product anions (Fig. 4,C and D). This indicates that with 4-linked disaccharides, the *m/z* 221 anion was essentially entirely derived from the intact nonreducing sugar, with the two-carbon fragment originating from the reducing monosaccharide. Possible fragmentation locations are indicated in the structural diagrams accompanying Figure 4.

The 2-linked disaccharides kojibiose and sophorose were also ^{18}O-labeled at the reducing carbonyl group, and dissociation of the resultant *m/z* 343 anions in the Paul trap was compared to the unlabeled compounds (Fig. 5). MS/MS spectra of the ^{18}O-labeled, *m/z* 343 precursor ions revealed *m/z* 223 product anions instead of the *m/z* 221 anions that were observed for the unlabeled compounds (Fig. 5, panels A–D). In the case of 2-linked disaccharides, it appeared feasible that the two-carbon fragment originated solely from carbons 1 and 2 of the reducing sugar, as postulated in the fragmentation diagrams in Figure 5.

To check whether it was possible that the *m/z* 223 anion might comprise the intact reducing sugar, with a two-carbon fragment derived from the nonreducing sugar, it was isolated and further dissociated an additional step (MS^3). Note that the fragmentation patterns of the *m/z* 223 ions derived from either α- or β-linked disaccharides having a linkage at the 2-position (Fig. 6A and B) were essentially the same as their respective 221 counterparts (Fig. 1C and D), with the sole difference being that the *m/z* 131 product ion was converted to an *m/z* of 133. However, to further verify that the two-carbon fragment was actually derived from the reducing portion of the 2-linked disaccharides, the synthetic α- and β-glucopyranosyl-glycolaldehydes were also exchanged at the carbonyl position with ^{18}O. These synthetic (*m/z* 223) compounds fragmented identically (Fig. 6C and D) to the ions isolated from their respective ^{18}O-labeled α- or β-linked disaccharides (Fig. 6A and B). Compiling all the ^{18}O-labeling studies, the key observations were (1) that the two-carbon fragment of *m/z* 221 (or 223) anions was always derived from the reducing sugar of disaccharides during dissociation in the negative-ion mode, and (2) that the ring form and anomeric configuration of the nonreducing sugar was maintained intact for *m/z* 221 anions. In the nomenclature of Domon and Costello,[31] reducing disaccharides fragmented to give *m/z* 221 cross-ring product anions solely of the A type, not the X type. It is worthy of note that for nonreducing disaccharides having an alkyl aglycon (methyl, ethyl, and butyl glycosides of lactose), X-type

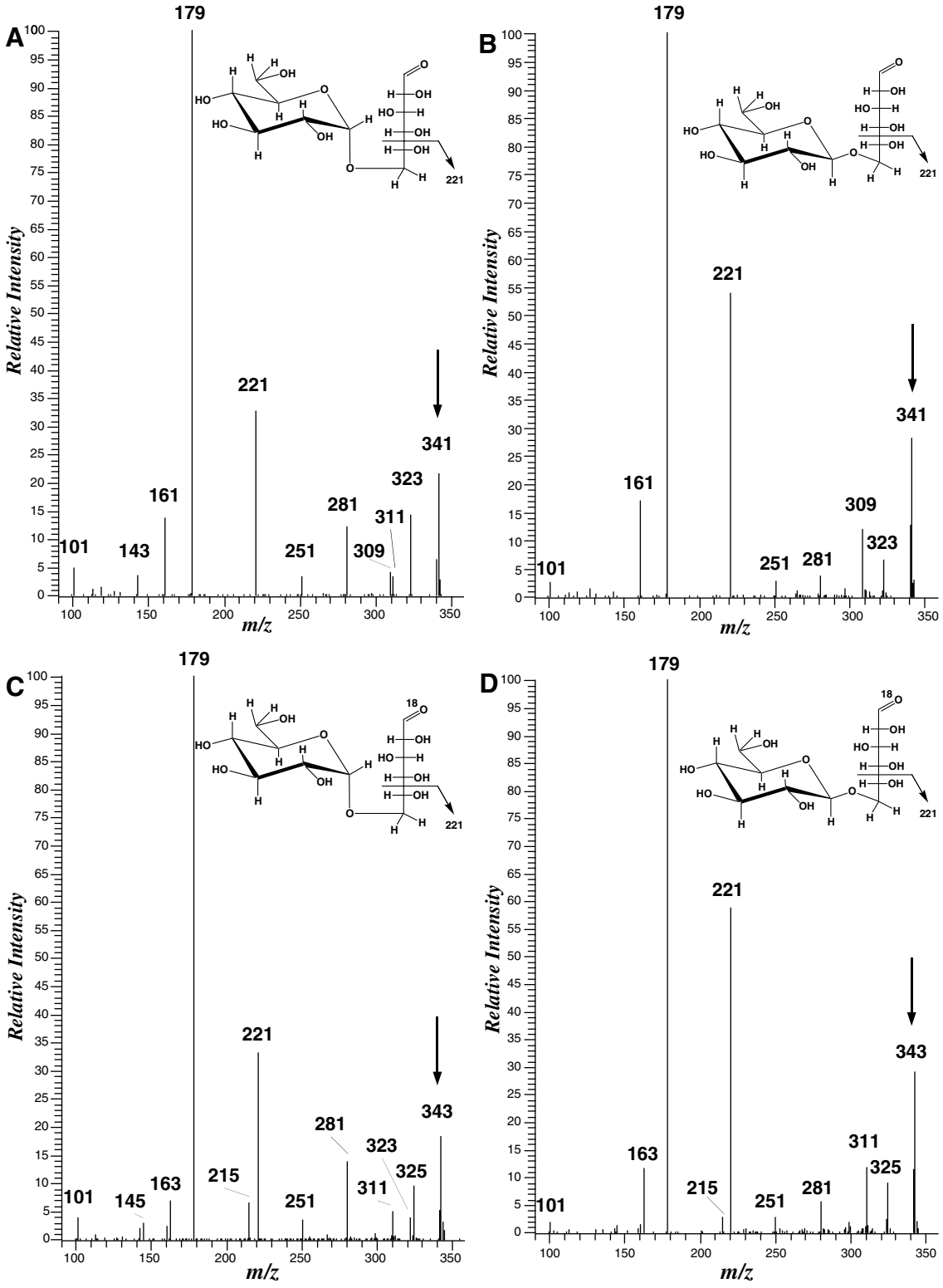


Figure 3. Fragmentation patterns of 6-linked disaccharides and comparison to their ^{18}O-labeled isotopomers by MS/MS in the negative-ion mode. Precursor ions (*m*/*z* 341 or 343) were isolated and dissociated under the same conditions in a Paul trap. Shown are the MS/MS spectra of: (A) isomaltose, α-D-Glc*p*-(1→6)-D-Glc; (B) gentiobiose, β-D-Glc*p*-(1→6)-D-Glc; (C) isomaltose ^{18}O-labeled at the reducing carbonyl position; (D) gentiobiose ^{18}O-labeled at the reducing carbonyl position. Fragmentation diagrams that indicate the origin of the *m*/*z* 221 product ions are shown above each spectrum. The open-chain form of the reducing sugar was drawn solely to indicate the fragmentation position; no implication regarding the relative abundance of either the open-chain or cyclic form(s) of the reducing sugar in the gas phase is intended, nor are any inferences drawn concerning dissociation mechanisms.

