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Synthesis and analysis of electrospray ionization-generated five-coordinate diastereomeric Ni–*N*-glycoside complexes using a quadrupole ion trap mass spectrometer

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Abstract

Five-coordinate Ni *N*-glycoside complexes, consisting of a single tridentate *N*-glycoside ligand and a bidentate *dap* ligand coordinated to a central Ni(II) ion, were synthesized for a series of diastereomeric monosaccharides. Following excitation of the precursor ions in a quadrupole ion trap, reproducibly distinct product ion spectra were obtained for each diastereomeric complex. Neutral losses resulted from cross-ring cleavages of the monosaccharide ring and/or losses of a bidentate *dap* ligand. Isotopic labeling studies confirmed which particular carbon centers were lost from the precursor ion as a result of the cross-ring cleavages. Synthesis of the Ni–*N* glycoside complex was achieved using as little as 21 μg of monosaccharide. Production of the representative five-coordinate precursor ion was obtained over a wide range of metal–ligand:monosaccharide molar ratios and product ion spectra remained reproducible over this range. (Int J Mass Spectrom 193 (1999) 153–160) © 1999 Elsevier Science B.V.

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

1. Introduction

The multitude of roles played by carbohydrates and carbohydrate-containing molecules (e.g. glycoproteins) in biological systems is becoming increasingly apparent [1]. For example, carbohydrates can serve as pathogens of infectious bacteria [2–4], perform integral functions in certain protein-folding events [5,6] and inflammation responses [7,8], or allow for highly specific recognition between cells [9,10]. It is often found that the carbohydrates associated with these various roles possess little or no similarity of composition. Conversely, it is also not unusual for carbohydrates differing by merely a single monomer unit [5] or functional group [7,11] to show vastly different activities in their biological functions. Thus the need becomes evident to possess methodologies capable of characterizing the structure of these important biomolecules.

There are several structural features present in carbohydrates, which allows for a unique determination of the primary sequence of these biopolymers. The structural features include the number and identity of diastereomeric monosaccharides, linkage position and configuration, and the location of substituents and branch points [1]. In this work, previous studies [12–17] are expanded upon, whereby the use of transition metal complexes of carbohydrates in con-

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junction with tandem mass spectrometry are used to characterize a specific structural feature of carbohydrates; the identity of the diastereomeric monosaccharide units. Data presented herein show that analysis can be performed with much lower sample quantities (micrograms) than has been previously achieved, and that the metal:monosaccharide molar ratio is less crucial to the production of the five-coordinate complex. This was not the case with the previously synthesized three-, four-, and six-coordinate complexes. More importantly, this work described herein presents the fundamental differences in the product ion spectra observed between these five-coordinate species and the three-, four- and six-coordinate complexes previously analyzed [17].

2. Experimental

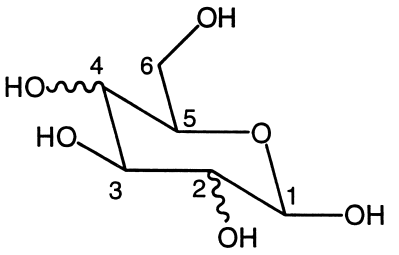
2.1. Instrumentation

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 1.9×10^{-3} Torr or less during all acquisitions. Samples were infused into the instrument at 3 μL/min. Ionization was performed at 4.5 kV. All spectra were acquired at a capillary temperature of 150 °C and all ion guide voltages were tuned so as to maximize the relevant precursor ion current. Precursor ions were isolated over a mass range of 1.7 Da prior to excitation. A rf voltage of 0.6 $V_{(p\text{-}p)}$ was applied to endcaps of the ion trap for 15 ms during the excitation period. Such excitation conditions were chosen to maximize the differences in the relative product ion intensities between the diastereomeric precursor ions.

2.2. Synthesis

Ni (dap)$_3$ · 2Cl [18]: Three molar equivalents of 1,3 diaminopropane (*dap*) were added slowly to a methanolic solution of $NiCl_2 \cdot 6H_2O$. Solids precipitated soon after stirring. The solid was purified by recrystallization from an isopropanol/methanol mixture.

