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# Influence of coordination number and ligand size on the dissociation mechanisms of transition metal-monosaccharide complexes

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**Abstract**

Several features of metal-carbohydrate complexes, in the form of deprotonated metal/*N*-glycoside ions, were varied in order to determine which types of complexes would best enable mass spectrometric differentiation of the stereochemical features of the coordinated monosaccharides. Metal complexes were generated by using transition metals such as nickel, copper, and zinc. Additionally, the size and coordination number of ligands in the complex, as well as the number of these ligands coordinated to the metal, were varied. By using a quadrupole ion trap mass spectrometer, multistage mass spectrometric experiments were performed on the electrospray-generated metal *N*-glycoside complexes. Several tricoordinate Ni *N*-glycoside systems were capable of differentiating the stereochemistry about the C-2 center in the monosaccharide ring, whereas the tricoordinate Cu/*en* system allowed for the differentiation of both the C-2 and C-4 stereocenters. No stereochemical differentiation was possible from four- or five-coordinate species with the exception of the four-coordinate Zn/*dien* complexes. Changes in the metal center or size of the *N*-glycoside ligand generated the greatest changes in the product ion spectra of the tricoordinate complexes. Such alterations in four- or five-coordinate complexes often did not result in greatly differing product ion spectra. Whereas the product ion spectra of most metal/*N*-glycoside complexes could be easily categorized according to structural features of the precursor ion, exceptional species such as the four coordinate [Zn(*dien*/monosaccharide)–H]$^+$ precursor ion, also capable of differentiating the stereochemical features about C-2 and C-4, can give unexpected stereochemical information. (Int J Mass Spectrom 182/183 (1999) 299–310) © 1999 Elsevier Science B.V.

*Keywords:* Transition metal; Monosaccharide; Electrospray ionization; Quadrupole ion trap; Stereochemistry.

## 1. Introduction

As the diverse composition and roles of carbohydrates in biological systems become increasingly apparent [1], so does the need for a rapid, sensitive methodology that would allow for the identification of the sequence and structural features of these important biomolecules. Tandem mass spectrometry (MS/MS) is attractive because it can be used to analyze small quantities of heterogeneous samples [2] such as those often encountered with biological samples. Numerous examples of the successful use of MS/MS can be cited where information has been obtained about the number of monomer units [3], substitution patterns (e.g.

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It is with great admiration and respect that we dedicate this research to the memory of Professor Ben S. Freiser.


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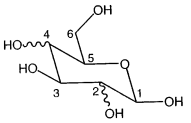
Table 1
Ring substituent configuration of *D*-aldohexoses




| Monosaccharide | C-2–OH | C-4–OH |
|---|---|---|
| glucose | equatorial | equatorial |
| mannose | axial | equatorial |
| galactose | equatorial | axial |
| talose | axial | axial |

occurrence of amine, *N*-acetylamine, or carboxylic acid functionalities) [3–5], linkage position or branching points [6–17] in a carbohydrate. However, structural characterization is not complete without distinguishing stereochemical features of the carbohydrate including those arising from the presence of differing diastereomeric monosaccharides (i.e. glucose, mannose, etc.). Mass spectrometric techniques may seem ill suited for an application requiring differentiation of stereochemical features of isomeric molecules, nevertheless, a large number of examples displaying the potential sensitivity of mass spectrometry toward stereochemical features of molecules are readily available, as demonstrated by the publication of an entire text on the subject [18].

Complete differentiation of all eight unsubstituted, diastereomeric D-monosaccharides would require an analytical technique that is capable of discriminating configurations of three stereocenters in a monosaccharide ring. A more pragmatic goal might involve the differentiation of the most biologically relevant unsubstituted monosaccharides: glucose, mannose, and galactose. Such a task still requires discerning the configurations of two stereocenters (Table 1). Previous work in our laboratory has utilized various metal-ligand systems (metal *N*-glycoside complexes) as a route to generating cationized carbohydrates [19–21]. It was often observed that the dissociation mechanisms of these metal-carbohydrate cations were reflective of specific stereochemical features of the coordinated carbohydrate. However, the use of some metal systems allowed for differentiation of no more than a single stereocenter [19,20].

Investigations into the formation of metal-carbohydrate complexes by different groups have been ongoing [22–25], with the complexes often applied to chromatographic and electrophoretic separation of carbohydrates. The ability of metal cations to act as separation agents in carbohydrate chromatography has been suggested to result from the presence of differing metal complexation sites on diastereomeric monosaccharides. These differing complexation sites are probably generated by variations in the number and position of axial and equatorial substituents on the monosaccharide. For example, the largest stability constants observed with cuprammonium complexes of carbohydrates occurred for those monosaccharides possessing an *axial, equatorial, axial* sequence of three oxygen atoms [26]. It may be generally concluded that the interactions between the metal and carbohydrate within a metal-carbohydrate complex will differ for a given metal depending on the structure of the complexation environment presented by the carbohydrate. This concept is clearly illustrated by the crystal structure of the distrontium complex of di-$\beta$-D-fructopyranose 2′,1:2,1′-dianhydride, where two structurally distinct coordination sites on a single sugar molecule yielded bidentate and tetradentate sites that were coordinated to different $Sr^{2+}$ cations [27]. The structure of metal-carbohydrate complexes can also vary greatly with the metal cation. Such structural differences are immediately apparent from the observation that the metal-carbohydrate stoichiometry in a methyl furanoside complex is 1:1 for the $Ca^{2+}$ complex and 1:2 for the $Pb^{2+}$ complex [28].