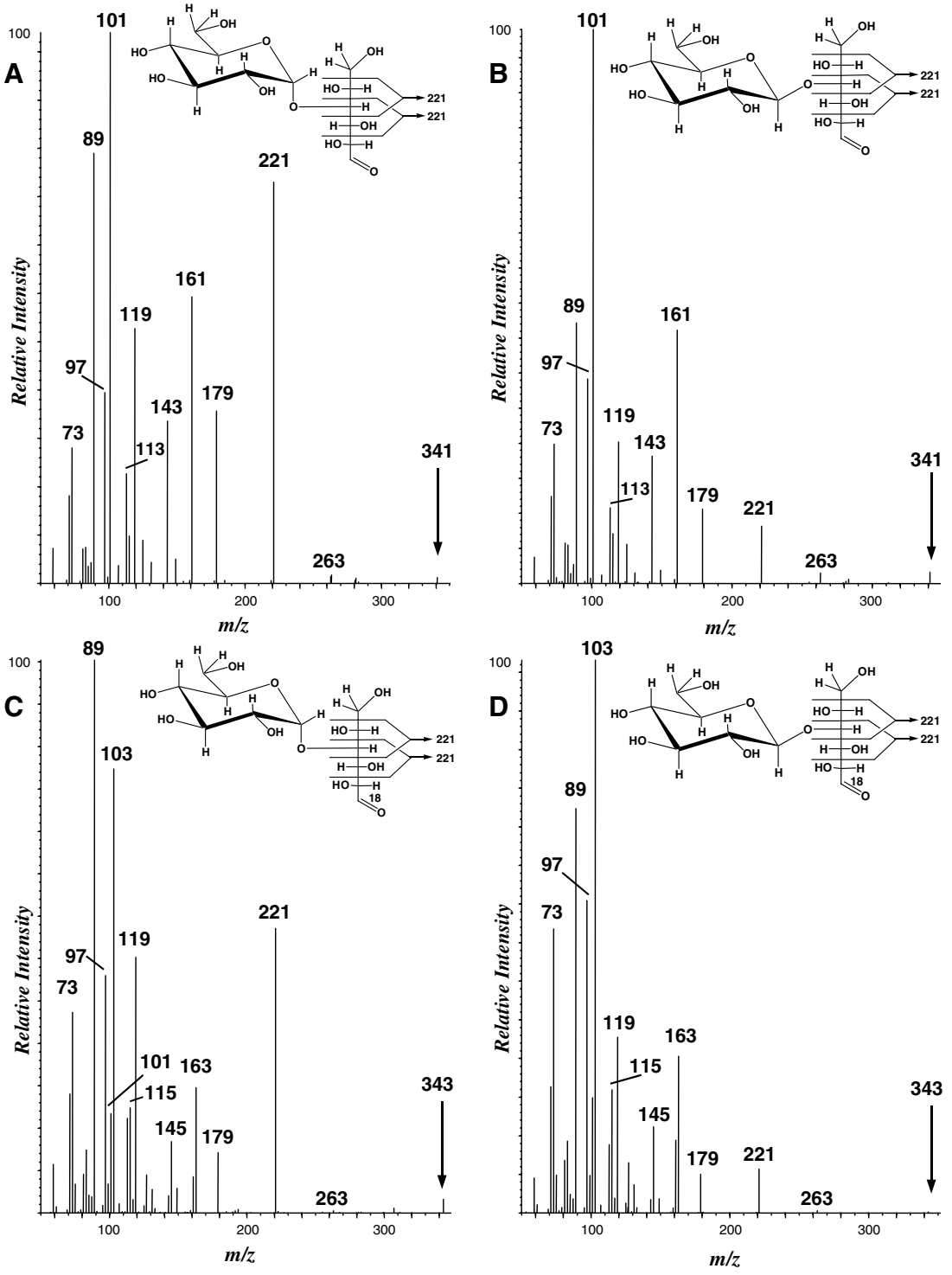


Figure 4. Dissociation of 4-linked disaccharides and comparison to their ^{18}O-labeled isotopomers by MS/MS in the negative-ion mode. Precursor ions (*m*/*z* 341 or 343) were selected and dissociated in the second quadrupole of a Sciex triple quadrupole instrument. Shown are the MS/MS spectra of: (A) maltose, α-D-Glc*p*-(1→4)-D-Glc; (B) cellobiose, β-D-Glc*p*-(1→4)-D-Glc; (C) maltose ^{18}O-labeled at the reducing carbonyl position; (D) cellobiose ^{18}O-labeled at the reducing carbonyl position. Fragmentation diagrams that indicate the possible origin(s) of the *m*/*z* 221 product ions are shown above each spectrum. The open-chain form of the reducing sugar was drawn only to illustrate fragmentation position(s); no implication regarding the relative abundance of either the open-chain or cyclic form(s) of the reducing sugar in the gas phase is intended, nor are any inferences intended concerning dissociation mechanisms.

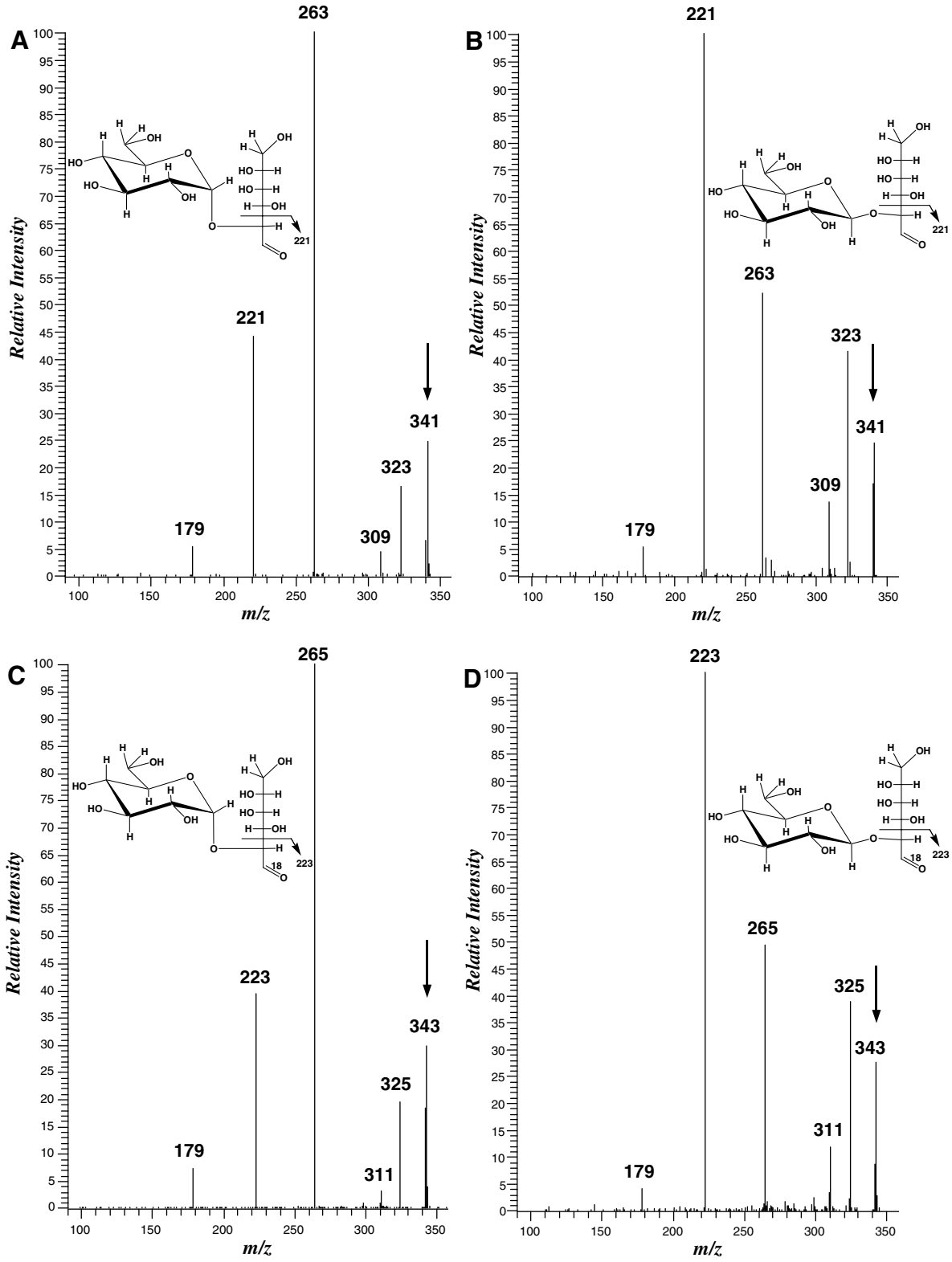


Figure 5. Dissociation of 2-linked disaccharides and comparison to their ^{18}O-labeled isotopomers by MS/MS in the negative-ion mode. Precursor ions (*m*/*z* 341 or 343) were isolated and dissociated under identical CID conditions in a Paul trap. Shown are MS/MS spectra of: (A) kojibiose, α-D-Glc*p*-(1→2)-D-Glc; (B) sophorose, β-D-Glc*p*-(1→2)-D-Glc; (C) kojibiose, ^{18}O-labeled at the reducing carbonyl position; (D) sophorose, ^{18}O-labeled at the reducing carbonyl position. Fragmentation diagrams that indicate the origins of the *m*/*z* 221 or 223 anions are included with each spectrum. As mentioned in Figs. 3 and 4, the open-chain form of the reducing sugar was drawn solely to illustrate the fragmentation position.