Table 1
Ring substituent configuration of D-aldohexoses



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

Metal/N–glycoside complexes: Labeled and unlabeled metal–*N*-glycoside complexes were synthesized using a microscaled adaptation of the procedure previously published by Yano and co-workers [19–22]. Although previous microscaling procedures involved measuring solid reagents [12–15,17], the low sample sizes utilized in this study necessitated preparing stock reagent solutions from which aliquots were taken, to assure accurate delivery of the desired reagent quantities. Unless noted otherwise, the following procedure was used to prepare all reaction solutions. Briefly, separate 47.5 mM stock solutions of the four monosaccharides listed in Table 1 were prepared in 90% MeOH. (10% water was utilized due to the lower solubility of some monosaccharides in pure methanol.) An additional set of 4.75 mM stock solutions was prepared for the four diastereomeric monosaccharides. A 95.0 mM methanolic stock solution of Ni (*dap*)$_3$ · 2Cl was also prepared.

Reaction mixtures possessing monosaccharide:Ni (*dap*)$_3$ · 2Cl molar ratio of 1:1 were prepared by mixing 25.0 μL of the 47.5 mM monosaccharide stock solution, 12.5 μL of the Ni (*dap*)$_3$ · 2Cl stock solution and 12.5 μL of MeOH. The additional methanol was added to achieve a total reaction volume of 50 μL. A final monosaccharide concentration of 23.5 mM (23.5 nmol/μL) was utilized. Such reaction conditions therefore utilized 214 μg of monosaccharide.

Reaction mixtures possessing monosaccharide:Ni $(dap)_3 \cdot 2Cl$ molar ratio of 1:10 were prepared by mixing 25.0 μL of the 4.75 mM monosaccharide stock solution, 12.5 μL of the Ni $(dap)_3 \cdot 2Cl$ stock solution and 12.5 μL of MeOH. A final monosaccharide concentration of 2.35 mM (2.35 nmol/μL) was utilized. Therefore, such reaction conditions utilized 21.4 μg of monosaccharide.

Samples were refluxed at ~70 °C for 20 min unless otherwise noted. All reaction mixtures were used immediately after heating to avoid any sample degradation. No purification was performed prior to analysis.

2.3. Electrospray ionization mass spectrometry (ESI-MS) analysis

Solutions for ESI-MS were prepared by diluting the reaction mixture 100-fold in 9:1 MeOH/H_2O. Final concentrations of all solutions were ~235 μM (235 pmol/μL) Ni unless otherwise noted.

2.4. Chemicals and materials

D-Glucose (A.C.S. reagent grade), D-mannose (99%) and 1,3-diaminopropane (99%) were obtained from Aldrich Chemical Co. (Milwaukee, WI). D-Galactose (99%), D-talose, high-performance liquid chromatography (HPLC) grade water and HPLC grade methanol were obtained from Sigma Chemical Co. (St. Louis, MO). D-glucose-6-$^2H_2$ (98% $^2H_2$), D-glucose-4-^{13}C (99% ^{13}C) and D-glucose-3-^{13}C (99% ^{13}C) were obtained from Omicron Biochemical Co. (South Bend, IN). Nickel dichloride hexahydrate was purchased from Fisher Chemical Co. (Fairborn, NJ). All materials were used as received without further purification.

3. Results and discussion

Fig. 1 shows a representative low-resolution mass spectrum of a reaction solution prepared with only 214 μg of D-mannose. Similar mass spectra were obtained from all other monosaccharide reaction mixtures. These reaction solutions contained at least two

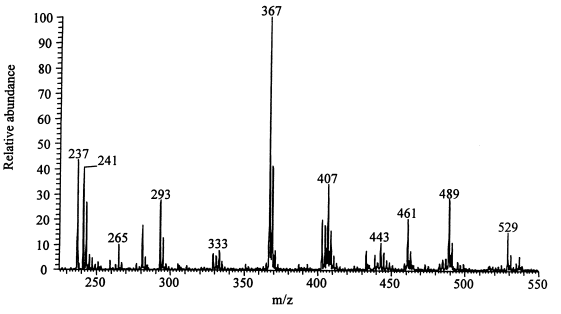
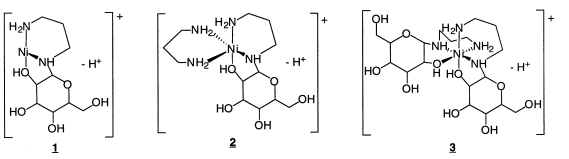


Fig. 1. Low-resolution mass spectrum of Ni $(dap)_3 \cdot 2Cl$/D-mannose reaction mixture (1:1 Ni/monosaccharide molar ratio, 214 μg monosaccharide). Total ion current = 7.66×10^5 (arbitrary units).