The goal of this work is to identify metal-carbohydrate complexes that may be used for the complete mass spectrometric differentiation of the diastereomeric monosaccharides listed in Table 1. An analysis of the behavior of the different complex stabilities and compositions reported for the condensed-phase metal-carbohydrate complexes allows for the formulation of a strategy to be used for identifying such complexes. First, changes in the *N*-glycoside ligands can be made that result in complexation sites that emphasize the dependence of the dissociation pathways upon the monosaccharide stereochemistry. Features of the *N*-glycoside ligands that may potentially be varied are

the size of the amine used to generate the *N*-glycoside ligand and the coordination number of the resulting ligand. Additionally, the number of ligands coordinated to the metal may be varied. Second, an attempt can be made to further "tune" the complex structure by taking advantage of a metal's preference for a specific coordination geometry. Thus, in the research presented herein, an assay has been undertaken that probes the effects of varying the metal center and ligand characteristics with an emphasis toward understanding how these changes influence the dissociation pathways of diastereomeric metal *N*-glycoside complexes. The various complexes were generated via electrospray ionization (ESI) and their product ion spectra obtained by using a quadrupole ion trap. Such instrumentation and its ability to perform multistage MS experiments ($MS^n$) have been shown to be useful in the stereochemical differentiation of a series of Zn complexes [21].

## 2. Experimental

### 2.1. Instrumentation

All experiments were carried out on a Finnigan LCQ quadrupole ion trap mass spectrometer (Finnigan MAT, San Jose, CA) equipped with an electrospray ionization source. The pressure inside the vacuum chamber was $1.9 \times 10^{-3}$ Torr or less during all acquisitions. Samples were infused into the instrument at 4 $\mu$L/min. Ionization was performed at either 4 kV for the Ni complexes or 5 kV for the Zn and Cu complexes. All spectra were acquired at a capillary temperature of 150 °C and all ion guide voltages were tuned so as to maximize the abundance of the relevant precursor ion current. A total of 30 scans were averaged to produce each spectrum. Precursor ions were isolated over a mass range of 1.5–1.7 Da prior to excitation. rf voltages between 0.45 and 1.00 $V_{(p-p)}$, depending on the precursor ion, were applied to endcaps of the ion trap for 30 ms during the excitation period. The amplitude of the rf voltage was chosen to optimize any stereochemically differentiating features of the product ion spectra. All subsequent acquisitions were performed at this preset rf voltage. Additionally, all acquisition parameters were identical for a given set of diastereomeric complexes. A criterion of 3% relative abundance has been utilized to determine the presence or absence of a product ion for all data collected on diastereomeric differentiation. Ions of lower relative abundance are not reproducible, or are included in the noise level background.

### 2.2. Synthesis

Transition metal-amine complexes that were used as reagents to generate the metal *N*-glycoside complexes were synthesized by using one of three amine ligands: 1,3-diaminopropane (*dap*), ethylenediamine (*en*), and diethylenetriamine (*dien*). All metal amine reagents were synthesized using previously published procedures. The preparations and purifications are described briefly below

#### 2.2.1. *Ni (dap)$_3$ · 2Cl* [29], *Ni (en)$_3$ · 2Cl* [30], *Zn (dap)$_3$ · 2Cl* [31], *Zn (en)$_3$ · 2Cl* [31]

Three molar equivalents of the appropriate amine were added slowly to a methanolic solution of either $NiCl_2 \cdot 6H_2O$ or $ZnCl_2$. Solids precipitated soon after stirring. Each solid was purified by recrystallization from an isopropanol/methanol mixture.

#### 2.2.2. *Cu (dap)$_2$ · 2Cl* [29], *Cu (en)$_2$ · 2Cl* [32], *Ni (dien)$_2$ · 2Cl* [33] and *Zn (dien)$_2$ · 2Cl* [34]

Two molar equivalents of the appropriate amine were added slowly to a methanolic solution of $CuCl_2 \cdot H_2O$. Solids precipitated soon after stirring. Each solid was purified by recrystallization from an isopropanol/methanol mixture.

#### 2.2.3. *Cu (dien) · 2Cl* [35]

One molar equivalent of the diethylenetriamine was added slowly to a methanolic solution of $CuCl_2 \cdot H_2O$. A solid precipitated soon after stirring. The solid was purified by recrystallization from an isopropanol/methanol mixture.

*2.2.4. Metal/N-glycoside complexes*

Labeled and unlabeled metal-*N*-glycoside complexes were synthesized by using a microscaled adaptation of the procedure previously published by Yano et al. [36–39]. Briefly, 4 mg of one of four diastereomeric D-aldohexoses (Table 1) and three molar equivalents of a Ni, Zn, or Cu amine complex were dissolved in 200 $\mu$L of methanol, yielding final concentrations of 0.04 M and $\sim$ 0.12 M, respectively. Samples were refluxed at 80 °C for 20 min unless otherwise noted. All reaction mixtures were kept at $\sim$ 5 °C until used. No purification was performed prior to analysis.

*2.3. ESI-MS analysis*

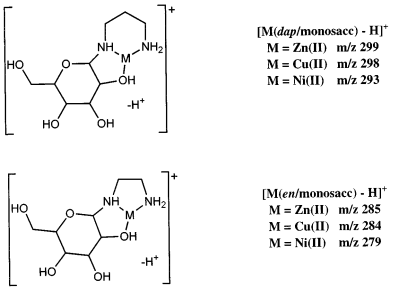
Solutions for ESI-MS were prepared by diluting the reaction mixture ($\sim$ 0.04 M) 100-fold by using 9:1 MeOH/$H_2O$. Final concentrations of all solutions were $\sim$ 400 $\mu$M.