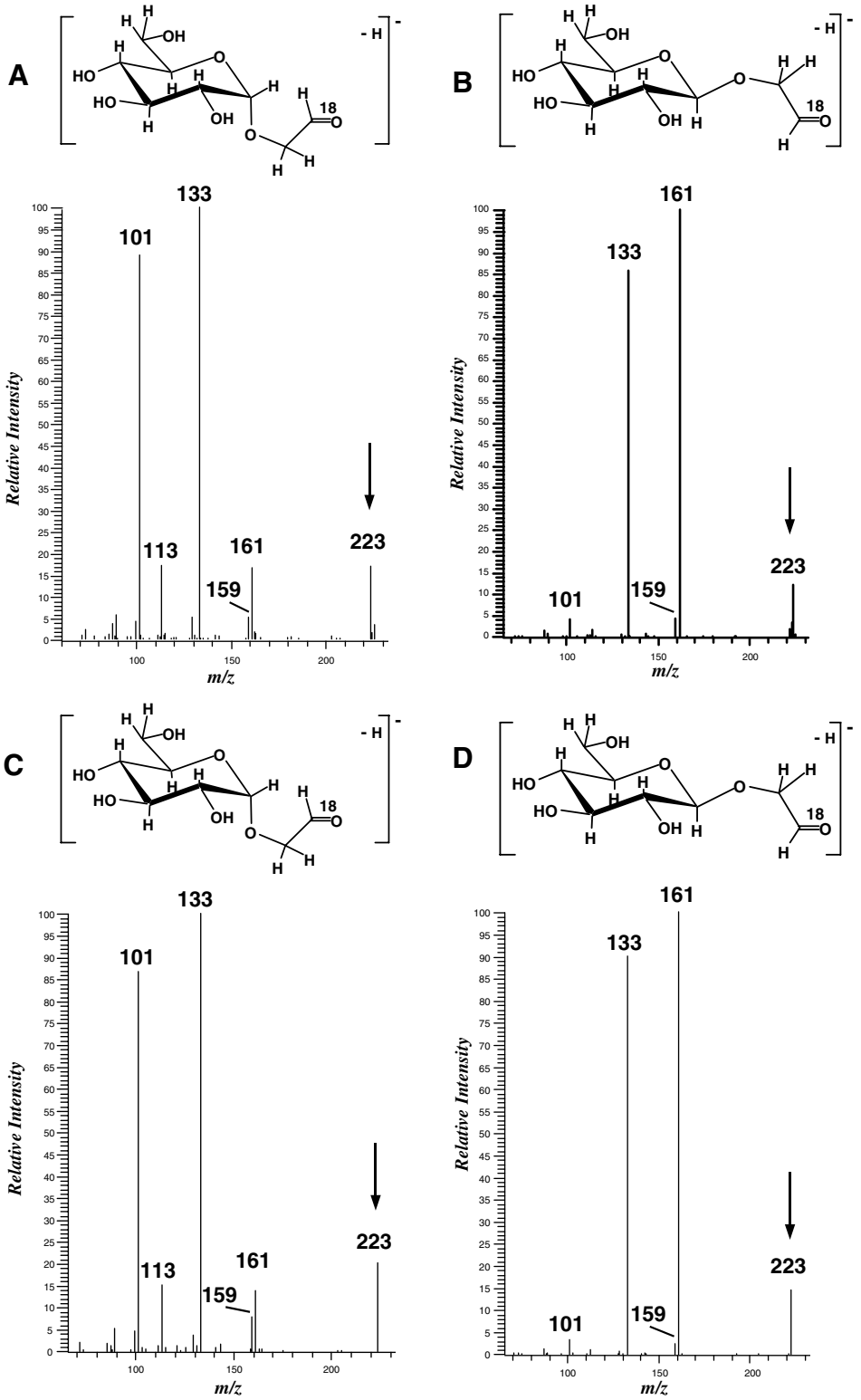


Figure 6. Comparison of dissociation patterns of ^{18}O-labeled product ions having *m*/*z* 223 derived from 2-linked disaccharides with those of synthetic glucosyl-glycolaldehydes ^{18}O-labeled at the glycolaldehyde carbonyl oxygen. For panels (A) and (B) are shown MS^3 spectra of the *m*/*z* 223 ions isolated from dissociation of: (A) kojibiose, α-D-Glc*p*-(1→2)-D-Glc, ^{18}O-labeled at the reducing carbonyl oxygen and (B) sophorose, β-D-Glc*p*-(1→2)-D-Glc, ^{18}O-labeled at the reducing carbonyl oxygen. In panels (C) and (D) are shown MS/MS spectra of synthetic glucopyranosyl-glycolaldehydes ^{18}O-labeled at the glycolaldehyde carbonyl oxygen: (C) α-D-glucopyranosyl-2-glycolaldehyde; (D) β-D-glucopyranosyl-2-glycolaldehyde.

ions having a two-carbon fragment derived from the nonreducing sugar have been reported in the negative-ion mode.[32] Consequently, the value of ^{18}O-labeling the carbonyl oxygen of reducing disaccharides in order to mass discriminate between potential isomeric product ions derived from portions of either monosaccharide cannot be overemphasized.

2.4. Disaccharide linkage positions can be assigned based on dissociation patterns in a Paul trap

As previously reported with sector instruments,[10–12] FTICR,[13] and in-source fragmentation during electrospray-ionization,[14,15] dissociation of disaccharides in the negative-ion mode yielded diagnostic fragmentation patterns that enabled linkage positions to be assigned. In the Paul trap, fragmentation patterns were also observed that were characteristic of specific linkage positions (Table 1). It is important to point out that the data in Table 1 were accumulated with enough scans to obtain high signal-to-noise ratios so that ions in the abundance range of 0.1–1% of the base product ion peak were clearly observable. Hence the criterion for the complete absence of certain ions ((−) in Table 1) was estimated as an intensity relative to the base product peak no greater than 0.1%, or 1:1000 the intensity.

It is also worthy of note that true MS/MS is completely different than in-source fragmentation[14,15] and that MS/MS utilizing CID in a Paul trap is different than MS/MS in sector, triple quadrupole, or FTICR instruments for two reasons. (1) In a Paul trap, precursor ions are excited at a unique resonance excitation frequency to induce CID events only in these ions based on their selective increase in axial velocity. Their product ions are not subject to additional dissociation as little or no additional vibrational energy is transferred to product ions.[8,33] This is fundamentally different than CID in sector, triple quadrupole, or FTICR instruments, where both precursor and product ions are subject to further fragmentation events. Therefore, product ions directly originate from precursor ions in a Paul trap rather than through successive intermediate fragments. (2) Vibrational energy input into selected ions in a Paul trap accrues through multiple successive low-energy collisions. This is also fundamentally different than the higher energy collisions that are typical of triple quadrupole instruments, and especially in sector instruments. For these reasons, the mass spectra of disaccharides in a Paul trap would be expected to be somewhat different than previous reports using the above instruments, and differences in product ion ratios were observed.

On a practical basis in the Paul trap, 3- and 6-linked disaccharides could be differentiated from 2- and 4-linked in the negative-ion mode because the *m*/*z* 263 ion was not present in mass spectra of 3- or 6-linked structures, even in trace quantities. The 6-linked disaccharides could be differentiated from the 3-linked ones based on the much greater abundance of the *m*/*z* 221 product ion (in the 3-linked examples there was just a trace), the fact that the *m*/*z* 251 ion was the most abundant product ion above *m*/*z* 179 in 3-linked structures, and the presence of a low abundance *m*/*z* 233 ion for 3-linked disaccharides that was not found, even in trace quantities, with any other linkage (also reported using CID in an FTICR instrument[13]). The 2- and 4-linked disaccharides were easily discriminated based on the much greater abundance of *m*/*z* 221 and 263 anions for 2-linked disaccharides and on the fact that the *m*/*z* 281 anion was the most abundant product ion above *m*/*z* 179 for 4-linked structures.

Regarding mechanistic aspects of disaccharide fragmentation, some previous hypotheses have suggested that disaccharide precursor ions in the negative-ion mode may fragment through successive losses of *m*/*z* (−60) or $C_2H_4O_2$ units.[11,12] In the positive-ion mode, Asam and Glish[8] clearly demonstrated in a Paul trap that a direct loss of $C_4H_8O_4$ can occur from precursor ions of 4- and 6-linked disaccharides, which they verified using double resonance experiments. Our experiments in the negative-ion mode also demonstrate that for 2-, 3-, 4-, and 6-linked disaccharides, a direct loss of $C_4H_8O_4$ occurs to give the *m*/*z* 221 ion in the Paul trap. However, we also sought to experimentally determine whether alternative fragmentation pathways could give rise to the *m*/*z* 221 ion under the fragmentation conditions available in a triple quadrupole instrument, using the precursor ion scan experiment (Table 2). In this experiment, the origins of product ions derived by potential multiple fragmentation events in Q2 of the triple quadrupole can be determined. Using 2-, 3-, 4-, and 6-linked disaccharides, it was evident that in the triple quadrupole instrument, multiple mechanisms give rise to the *m*/*z* 221 anion. For all the linkages, the direct loss of

Table 1. Product ions generated in the LCQ Paul trap from disaccharide precursor ions (*m*/*z* 341) having different linkages[a]

Disaccharide linkage[b]	Diagnostic product ion (*m*/*z*), relative abundance[c]					
	323	281	263	251	233	221
1→2	+	LA	+	LA	–	+
1→3	LA	LA	–	+	LA	TR
1→4	LA	+	LA	LA	–	LA
1→6	+	+	–	LA	–	+

[a] Disaccharides were solely glucose-containing (see text), examined in the negative-ion mode with nanospray infusion.

[b] Linkages of both anomeric configurations were examined.

[c] Diagnostic product ions were those of higher MW than a monosaccharide (*m*/*z* 179), with multiple scans averaged so that noise levels were <0.1% of the base product peak. As compared to the intensity of the base product peak, (+) = abundance >5%; LA = low abundance, 1–5%; TR = trace, 0.1–1.0% abundance; (−) = <0.1% abundance.

Table 2. Disaccharide anions that give rise to the *m/z* 221 ion via multiple high-energy collision events in a Sciex triple quadrupole instrument: precursor ion scans[a]

Disaccharide linkage[b]	Anions giving rise to *m/z* 221 anion, and approximate relative abundance[c]
1→2	341 (>95%); 311 (<5%); 281 (<5%)
1→3	341 (>50%); 311 (<50%)
1→4	341 (<50%); 281 (>50%)
1→6	341 (>80%); 323 (<5%); 311 (<10%); 281 (<20%)

[a] Precursor ion scans were carried out in the negative-ion mode on the *m/z* 221 product ion under conditions where the Sciex collision energy was set to −15 V. Initial precursor ions were disaccharide anions (*m/z* 341). Disaccharides were solely glucose-containing (see text), introduced through electrospray infusion.

[b] Linkages of both anomeric configurations were examined.

[c] Precursor ion scanning in triple quadrupole instruments characterizes multiple ions that can give rise to a given product ion by multiple stepwise dissociation events in Q2. Abundances (> or < the values indicated) are presented as such because experiments were independently performed with both α and β anomers, where the values represent mininum (>) or maximum (<) quantities of multiple-collision precursor species for both anomers.

$C_4H_8O_4$ was in fact a facile dissociation process to directly generate the *m/z* 221 product ion from the *m/z* 341 precursor ion (Table 2). For 2-, 3-, and 6-linkages, an initial loss of CH_2O (−30) also occurred concurrently to generate an *m/z* 311 intermediate, which then underwent a second direct collisional loss of $C_3H_6O_3$ (−90) to produce the *m/z* 221 product ion. For the 2-, 4-, and 6-linkages, two successive losses of $C_2H_4O_2$ (−60) also occurred concurrently via the intermediacy of the *m/z* 281 product ion to generate the *m/z* 221 anion, this being the most facile pathway only for 4-linked disaccharides. Finally, for 6-linked disaccharides alone, a loss of a water molecule (−18) occurred to generate an *m/z* 323 intermediate, which then underwent a collisional loss of $C_4H_6O_3$ (−102) to generate the *m/z* 221 product ion via a fourth independent pathway. Conclusions regarding disaccharide fragmentation both in the Paul trap and triple quadrupole were (1) A direct loss of $C_4H_8O_4$ occurs in the Paul trap for disaccharides of all linkages. (2) A direct loss of $C_4H_8O_4$ occurs in a triple quadrupole instrument for all linkages, but at least three other mechanisms can give rise to multiple successive fragmentation pathways, depending on the linkage position. We have refrained from drawing mechanisms because first, they may be unique to disaccharides having specific stereochemistries, linkages, and anomeric configurations, second, they clearly are unique to specific instruments, and third, isotopomers will probably be required to mass-discriminate ions so that the origins of each product ion can be confidently determined.

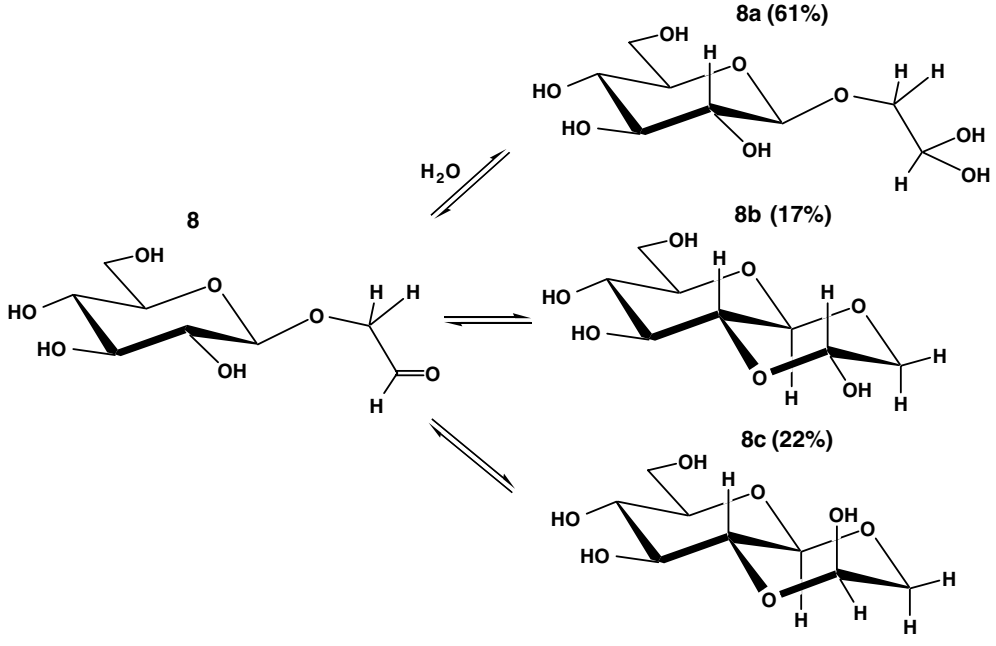
2.5. Mass spectral reproducibility

Spectral reproducibility is crucial for the identification of ions as 'spectral fingerprints'. This is especially germane when isobaric variants need to be identified, particularly from truly unknown molecules. Shown in Figure 1A is the variability of the spectrum of α-D-glucopyranosyl-glycolaldehyde. Error bars show standard deviation of peak heights over the collection of 20 independent spectra obtained over a two-month period. Spectral reproducibility could be maintained from day-to-day and over several months by keeping four parameters carefully controlled. The first was the isolation width, which was kept constant for analysis of the *m/z* 221 anions (3 Finnigan 'amu') and moderately wide purposefully to improve reproducibility. The second issue was the calibration of the resonance excitation frequency. Normally after calibration of the instrument, this frequency drifts over time, and appears to drift at a different rate in the LCQ than that of the mass measurement frequencies. If this drifts by 0.2–0.5 amu, it will slightly affect the ratios of resultant mass spectra, and either requires recalibration, or can be finely adjusted manually to optimize the total product ion current by slightly shifting the excitation resonance frequency.