*2.4. Chemicals and materials*

D-Glucose (A. C. S. reagent grade), D-mannose (99%), 1,3-diaminopropane (99%), ethylenediamine (99%), and diethylenetriamine (99%) were obtained from Aldrich Chemical Co. (Milwaukee, WI). D-Galactose (99%), D-talose, and HPLC grade water and methanol were obtained from Sigma Chemical Co. (St. Louis, MO). D-mannose-6-$^2H_2$ (98% D) and D-mannose-3-$^{13}$C (99% $^{13}$C) were obtained from Omicron Biochemical Co. (South Bend, IN). Zinc dichloride, nickel dichloride hexahydrate, and A. C. S. grade methanol were purchased from Fisher Chemical Co. (Fairborn, NJ). Copper dichloride dihydrate was purchased from Mallinckrodt, Inc. (Paris, KN). All materials were used as received without further purification.

## 3. Results and discussion

The original reports of the synthesis of these types of metal *N*-glycoside complexes typically discussed only one product complex that had been isolated.




Fortunately, in the performance of the desired studies on the various metal *N*-glycoside complexes of interest, it was not necessary to specifically synthesize and isolate only the desired complex prior to the preparation of any analyte solution. The low resolution mass spectra of a given reaction mixture always showed the presence of several types of metal *N*-glycoside complexes. The appropriate precursor ions were merely isolated in the ion trap prior to any $MS^n$ experiments.

Epimerization of the reactant monosaccharides was also a concern for all reactions. It has been shown that D-glucose can undergo rapid epimerization in the presence of metal cations and amine bases, even under mild reflux conditions [37]. However, such transformations of the monosaccharides were observed with transition metal cations only when using methylated amines (i.e. *N*, *N′*-dimethylethylenediamine). None of the amine ligands used in this study were methylated. Epimerization of monosaccharides in the presence of the transition metal cations and the amine ligands used in this study has not been observed previously.

*3.1. Tricoordinate complexes*

Scheme 1 displays the various types of tricoordinate, deprotonated species that were investigated in this study. All complexes consisted of a single tridentate *N*-glycoside ligand coordinated to a doubly charged metal ion. The ligands possess a deprotonation site, yielding the singly charged species of interest. The tridentate *N*-glycoside ligands were generated by using either *en* or *dap*. Metal *N*-glycoside complexes of all four monosaccharides (Table 1) were

generated for all tricoordinate metal/*en* and metal/*dap* systems.

Ions consistent with the monoisotopic, deprotonated tricoordinate species were present in the low-resolution mass spectra of all reaction mixtures. The presence of isobaric interferences was not obvious in most low-resolution mass spectra as suggested by the typically excellent agreement between the theoretical and experimental isotope patterns of the complexes. However, for the Cu/*en*/talose and all Cu/*dap* reaction mixtures, the low-resolution mass spectra clearly indicated the presence of isobaric interferences. In such cases, the deprotonated species was alternately generated via a corresponding HCl adduct present in all reaction mixtures. Collision induced dissociation (CID) of these adducts resulted primarily in the loss of HCl, yielding the desired deprotonated species:

$$[M + Cl]^+ \overset{CID}{\rightarrow} M + HCl$$

The product ion spectra of the deprotonated species of such complexes were thus generated in $MS^3$ experiments.

It was of great concern that this alternate method of producing the deprotonated species would result in the formation of a different precursor ion than would have otherwise been produced in-source in the absence of the isobaric interferences. To alleviate such concerns, product ion spectra of the in-source generated deprotonated species ($MS^2$ experiments) were compared to the product ion spectra of similar complexes generated via CID of the HCl adduct ($MS^3$ experiments) in reaction mixtures where such isobaric interferences were not detected. The product ion spectra obtained in the $MS^2$ and $MS^3$ experiments were identical to each for all reaction mixtures where such comparisons could be made. Therefore, it was assumed that when isobaric interferences were likely to be present along with the deprotonated species, relevant product ion spectra could be obtained by using the precursor ion generated via the HCl adduct.

Although the product ion spectra varied somewhat with different metal centers and ligand sizes, similar types of neutral losses were observed from all tricoordinate species. Most product ions resulted from

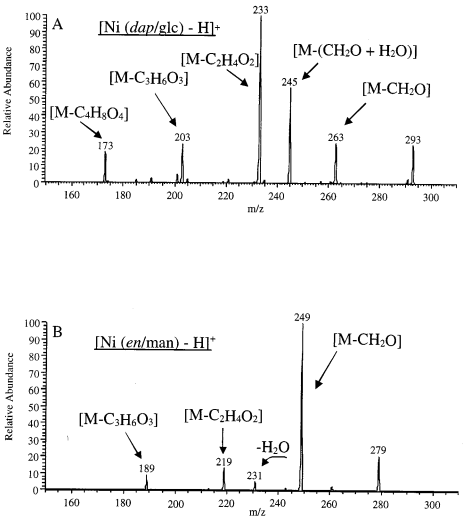



Fig. 1. Product ion spectra ($MS^2$) of tricoordinate deprotonated Ni *N*-glycoside precursor ions: (A) [Ni (*dap*/glucose)–H]$^+$ precursor ion (*m*/*z* 293); (B) [Ni (*en*/mannose)–H] + precursor ion (*m*/*z* 279). *M* represents the precursor ion in each spectrum.

cross-ring cleavages in the monosaccharide moiety (neutral loss of $C_nH_{2n}O_n$, where *n* ranges from 1 to 4) [8,9,40–42] and/or losses of one or more water molecules. Similar product ion spectra have also been reported for the tricoordinate Ni/*dap* precursor ions by using either a sector instrument fast-atom bombardment (FAB-generated complexes) [19] or a triple quadrupole mass spectrometer (ESI-generated complexes) [20].