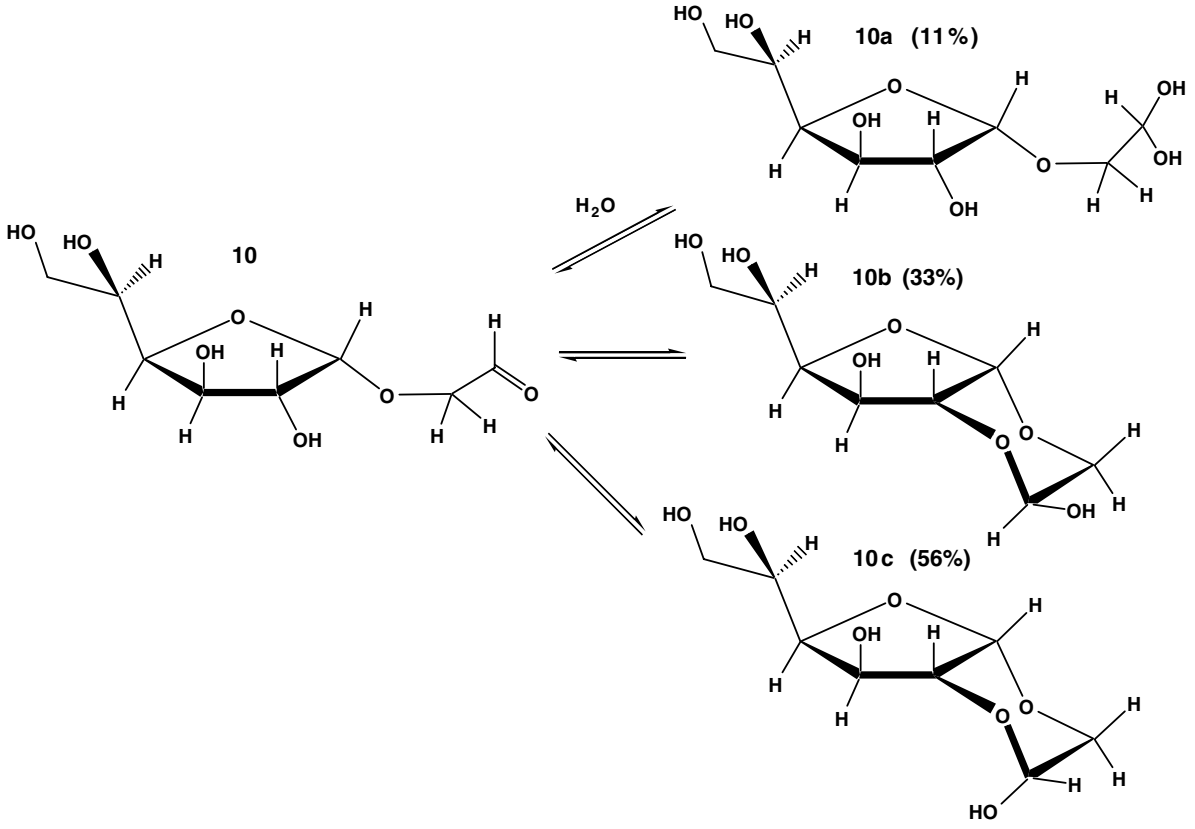
The third issue involves carefully defining the energy input into an ion, where the ratio of the most abundant product ion (base) peak to the remaining precursor ion peak was chosen as the most reliable criterion for reproducible energy deposition into the ions. We selected synthetic α-D-glucopyranosyl-glycolaldehyde as an internal 'energy-input tuning ion' and routine standard, in a similar fashion to,[34] and kept its base product ion:precursor ion ratio (100:18) as constant as possible over the long term to ensure consistent energy deposition into this ion and other isomeric *m/z* 221 precursor ions. Hence, over a period of months, the collision energy needs to be slightly adjusted to attain the same ratio of base product ion:precursor ion. The fourth issue was monitoring the number of ions in the trap. Variation of dissociation behavior with numbers of ions was investigated by varying in a stepwise manner the number of ions in the trap (from 3×10^6 to 5×10^3) under otherwise identical hardware settings and calibration conditions. The overall number of ions in the trap was found to slightly affect dissociation patterns, irrespective of other parameter settings. Consequently, the levels of ions in the trap need to be approximately the same for comparison of a standard to an unknown (within an order of magnitude) to have the best reproducibility. It is worth noting that for these ions, the differences in dissociation between the four isomers were so marked that this was not a significant factor in their discrimination.

2.6. Properties of the *m/z* 221 anions in the gas phase: evidence for possible open-chain and cyclic structures based on ^{1}H NMR studies of the glucosyl-glycolaldehydes in solution

As indicated in Scheme 1, four potential classes of gas-phase anions for individual glucosyl-glycolaldehydes have previously been postulated, with charge located potentially on the two-carbon fragment of the former reducing sugar or on alkoxy anions of hydroxyl groups of the hexose portion of the ion, where the reducing fragment may be either an aldehyde or enol tautomer. Even though we have synthesized the glucosyl-glycolaldehydes and demonstrated their mass spectra to be the same as those derived from disaccharide dissociation, transient proton transfers over rapid time scales could occur, especially under resonance excitation conditions in the trap when selected ions build up high internal energy as they approach their dissociation threshold. Consequently, none of the potential structures shown in Scheme 1 can be ruled out in equilibria using MS methods alone. Their gas-phase structures may be even more complicated, because we have observed three forms for two of the glucosyl-glycolaldehydes (the β-glucopyranoside and the α-glucofuranoside, **8** and **10**) by ^{1}H NMR spectroscopy in D_2O solution (Schemes 3 and 4, and compound characterization data under the synthesis section). The other two glucosyl-glycolaldehydes **6** and **12** were only found in solution as their hydrates. Compounds **8** and **10** exist in an open-chain form as a hydrate, and as two cyclic, six-membered ring forms through hemiacetal formation via ring closure of the aldehyde with OH-2 of the sugar, in different proportions as determined by integration of the NMR spectra (Schemes 3 and 4, Table 3). These cyclic forms have significantly different *J*-couplings between the glycolaldehyde H-1′ and its own H-2′a and H-2′b, as opposed to the open-chain hydrate. Also, differences were seen in the *J*-couplings of the α-glucofuranoside ring protons for cyclic forms **10b** and **10c** (Scheme 4, Table 3), whereas the open-chain hydrate **10a** had *J*-couplings similar to that of α-allylglucofuranoside (described later in this report) or α-methylglucofuranoside.[35] This was not observed for the cyclic forms of the β-glucopyranoside-glycolaldehyde **8b** and **8c** (Scheme 3, Table 3), indicating that for the α-glucofuranosides, some minor change in the five-membered ring conformation takes place when hemiacetal formation occurs between the glycolaldehyde carbonyl and the sugar OH-2. Interestingly, a weak $^4J_{H,H}$ (<1.0 Hz) was also observed between the glycolaldehyde H-1 proton and the sugar ring H-2 proton for structure **8c**, a coherent through-bond coupling similarly sometimes observed between protons across glycosidic linkages.[36] The open-chain hydrate could not, of course, contribute to the *m/z* 221 ion unless it loses water, but the cyclic forms are isobaric with the open-chain aldehydo species and any of its potential tautomers shown in Scheme 1. It is worth noting that some of the hydrate (*m/z* 239, ~20% as abundant as *m/z* 221) was observed in the gas phase under our ionization conditions (nanospray from methanol). These data indicate that the *m/z* 221 anions in the gas phase might exist in additional cyclic forms, via hemiacetal formation as is typical of reducing sugars. Cyclization may endow the ions with unique gas-phase equilibria that in part contribute to the spectral differences observed between the different anomers and ring forms.



Scheme 3.



Scheme 4.

Table 3. ^{1}H NMR data (500 MHz) for α- and β-D-glucopyranosyl- and glucofuranosyl-glycolaldehyde hydrates and their cyclic forms **6**, **8**, **10**, and **12**

Proton signal	6	8a	8b	8c	10a	10b	10c	12
Chemical shift[a]								
H-1	4.957 d	4.499 d	4.554 d	4.507 d	5.217 d	5.415 d	5.491 d	5.034 s
H-2	3.554 dd	3.316 dd	3.658 dd	3.316 dd	4.184 t	4.246 dd	4.253 dd	4.218 br s
H-3	3.744 t	3.497 t	3.633 t	3.69[c]	4.34 t	4.335 d	4.304 d	4.258 d
H-4	3.411 t	3.393 t	3.76[c]	3.472 t	4.147 dd	4.407 dd	4.335 dd	4.195 dd
H-5	3.72[c] ddd	3.461 ddd	3.86[c]	3.589 ddd	3.88 ddd	3.882 ddd	3.884 ddd	3.990 ddd
H-6a	3.858 dd	3.915 dd	4.231 dd	3.91[c] dd	3.807 dd	3.833 dd	3.837 dd	3.857 dd
H-6b	3.758 dd	3.725 dd	3.819 dd	3.76[c] dd	3.66 dd	3.679 dd	3.677 dd	3.702 dd
H-1′	5.220 t	5.203 t	5.064 dd	5.145 br s[d]	5.190 t	4.976 dd	5.121 br s[d]	5.164 t
H-2′a	3.703 dd	3.861 dd	4.051 dd	3.984 d	3.742 dd	3.667 dd	4.004 dd	3.677 dd
H-2′b	3.511 dd	3.661 dd	3.819 dd	3.948 dd	3.575 dd	3.560 dd	3.517 dd	3.489 dd
Coupling constant[b]								
$J_{1,2}$	3.8	7.9	7.7	7.8	4.2	2.5	2.7	⩽1.0
$J_{2,3}$	9.8	9.3	9.8	9.8	3.4	1.2	1.1	⩽1.0
$J_{3,4}$	9.3	9.1	9.6	n.d.[e]	4.8	3.1	3.1	4.6
$J_{4,5}$	9.6	9.6	n.d.[e]	9.9	7.9	9.0	8.8	9.1
$J_{5,6a}$	1.9	2.3	n.d.[e]	2.1	3.0	2.3	2.4	2.7
$J_{5,6b}$	5.4	5.8	5.4	5.4	n.d.[e]	6.0	6.1	6.0
$J_{6a,6b}$	−12.0	−12.4	−12.5	−13.3	−12.0	−12.0	−12.0	−12.1
$J_{1',2'a}$	4.5	4.6	2.7	⩽1.0	4.4	2.4	2.1	4.5
$J_{1',2'b}$	5.2	5.2	9.1	1.8	5.1	8.8	1.5	5.1
$J_{2'a,2'b}$	−10.6	−10.7	−11.8	−12.6	−10.8	−11.2	−12.3	−10.7

[a] Chemical shifts are in parts per million with spectra recorded at 25 °C in D_2O relative to internal acetone at $\delta = 2.225$ ppm.
[b] In Hertz.
[c] Chemical shifts could only be determined to two decimal places due to spectral overlap.
[d] br s = broad singlet.
[e] n.d. = coupling constant not determined due to spectral overlap.

2.7. Preparation of glucosyl-glycolaldehydes

The glucopyranosyl- and glucofuranosyl-glycolaldehydes were prepared according to Scheme 5, with details presented in Section 3. Glycosidation to generate the allyl glycosides was essentially complete, but yielded all four allyl glycoside products that required separation by HPLC. Ozonolysis was clean and furnished the glycolaldehyde products essentially quantitatively as judged by NMR, bubbling O_3-rich oxygen into a sample in D_2O over a 6–8 min time period. The use of D_2O made quantitative reaction times straightforward to optimize as an aliquot of the sample could be rapidly analyzed nondestructively by NMR over different reaction times. Characterization of compounds by NMR and MS/MS is either tabulated in Table 3 or presented along with the title compounds.

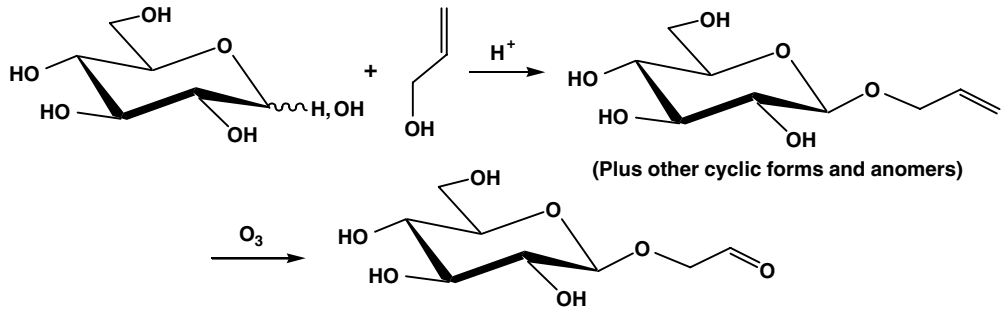
2.8. Concluding remarks

The *m*/*z* 221 anions derived from glucose-containing disaccharides were demonstrated, by comparison of their mass spectra to chemically synthesized standards, to be glucosyl-glycolaldehydes. Dissociation spectra of all four isomeric variants revealed two important features: (1) The anomeric configuration of the ions can be easily differentiated. (2) The ring forms (pyranoside or furanoside) are easily discriminated. Cleavage of 2-, 4-, and 6-linked glucose-containing disaccharides occurred to give *m*/*z* 221 anions that were comprised solely of the intact nonreducing sugar and a two-carbon fragment derived from the reducing sugar, clearly established by ^{18}O labeling of the carbonyl oxygen of their reducing glucose residues. This has been hypothesized[11,12,16] but not previously firmly established in the negative-ion mode by isotopically labeling one specific sugar of disaccharides to mass discriminate between their monosaccharides after dissociation. This structural information should be valuable in the studies of larger oligosaccharides where first disaccharide anions of *m*/*z* 341 and second their *m*/*z* 221 product ions may be selected after multiple dissociation/isolation events. It is of value to be able to assign the anomeric configuration, ring form, and location of the *m*/*z* 221 anion to the nonreducing position, in addition to the information about linkage position that could be assigned previously based on disaccharide ion (*m*/*z* 341) fragmentation in the negative-ion mode.[10–15] While it is premature to generalize these observations to other disaccharides with different monosaccharide stereochemistries, it is worthy of note that *m*/*z* 221 anions have been observed as products from other 2-, 4-, and 6-linked disaccharides.[10–15] For 3-linked disaccharide precursor ions, only trace amounts of the *m*/*z* 221 product ions were observed following dissociation in a Paul trap, in quantities too small to practically isolate and generate MS^3 spectra.

3. Experimental

3.1. Mass spectrometry

Mass spectrometry was performed to MS^2 or MS^3 using either a Thermoquest (Finnigan) classic LCQ instrument of Paul trap design equipped with a Finnigan nanospray source or MS^2 using a Sciex API 3000 equipped with an electrospray-ionization source in the negative-ion mode. The LCQ was operated using automatic gain control, with a typical needle voltage of 2.8 kV and a heated capillary temperature of 200 °C, with sample introduced at a flow rate of 0.4 μL/min, except in the analysis of sugar-glycolaldehydes where the temperature was lowered to 120 °C. The MS^n experiments were carried out with a relative collision energy that ranged from 27% to 34% and an activation *q* value of 0.25. The activation time was set at 30 ms. A total of 20–100 scans were accumulated for each spectral acquisition. The triple quadrupole instrument was operated at collision gas levels in the second quadrupole of 10 CAD units (Sciex), collision energies ranging from −5 to −50 V (data in Fig. 4 at −30 V, and precursor ion scans in Table 2 at −15 V), a needle voltage of −3500 V, temperature of the countercurrent gas at 250 °C, declustering potential of −15 V, focusing potential of −75 V, with an infusion flow of 70 μL/min. Product-ion scans were taken from *m*/*z* 50–400 over 3 s. Spectra were



Scheme 5.

acquired with unit mass resolution in Q1 and Q3. Samples were introduced into the source of either instrument in MeOH or MeOH–water mixtures having up to 5% water or ^{18}O water. High-resolution MS measurements were carried out in the negative-ion mode on a Thermoquest 7 T FTICR instrument interfaced to a front end linear ion trap equipped with a nanospray source (Finnigan LTQ-FTICR), located in the research resources center at the University of Illinois at Chicago medical center.

3.2. Nuclear magnetic resonance (NMR) spectroscopy

NMR spectroscopy was carried out at a field strength of 500 MHz (^1H) on a Varian Inova instrument. Acquisitions were performed on synthetic compounds in D_2O at 25 °C with a trace of acetone as an internal chemical shift standard at $\delta = 2.225$ ppm, and are reproducible to within 0.002 ppm. ^1H–^1H J-couplings are accurate to within 0.2 Hz. Assignments were made either through decoupling in 1D experiments or through 2D gCOSY[37] correlations. In some cases, a presaturation pulse was applied to diminish the HOD peak intensity.