The dissociation of the tricoordinate Ni/*dap* precursor ions resulted in the formation of a large variety of product ions as represented by the product ion spectrum of the glucose complex in Fig. 1(A). One notable product ion, the *m*/*z* 245 ion (combined neutral loss of $CH_2O$ and $H_2O$) was useful for stereochemical characterization. The *m*/*z* 245 product ion was fairly intense for both complexes possessing equatorial C-2 hydroxyl substituents as indicated in Fig. 1(A) for the glucose complex. Conversely, complexes possessing axial C-2 substituents were characterized by a low intensity ($< 5\%$ of the base peak) of this same product ion.

A number of product ions were also detected following the dissociation of some Ni/*en* complexes

as represented by the product ion spectrum of [Ni (*en*/mannose)–H]$^+$ in Fig. 1(B). Unlike the Ni/*dap* complexes, the intensity of the product ion resulting from the combined losses of $CH_2O$ and $H_2O$ (*m*/*z* 231) was not sensitive to the stereochemical features of the complexed monosaccharides. Instead, it was observed that, at the collision energies utilized, product ions at a mass-to-charge ratio smaller than *m*/*z* 219 (e.g. *m*/*z* 189) were observed only for complexes possessing C-2 axial substituents. Such lower mass product ions were entirely absent from the product ion spectra of those complexes possessing equatorial C-2 substituents.

Both tricoordinate Ni complexes can be used to differentiate the orientation of the C-2 hydroxyl group of a monosaccharide. However, such structural sensitivity manifests itself differently depending on the ligand size (i.e. *en*- versus *dap*-based *N*-glycosidic ligands). One feature shared by the dissociation processes of all tricoordinate Ni complexes is the inability to differentiate the configurations of the C-4 substituent of any of the monosaccharides. The ability to differentiate the substituent orientations about both stereocenters (C-2 and C-4) is necessary to distinguish all four diastereomeric monosaccharides.

A representative product ion spectrum of a tricoordinate Zn/*dap* complex is shown in Fig. 2(A). As was observed with the Ni/*dap* system, a number of product ions were present. Unfortunately, the general features of the product ion spectra of the four diastereomeric complexes were not significantly varied to allow for any stereochemical differentiation. Most notably, the intensity of the *m*/*z* 251 product ion (combined losses of $CH_2O$ and $H_2O$) was not sensitive to the stereochemical features of the C-2 substituent as it was in the comparable Ni/*dap* complexes.

The formation of product ions from the tricoordinate Zn precursor ions was also dependent upon the size of the coordinated ligand. Fewer product ions were generated from the dissociation of the smaller Zn/*en* complexes as shown by a representative product ion spectrum in Fig. 2(B). Product ions at a mass-to-charge ratio less than 225 were very weak (< 1% of the base peak) for all four Zn/*en* complexes. The product ion spectra of the tricoordinate Zn/*en*

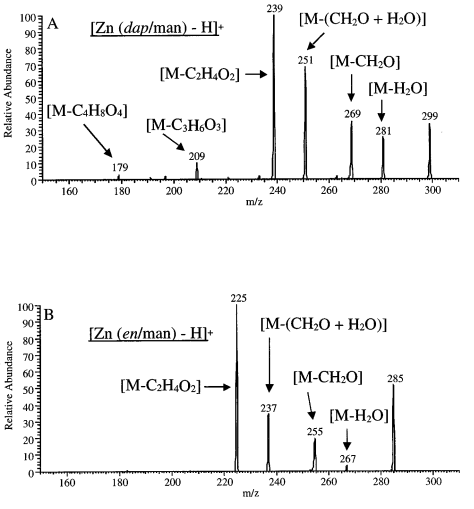



Fig. 2. Product ion spectra ($MS^2$) of tricoordinate deprotonated Zn *N*-glycoside precursor ions: (A) [Zn (*dap*/mannose)–H]$^+$ precursor ion (*m*/*z* 299); (B) [Zn (*en*/mannose)–H] + precursor ion (*m*/*z* 285). *M* represents the precursor ion in each spectrum.

precursor ions also did not include any apparent features allowing for stereochemical differentiation.

The product ion spectra of all tricoordinate Cu/*dap* complexes were very simple, possessing a single prominent product ion at *m*/*z* 178 ($C_4H_8O_4$). This is demonstrated by the product ion spectrum of the [Cu (*dap*/mannose)–H]$^+$ precursor ion [Fig. 3(A)]. It is clear that such uncomplicated product ion spectra do not possess features that would allow for any stereochemical differentiation.

The product ion spectra of the tricoordinate Cu/*en* complexes present a stark contrast to the uninformative spectra obtained with the Cu/*dap* tricoordinate species. The product ion spectrum of the [Cu (*en*/mannose)–H]$^+$ precursor ion [Fig. 3(B)] is an example of the increased diversity of the dissociations that occur from this smaller Cu *N*-glycoside complex. Inspection of the spectra obtained under identical experimental conditions from all four diastereomeric Cu/*en* complexes revealed unique product ion spectra for each of these precursor ions. Table 2 summarizes the various product ions that were generated from the different tricoordinate Cu/*en* species.

It is clear that the types of neutral losses obtained from the dissociation of deprotonated, tricoordinate

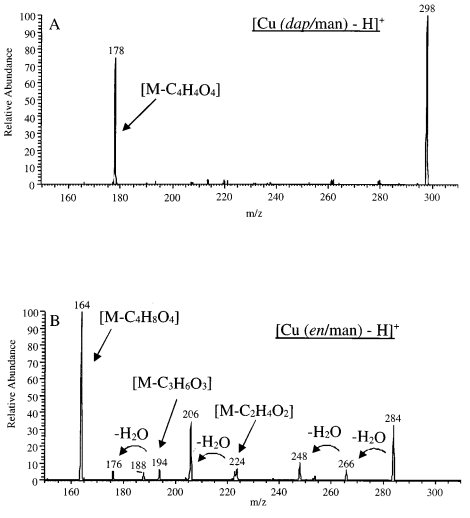
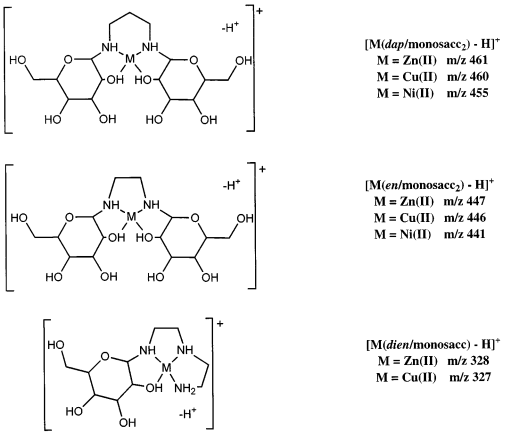



Fig. 3. Product ion spectra ($MS^3$) of tricoordinate deprotonated Cu *N*-glycoside precursor ions: (A) [Cu (*dap*/mannose)–H]$^+$ precursor ion (*m*/*z* 298); (B) [Cu (*en*/mannose)–H] + precursor ion (*m*/*z* 284). *M* represents the precursor ion in each spectrum. The deprotonated precursor ions were generated via a HCl adduct (see text).

metal/*N*-glycoside complexes vary when comparing the *dap*-based *N*-glycoside ligands to the smaller *en*-based *N*-glycoside ligands. Additionally, changing the metal center also affected the ability to achieve stereochemical differentiation from the product ion spectra of tricoordinate complexes. The orientation of only the C-2 substituent was discernible from the dissociation mechanisms of the Ni/*en* and Ni/*dap* complexes, whereas a full differentiation of the four diastereomeric monosaccharides was achieved with the Cu/*en* complexes. Contrasting these results, the product ion spectra of all tricoordinate Zn complexes




were devoid of features indicative of a clear stereochemical differentiation.

### 3.2. Four-coordinate complexes

All deprotonated, four-coordinate complexes consisted of a single tridentate *N*-glycoside ligand coordinated to a doubly charged metal ion as shown in Scheme 2. Two classes of *N*-glycoside ligands were utilized: *dien*-based ligands having a 1:1 amine/monosaccharide molar ratio, and *en*- or *dap*-based ligands having a 1:2 molar ratio. The ligands possess a deprotonation site, yielding the singly charged species of interest. The four-coordinate complexes could be generated from all reaction mixtures by using the four monosaccharides in Table 1, except for the Ni/*dien* reaction mixtures. Low-resolution mass spectra of the Ni/*dien* reaction mixtures often did not show any Ni *N*-glycoside ions sufficiently intense for $MS^2$ experiments, even after extended reflux times

Table 2
Product ions from [Cu (*en*/monosaccharide–H] + precursor ions (*m*/*z* 298) ("X" represents product ions that were observed at relative intensities greater than 3% of the base peak)

| | Product ions | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *m*/*z* 266 | *m*/*z* 253 | *m*/*z* 248 | *m*/*z* 224 | *m*/*z* 206 | *m*/*z* 194 | *m*/*z* 188 | *m*/*z* 176 | *m*/*z* 164 |
| glucose | ··· | ··· | ··· | X | X | ··· | X | ··· | X |
| galactose | X | X | ··· | X | X | ··· | X | ··· | X |
| mannose | X | ··· | X | X | X | X | X | X | X |
| talose | X | ··· | X | ··· | ··· | X | ··· | ··· | X |