3.3. Synthesis of allyl glucosides

The allyl glucosides **5**, **7**, **9**, and **11** (Scheme 2) were prepared by Fischer-type glycosidation. D-Glucose (100 mg) was dissolved in 10 mL of allyl alcohol by heating to about 90 °C. The sample was cooled to room temperature, trifluoroacetic acid (Aldrich, 0.385 mL) was added to 1 M, and the mixture was heated in a Teflon-capped tube under argon to 105 °C for 26 h. The sample was concentrated under high vacuum, and dissolved in 0.5 mL water-followed by 4.5 mL acetonitrile. The resultant allyl glycosides of all four configurations and ring forms were separated by HPLC, first in about 10 batches on a column (2.15 × 60 cm) of glycopak N (Waters) eluted with 95:5 (v/v) acetonitrile (Mallinckrodt, ChromAR grade)–water at 5 mL/min, with detection by UV at 200 nm using a Waters 486 variable-wavelength detector. This purified both glucose allyl pyranosides to homogeneity and separated them from the furanosides. The furanosides were completely separated (after concentration) on a column of Shodex DC-613 (6 × 150 mm) converted to the Cs form,[38] eluted with 95:5 (v/v) acetonitrile–water at 0.6 mL/min, detecting at 200 nm. The allyl α- and β-D-glucopyranosides **5** and **7** have been previously prepared and assigned using ^1H MR at 270 MHz.[39] Our data were essentially identical to that reported, except that at 500 MHz, small (1.1–1.3 Hz) couplings were also observed between the allyl H-1′ and H-3′ sets of protons, and approximately a −1.5 Hz coupling was observed between the H-3′a and H-3′b protons themselves. MS/MS spectra of compounds **5** and **7**, negative-ion, Paul trap, precursor m/z 219 $[M-H]^-$, m/z, relative abundance as % of base peak bracketed: for **5**, 71 (13%), 89 (14%), 101 (54%), 113 (21%), 143 (4%) 159 (10%), 161 (100%, base), 219 (10%, precursor); for **7**, 71 (4%), 101 (18%), 113 (8%), 143 (2%), 159 (7%), 161 (100%, base), 219 (9%, precursor). HRMS: $[M-H]^-$, for **5**, m/z 219.087 for $C_9H_{15}O_6$; Calcd 219.086; for **7**, m/z 219.087 for $C_9H_{15}O_6$; Calcd 219.086. The allyl glucofuranosides have not been previously prepared. NMR assignments of α and β anomeric configuration were based on similarity of the sugar ring J-couplings to the previously assigned methyl glucofuranosides.[35] For **9**, allyl α-D-glucofuranoside, ^1H NMR (D_2O): allyl group, δ 4.274 (dddd, 1H, $J_{1'a,1'b}$ −12.9, $J_{1'a,2'}$ 5.6, $J_{1'a,3'a}$ 1.3, $J_{1'a,3'b}$ 1.3 Hz, H-1′a), 4.149 (dddd, 1H, $J_{1'b,2'}$ 6.2, $J_{1'b,3'a}$ 1.2, $J_{1'b,3'b}$ 1.2 Hz, H-1′b), 5.969 (dddd, 1H, $J_{2',3'a}$ 17.3, $J_{2',3'b}$ 10.5 Hz, H-2′), 5.350 (dddd, 1H, $J_{3'a,3'b}$ −1.5 Hz, H-3′a), 5.262 (dddd, 1H, H-3′b), glucoside, 5.234 (d, 1H, $J_{1,2}$ 4.3 Hz, H-1), 4.169 (dd, 1H, $J_{2,3}$ 3.4 Hz, H-2), 4.316 (dd, 1H, $J_{3,4}$ 4.6 Hz, H-3), 4.126 (dd, 1H, $J_{4,5}$ 8.0 Hz, H-4), 3.884 (ddd, 1H, $J_{5,6a}$ 3.0, $J_{5,6b}$ 6.4 Hz, H-5), 3.807 (dd, 1H, $J_{6a,6b}$ −12.0 Hz, H-6a), 3.657 (dd, 1H, H-6b). MS/MS: negative-ion, Paul trap, precursor m/z 219 $[M-H]^-$; m/z, relative abundance as % base peak bracketed: 85 (1.3%), 101 (91%), 113 (4%), 161 (100%, base), 219 (6%, precursor). HRMS: $[M-H]^-$, for **9**, m/z 219.087 for $C_9H_{15}O_6$, Calcd 219.086. For **11**, allyl β-D-glucofuranoside, ^1H NMR (D_2O): allyl group, δ 4.209 (dddd, 1H, $J_{1'a,1'b}$ −12.8, $J_{1'a,2'}$ 5.6, $J_{1'a,3'a}$ 1.1, $J_{1'a,3'b}$ 1.1 Hz, H-1′a), 4.080 (dddd, 1H, $J_{1'b,2'}$ 6.3, $J_{1'b,3'a}$ 1.0, $J_{1'b,3'b}$ 1.0 Hz, H-1′b), 5.947 (dddd, 1H, $J_{2',3'a}$ 17.3, $J_{2',3'b}$ 10.4 Hz, H-2′), 5.344 (dddd, 1H, $J_{3'a,3'b}$ −1.5 Hz, H-3′a), 5.271 (dddd, 1H, H-3′b), glucoside, 5.031 (s, 1H, $J_{1,2}$ 1.0 Hz, H-1), 4.176 (br s, 1H, $J_{2,3}$ 1.0 Hz, H-2), 4.262 (d, 1H, $J_{3,4}$ 4.6 Hz, H-3), 4.176 (overlapped with H-2, dd, 1H, $J_{4,5}$ 8.9 Hz, H-4), 3.990 (ddd, 1H, $J_{5,6a}$ 2.7, $J_{5,6b}$ 6.0 Hz, H-5), 3.859 (dd, 1H, $J_{6a,6b}$ −12.0 Hz, H-6a), 3.696 (dd, 1H, H-6b). MS/MS: negative-ion, Paul trap, precursor m/z 219 $[M-H]^-$, m/z, relative abundance as % base peak bracketed: 85 (1.4%), 101 (100%, base), 113 (7%), 161 (75%), 219 (7%, precursor). HRMS: $[M-H]^-$, for **11**, m/z 219.086 for $C_9H_{15}O_6$; Calcd 219.086.

3.4. Synthesis of glucosyl-glycolaldehydes

The glucopyranoside-glycolaldehyde products **6** and **8** (Scheme 2) have been reported,[39–42] but in only one paper was the NMR spectrum of the α-D-glucopyranosyl-glycolaldehyde published but not fully assigned (in Supplementary data of Ref. 40). In the other papers, neither NMR nor MS data were reported as the sugar-glycolaldehydes were directly converted to Schiff base products.[39,41,42] The glucosyl-glycolaldehydes **6**, **8**, **10**, and **12** were prepared directly from the purified allyl glycosides. Allyl glycosides **5**, **7**, **9**, or **11** (10 mg) were

dissolved in 2 mL of D_2O; O_3, produced from pure O_2 gas using a commercial ozone generator, was bubbled through the sample for 6–8 min. The samples were immediately analyzed by NMR spectroscopy to check for reaction completion, freeze-dried to remove formaldehyde, and then stored in aqueous solution. Ozonolysis was efficient and clean, with essentially quantitative reaction yields during this (optimized) time period. Similar high reaction yields (albeit under different conditions) have been previously reported.[39] Aliquots were removed for NMR spectroscopy and MS analyses. NMR spectroscopy demonstrated that the glucosyl-glycolaldehyde products were either virtually entirely in the open-chain form as the hydrate (**6** and **12**), or were present in three forms having different proportions. For **8** and **10**, two additional six-membered cyclic forms were present (**8a**–**c** and **10a**–**c**, Schemes 3 and 4), through hemiacetal formation between the sugar OH-2 and the glycolaldehyde carbonyl. For **6**, α-D-glucopyranosyl-2-glycolaldehyde, NMR, hydrate: Table 3. MS/MS, Figure 2A, negative-ion, Paul trap, precursor *m/z* 221, $[M-H]^-$, *m/z*, relative abundance as % base peak bracketed: 161 (19%), 131, (100%, base), 113 (18%), 101 (87%), 221 (precursor, 16%) other ions less than 3%: 159, 129, 111, 99. HRMS: $[M-H]^-$, for **6**, *m/z* 221.067 for $C_8H_{13}O_7$; Calcd 221.066. For **8**, β-D-glucopyranosyl-2-glycolaldehyde, three forms; % abundance of **8a**:**8b**:**8c** 61:17:22, based on integration of H-1′ signals. NMR: Table 3. MS/MS, Figure 2B, negative-ion, Paul trap, precursor *m/z* 221, $[M-H]^-$, *m/z*, relative abundance as % base peak bracketed: 161 (100%, base), 159 (5%), 131 (89%), 101 (6%), 87 (3%), 221 (precursor, 19%). HRMS: $[M-H]^-$, for **8**, *m/z* 221.067 for $C_8H_{13}O_7$; Calcd 221.066. For **10**, α-D-glucofuranosyl-2-glycolaldehyde, three forms; % abundance of **10a**:**10b**:**10c** 11:33:56, based on integration of H-1′ signals. NMR: Table 3. MS/MS, Figure 2C, negative-ion, Paul trap, precursor *m/z* 221, $[M-H]^-$, *m/z*, relative abundance as % base peak bracketed: 177 (3%), 173 (7%), 161 (81%), 159 (73%), 113 (11%), 101 (100%, base), 99 (81%), 221 (precursor, 15%). HRMS: $[M-H]^-$, for **10**, *m/z* 221.066 for $C_8H_{13}O_7$; Calcd 221.066. For **12**, β-D-glucofuranosyl-2-glycolaldehyde, NMR, hydrate: Table 3. MS/MS, Figure 2D, negative-ion, Paul trap, precursor *m/z* 221, $[M-H]^-$, *m/z*, relative abundance as % base peak bracketed: 161 (100%, base), 101 (10%), 99 (3%), 221 (precursor, 13%). HRMS: $[M-H]^-$, for **12**, *m/z* 221.066 for $C_8H_{13}O_7$; Calcd 221.066.