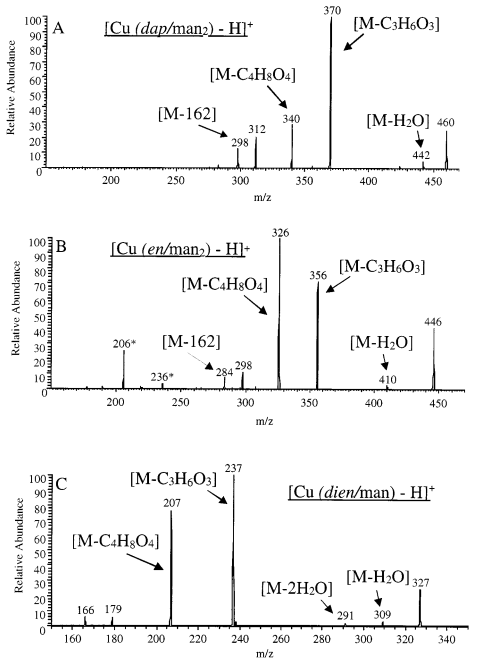



Fig. 4. Product ion spectra ($MS^2$) of four-coordinate deprotonated Cu *N*-glycoside precursor ions: (A) *dap*-based ligand possessing 1:2 amine/monosaccharide molar ratio (*m*/*z* 460); (B) *en*-based ligand possessing 1:2 amine/monosaccharide molar ratio (*m*/*z* 446); (C) *dien*-based ligand possessing 1:1 amine/monosaccharide ratio (*m*/*z* 327). *M* represents the precursor ion in each spectrum. Product ions marked with "*" result from cleavages in both monosaccharide rings.

(~ 12 h). Evidence of isobaric interferences with the four-coordinate species was not found in the low-resolution mass spectra as suggested by the typically excellent agreement between the theoretical and experimental isotope patterns of the complexes. Therefore, all product ion spectra were generated directly from the deprotonated precursor ions produced in the source.

Product ion spectra of representative four-coordinate Cu *N*-glycoside complexes possessing different amine/monosaccharide molar ratios and ligand sizes are shown in Fig. 4. The product ion spectra of four-coordinate Ni and Zn complexes (data not shown) were qualitatively similar to those in Fig. 4 in that identical neutral losses were observed with the different metal centers. Also evident from Fig. 4, the product ion spectra of different classes of four-coordinate precursor ions were remarkably similar to each other; i.e. changes in the ligand size or amine/monosaccharide molar ratio had little effect on the neutral losses observed. A notable exception to these similarities occurred for the previously studied Zn/*dien* complexes [21], as discussed below.

Two features occurring consistently in the product ion spectra of most four-coordinate complexes are the product ions resulting from neutral losses of 90 Da ($C_3H_6O_3$) and 120 Da ($C_4H_8O_4$). These 90 and 120 Da losses are most likely similar to those mentioned above, which involve cross-ring cleavages in the monosaccharide ring. Other types of cross-ring cleavages were relatively weak or absent. Losses of one or two water molecules were often observed, especially from the Zn and Cu complexes. Concurrent losses of $C_nH_{2n}O_n$ and additional water molecules (e.g. $CH_2O$ and $H_2O$), having been shown above to be useful for stereochemical differentiation of some tricoordinate complexes, were absent.

Large mass neutral losses (180, 210, and 240 Da) were present only in the product ion spectra of complexes containing ligands with a 1:2 amine/monosaccharide molar ratio. Such neutral losses are likely to result from a loss of 90 or 120 Da from one monosaccharide ring and an additional loss of 90 or 120 Da from the second monosaccharide ring. Cleavage of the *N*-glycoside bond, resulting in the loss of a neutral anhydromonosaccharide (162 Da) was also observed only for complexes possessing this class of ligands.

Unique product ions, characteristic of the configuration of a stereocenter, were not observed as shown by the similarity amongst the product ion spectra of most of the diastereomeric, four-coordinate complexes. Therefore, stereochemical differentiation was not possible amongst most four-coordinate complexes. Unlike the tricoordinate ions, changes in the metal center and ligand size typically did not significantly alter the product ion spectra of the four-coordinate species.

One notable exception amongst the four-coordinate complexes is the Zn/*dien* species. As has been previously reported in detail [21], characteristic product ion spectra of the four diastereomeric precursor

Table 3
Product ions from [Zn (*dien*/monosaccharide)–H] + precursor ions (*m*/*z* 328) ("X" represents product ions that were observed at relative intensities greater than 3% of the base peak)

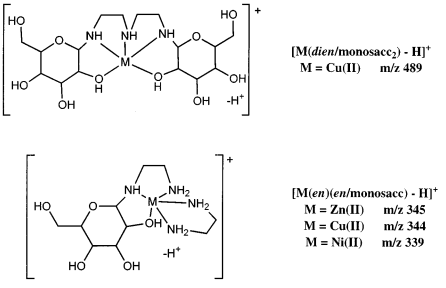
| | Product ions | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *m*/*z* 310 | *m*/*z* 298 | *m*/*z* 292 | *m*/*z* 280 | *m*/*z* 268 | *m*/*z* 238 | *m*/*z* 226 | *m*/*z* 208 |
| glucose | ··· | X | ··· | X | X | ··· | X | X |
| galactose | X | X | ··· | X | X | X | X | X |
| mannose | X | X | ··· | X | X | X | ··· | X |
| talose | X | X | X | ··· | X | X | ··· | X |

ions can be obtained from the four-coordinate [Zn (*dien*/monosaccharide)–H]$^+$ complexes, as summarized in Table 3.

### 3.3. Five-coordinate complexes

The two types of deprotonated, five-coordinate complexes studied are shown in Scheme 3. One group of complexes consisted of a single pentadentate *dien*-based *N*-glycoside ligand (1:2 amine/monosaccharide molar ratio) coordinated to a doubly charged metal ion. The second group of complexes consisted of a doubly charged metal ion coordinated by two ligands: a tridentate *en*-based *N*-glycoside ligand and a bidentate amine ligand. All complexes possess a deprotonation site, yielding the singly charged species of interest.

Examples of single-ligand, five-coordinate complexes were limited only to the species obtained from the Cu/*dien* reaction mixtures. Complexes of this sort were generated for all four diastereomeric monosaccharides (Table 1). Although other types of Zn *N*-glycoside complexes were produced from the Zn/*dien* reaction mixtures (i.e. [Zn (*dien*/monosaccharide)–H]$^+$), the five coordinate species were not sufficiently intense to allow for MS$^n$ experiments. The problems encountered with generating any Ni *N*-glycoside complexes from the Ni/*dien* reaction mixtures have been discussed above. The deprotonated, single-ligand Cu *N*-glycoside precursor ions were those generated in-source and not via a HCl adduct.



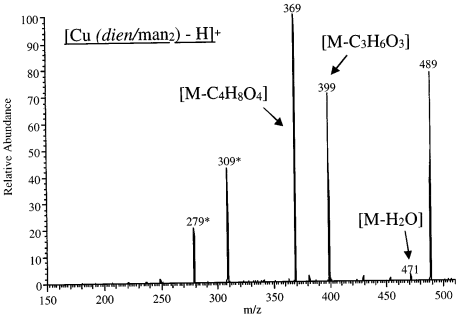




Fig. 5. Product ion spectrum (MS$^2$) of five-coordinate [Cu (*dien*/mannose$_2$)–H]$^+$ precursor ion (*m*/*z* 489). *M* represents the precursor ion in each spectrum. Product ions marked with "*" result from cleavages in both monosaccharide rings.

A representative product ion spectrum of a single-ligand, five-coordinate complex is shown in Fig. 5. Such a spectrum is very reminiscent of the product ion spectra of the four-coordinate complexes possessing ligands with a 1:2 amine/monosaccharide molar ratio [Fig. 4(A) and (B)]. Cross-ring cleavages within a single monosaccharide ring resulting in neutral losses of 90 Da ($C_3H_6O_3$) or 120 Da ($C_4H_8O_4$) are the most prominent dissociation pathways. Concurrent losses from the second monosaccharide ring (product ions marked with a "*") were also present. As was the case for most four-coordinate complexes, characteristic product ions were not obtained from the four diastereomeric single-ligand, five-coordinate systems.

Difficulties were also encountered when attempting to make many of the two-ligand, five-coordinate precursor ions. The low-resolution mass spectra indicated that such species were not produced for many of the metal/*dap* reaction mixtures. However, the two-ligand complexes could be generated from most Ni/*en*, Cu/*en*, and Zn/*en* reaction mixtures; only the [Cu (*en*)(*en*/glucose)–H]$^+$ precursor ion could not be formed. All deprotonated, two-ligand, five-coordinate

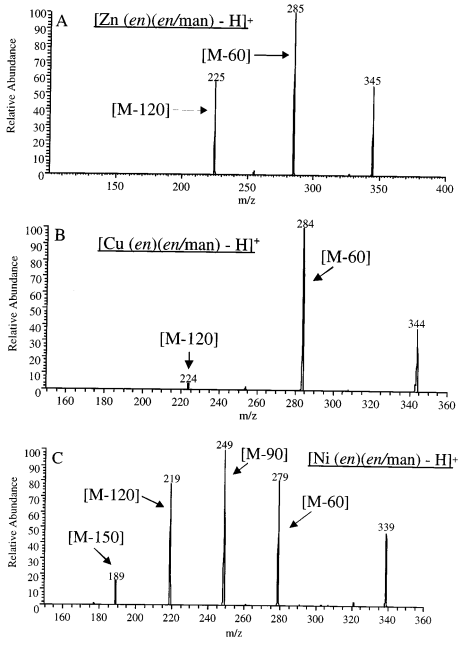



Fig. 6. Product ion spectrum ($MS^2$) of two-ligand, five-coordinate precursor ions: (A) [Zn (*en*)(*en*/mannose)–H]$^+$ precursor ion (*m*/*z* 345); (B) [Cu (*en*)(*en*/mannose)–H]$^+$ precursor ion (*m*/*z* 344); (C) [Ni (*en*)(*en*/mannose)–H]$^+$ precursor ion (*m*/*z* 339). *M* represents the precursor ions in each spectrum.

precursor ions utilized in these experiments were obtained directly from those generated in-source and not via CID of the corresponding HCl adduct.

Fig. 6(A) and (B) show representative product ion spectra of the [Zn (*en*)(*en*/mannose)–H]$^+$ and [Cu (*en*)(*en*/mannose)–H]$^+$ precursor ions, respectively. Similar spectra were obtained from the corresponding precursor ions from all other Zn/*en* and Cu/*en* reaction mixtures. Prominent product ions resulting from the loss of 60 and 120 Da were always present. Two assignments exist for each of these neutral losses. The 60 Da loss may be assigned to a neutral $C_2H_4O_2$ molecule following the typical cross-ring cleavages in the monosaccharide ring. However, because the bidentate *en* ligand also has a molecular weight of 60 Da, the loss of the amine ligand cannot be ruled out. Similarly, the 120 Da losses may result from either a cross-ring cleavage in the monosaccharide ring (loss of $C_4H_8O_4$) or a concurrent loss of the *en* ligand and $C_2H_4O_2$.