3.5. ^{18}O-Labeling of the carbonyl of reducing disaccharides and glucosyl-glycolaldehydes

Labeling of the carbonyl oxygen of the reducing sugar of disaccharides was carried out essentially as described.[5] Briefly, the disaccharide was labeled with ^{18}O by adding 0.5 μL of acetyl chloride to 1 mg of the disaccharide dissolved in 100 μL of $H_2{}^{18}O$ (Isotec). The sample was kept in a sealed vial for 3 weeks. It was lyophilized and redissolved in 100 μL of $H_2{}^{18}O$ for use as a stock solution, stored frozen, and aliquots were diluted at least 100-fold in MeOH prior to analysis. The ^{18}O-labeled glucopyranosyl-glycolaldehydes **6** and **8** were prepared by directly dissolving them in 100 μL of $H_2{}^{18}O$ without the use of acetyl chloride, whereupon the carbonyl group was replaced with ^{18}O after 24 h. The ^{18}O-labeled precursor ions were isolated in the gas phase free from any residual unlabeled precursor ions having a 2 Da mass difference prior to dissociation.

Acknowledgments

We thank Dr. Robert Murphy for use of his ozone generator and Dr. Yan Wang for running high-resolution MS at the University of Illinois at Chicago. This work was supported in part by a grant from the National Science Foundation (CHE-0137986). Funds from the NSF, NIH, Howard Hughes medical foundation, and the Keck foundation supported instrumentation used in this research (NMR and mass spectrometers).

References

1. Zaia, J. *Mass Spectrom. Rev.* **2004**, *23*, 161–227.
2. Park, Y. M.; Lebrilla, C. B. *Mass Spectrom. Rev.* **2005**, *24*, 232–264.
3. Ashline, D.; Singh, S.; Hanneman, A.; Reinhold, V. *Anal. Chem.* **2005**, *77*, 6250–6262.
4. Zhou, Z.; Ogden, S.; Leary, J. A. *J. Org. Chem.* **1990**, *55*, 5444–5446.
5. Hofmeister, G. E.; Zhou, Z.; Leary, J. A. *J. Am. Chem. Soc.* **1991**, *113*, 5964–5970.
6. Staempfli, A.; Zhou, Z.; Leary, J. A. *J. Org. Chem.* **1992**, *57*, 3590–3594.
7. Dongre, A. R.; Wysocki, V. H. *Org. Mass Spectrom.* **1994**, *29*, 700–702.
8. Asam, M. R.; Glish, G. L. *J. Am. Soc. Mass Spectrom.* **1997**, *8*, 987–995.
9. Polfer, N. C.; Valle, J. J.; Moore, D. T.; Oomens, J.; Eyler, J. R.; Bendiak, B. *Anal. Chem.* **2006**, *78*, 670–679.
10. Ballistreri, A.; Montaudo, G.; Garozzo, D.; Giuffrida, M.; Impallomeni, G. *Rapid Commun. Mass Spectrom.* **1989**, *3*, 302–304.
11. Garozzo, D.; Giuffrida, M.; Impallomeni, G.; Ballistreri, A.; Montaudo, G. *Anal. Chem.* **1990**, *62*, 279–286.
12. Dallinga, J. W.; Heerma, W. *Biol. Mass Spectrom.* **1991**, *20*, 215–231.
13. Carroll, J. A.; Ngoka, L.; Beggs, C. G.; Lebrilla, C. B. *Anal. Chem.* **1993**, *65*, 1582–1587.
14. Garozzo, D.; Impallomeni, G.; Spina, E.; Green, B. N.; Hutton, T. *Carbohydr. Res.* **1991**, *221*, 253–257.
15. Mulroney, B.; Traeger, J. C.; Stone, B. A. *J. Mass Spectrom.* **1995**, *30*, 1277–1283.
16. Mulroney, B.; Peel, J. B.; Traeger, J. C. *J. Mass Spectrom.* **1999**, *34*, 856–871.

17. Mendonca, S.; Cole, R. B.; Zhu, J.; Cai, Y.; French, A. D.; Johnson, G. P.; Laine, R. A. *J. Am. Soc. Mass Spectrom.* **2005**, *14*, 63–78.
18. Domon, B.; Muller, D. R.; Richter, W. J. *Int. J. Mass Spectrom. Ion Processes* **1990**, *100*, 301–311.
19. Peltier, J. M.; MacLean, D. B.; Szarek, W. A. *Rapid Commun. Mass Spectrom.* **1991**, *3*, 446–449.
20. Seymour, F. R. *Meth. Carbohydr. Chem.* **1993**, *9*, 59–85.
21. Bendiak, B.; Fang, T. T. *Carbohydr. Res.* **2000**, *327*, 463–481.
22. Lindberg, B.; Lönngren, J. *Methods Enzymol.* **1978**, *50*, 3–33.
23. Gray, G. R. *Methods Enzymol.* **1990**, *193*, 573–586.
24. Smith, G.; Leary, J. A. *J. Am. Soc. Mass Spectrom.* **1996**, *7*, 953–957.
25. Jiang, Y.; Cole, R. B. *J. Am. Soc. Mass Spectrom.* **2005**, *16*, 60–70.
26. Xue, J.; Song, L.; Khaja, S. D.; Locke, R. D.; West, C. M.; Laine, R. A.; Matta, K. L. *Rapid Commun. Mass Spectrom.* **2004**, *18*, 1947–1955.
27. Ray, T. C.; Smith, A. R. W.; Wait, R.; Hignett, R. C. *Eur. J. Biochem.* **1987**, *170*, 357–361.
28. Freire, C. S. R.; Silvestre, A. J. D.; Silva, A. M. S.; Neto, C. P.; Domingues, P. *Holzforschung* **2004**, *58*, 501–503.
29. Mukhamed'yarova, M. M.; Chumbalov, T. K. *Chem. Nat. Compd.* **1977**, *13*, 243.
30. Rendina, M. A. C.; Giuffra, S. E.; Galeffi, C.; Monache, E. M. D. *Atti Della Accademia Nazionale Dei Lincei Rendiconti-Classe D, Scienze Fisiche-Matematiche e Naturali* **1971**, *50*, 29–32.
31. Domon, B.; Costello, C. E. *Glycoconjugate J.* **1988**, *5*, 397–409.
32. Carroll, J. A.; Willard, D.; Lebrilla, C. B. *Anal. Chim. Acta* **1995**, *307*, 431–447.
33. McClellan, J. E.; Quarmby, S. T.; Yost, R. A. *Anal. Chem.* **2002**, *74*, 5799–5806.
34. Bristow, A. W. T.; Nichols, W. F.; Webb, K. S.; Conway, B. *Rapid Commun. Mass Spectrom.* **2002**, *16*, 2374–2386.
35. Angyal, S. J. *Carbohydr. Res.* **1979**, *77*, 37–50.
36. Otter, A.; Bundle, D. R. *J. Magn. Reson. Ser. B* **1995**, *109*, 194–201.
37. Hurd, R. E. *J. Magn. Reson.* **1990**, *87*, 422–428.
38. Martensson, S.; Levery, S. B.; Fang, T. T.; Bendiak, B. *Eur. J. Biochem.* **1998**, *258*, 603–622.
39. Holme, K. R.; Hall, L. D. *Carbohydr. Res.* **1992**, *225*, 291–306.
40. Bendiak, B.; Salyan, M. E.; Pantoja, M. *J. Org. Chem.* **1995**, *60*, 8245–8256.
41. Bernstein, M. A.; Hall, L. D. *Carbohydr. Res.* **1980**, *78*, C1–C-3.
42. Weingarten, S.; Thiem, J. *Synlett* **2003**, *7*, 1052–1054.