Isotopic labeling experiments were performed to identify the molecular formula of the 60 and 120 Da neutral losses. *N*-glycoside complexes of Cu and Zn were synthesized by using either mannose-6-$D_2$ or mannose-3-$^{13}$C. Such labeled compounds were chosen because it has been shown that the $C_2H_4O_2$ neutral loss may result from losses of either C-3 and C-4, or C-5 and C-6 [42]. Losses of other combinations of carbon centers (i.e. C-4 and C-5) or carbon centers possessing substituents coordinated directly to the metal center (i.e. C-1 or C-2) are likely to involve large barriers and have not been previously observed in the product ion spectra of metal *N*-glycoside complexes [20,21,42]. These labeled complexes can thus be used to distinguish neutral losses consisting of *en* or $C_2H_4O_2$. A neutral loss of 60 Da was observed in the product ion spectra of all the labeled [metal (*en*)(*en*/mannose)–H]$^+$ complexes (metal = Cu, Zn). Such results conclusively show that the 60 Da loss from the two-ligand, five-coordinate complexes was because of the loss of only the bidentate *en* ligand from the precursor ion.

The loss of 120 Da was also easily assigned. Neutral losses of 122 Da were present in the product ion spectra of the mannose-6-$D_2$ complexes, indicating that C-6 and its labeled atoms are lost in this dissociation pathway. However, neutral losses of only 120 Da were observed from the mannose-3-$^{13}$C complexes, which showed that C-3 was never lost from the precursor ion following this dissociation. It has been previously demonstrated that the loss of $C_4H_8O_4$ from Zn *N*-glycoside complexes resulted from the losses of C-3, C-4, C-5, and C-6. Because C-3 is retained by the product ion, the 120 Da loss can only be due to a concurrent loss of the bidentate *en* ligand and $C_2H_4O_2$.

The product ion spectra of the five-coordinate complexes generated from the Ni/*en* reaction mixtures also displayed prominent losses of 60 and 120 Da, as represented by the [Ni (*en*)(*en*/mannose)–H]$^+$ complex in Fig. 6(C). Several additional product ions (*m*/*z* 249 and *m*/*z* 189) were also present. As was observed with the Zn and Cu complexes, no stereochemically characteristic product ions were generated from any of the four diastereomeric precursor ions.

Isotopically labeled Ni/*en* reaction mixtures were also prepared by using mannose-6-$D_2$ and mannose-3-$^{13}C$. Again, the losses of 60 and 120 Da could be assigned to the loss of only the *en* ligand, and a concurrent loss of *en* and $C_2H_4O_2$, respectively. The labeling experiments that were performed did not allow for the unequivocal assignment of the other neutral losses from the Ni complex. Experiments that will allow for a conclusive assignment are currently in the planning stages for all metal-coordinated complexes. However, the loss of 150 Da ($m/z$ 189) is likely to be because of a successive loss of $C_3H_6O_3$ (90 Da) following a cross-ring cleavage and *en* ligand (60 Da). Cross-ring cleavages in the monosaccharide ring leading to the formation of the 150 Da neutral loss, $C_5H_{10}O_5$, have not been observed from these *N*-glycoside complexes. The loss of 90 Da may represent either the loss of $C_3H_6O_3$ or simultaneous losses of $CH_2O$ and the *en* ligand.

Although a complete survey of all relevant five-coordinate species could not be performed, some general trends in the dissociation pathways could still be discerned. First, the types of neutral losses generated were largely dependent upon the number of ligands coordinated to the metal. The single-ligand, five-coordinate species generated product ion spectra that were very similar to those of the four-coordinate species. The product ion spectra of the two-ligand, five-coordinate precursor ions varied for different metal complexes. Both the Cu and Zn complexes generated fewer product ions than the Ni complexes. However, the dissociation pathways of all such complexes were dominated by mechanisms involving loss of the bidentate amine ligand coordinated to the metal. Although the product ion spectra of both types of five-coordinate precursor ions were quite different, neither type of complex was capable of generating product ions that were characteristic of any of the stereochemical features of the coordinated monosaccharide.

## 4. Conclusions

Several features of deprotonated metal/*N*-glycoside complexes were varied in order to determine which types of complexes would best enable stereochemical differentiation of the coordinated monosaccharides. When possible, different coordination numbers, metal centers, and types of ligands were utilized. This work has demonstrated that the monosaccharides are typically best differentiated by MS/MS of the tricoordinate complexes, with the exception of the four coordinate Zn/*dien* species. The tricoordinate Cu(*en*) *N*-glycoside complexes displayed characteristic product ion spectra for each of the four diastereomeric monosaccharides. Therefore, these complexes allowed for the differentiation of the stereochemical features about the C-2 and C-4 centers of the monosaccharides. Differentiation of the stereochemical features about a single carbon center, C-2, was achieved by the tricoordinate Ni *N*-glycoside systems. However, the information obtained from a single stereocenter is insufficient to differentiate the four diastereomeric monosaccharides in Table 1. Changes in the metal center or size of the *N*-glycoside ligand generated the greatest changes in the product ion spectra of the tricoordinate complexes. Such alterations in four- or five-coordinate complexes typically did not result in greatly differing product ion spectra.

Although the product ion spectra of most metal/*N*-glycoside complexes could be easily categorized according to structural features of the precursor ion, exceptional species such as the four coordinate [Zn(*dien*/monosaccharide)–H]$^+$ precursor ion can give unexpected stereochemical information. It is presently unclear whether factors such as complex geometry, electronic configuration or sterics, or a combination of the three, dictate the dissociation pathways of the metal *N*-glycoside complexes, thus allowing for the differentiation of diastereomeric monosaccharides. Further detailed studies of the effects of these factors will be necessary to answer such questions.

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