times less carbohydrate than previously reported for the conventional synthesis of related metal–*N*-glycoside complexes [14]. A prominent series of singly charged ions (*m/z* 293, 367, and 529) resulted from the deprotonation of various Ni(II) complexes. These deprotonated ions have been previously assigned to three-coordinate (1), five-coordinate (2), and six-coordinated (3) complexes, respectively [15]. At the present time, specific structural details concerning the *N*-glycoside ligand are not available. The *N*-glycoside ligand can be assumed to act as a tridentate ligand, coordinating to the metal center in a T-shaped fashion as has been previously observed from the x-ray crystal structure of 3 [19–22] and predicted for 1 using density functional theory calculations [15]. A β configuration about the anomeric carbon center can also be assumed since *N*-glycoside complexes possessing α configurations have not been observed [19–23]. Additionally, an intense ion at *m/z* 237, assigned to the protonated, metal-free *N*-glycoside ligand, is present. Other ions such as those at *m/z* 241, 407, 461, and 489 are attributed to other Ni/*dap*-containing species that lack monosaccharides.



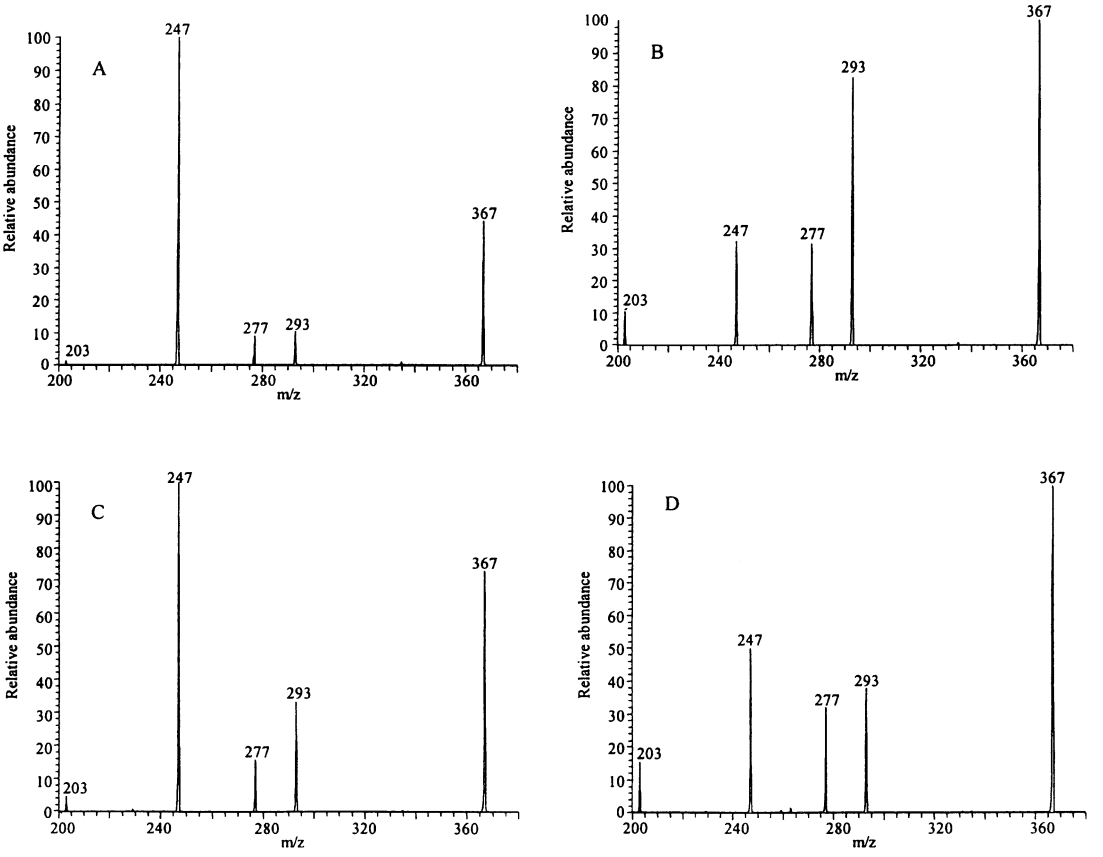


Fig. 2. Product ion spectra (MS^2) of *m/z* 367 [Ni (*dap*)(*dap*/monosaccharide)–H]$^+$ precursor ions generated using 214 μg of monosaccharide: (A) D-glucose complex; (B) D-mannose complex; (C) D-galactose complex; and (D) D-talose complex.

Fig. 2 shows the product ion spectra of the four diastereomeric *m/z* 367 precursor ions ([Ni (*dap*)(*dap*/monosaccharide)–H]$^+$) obtained under identical conditions. A *m/z* 293 product ion (neutral loss of 74 Da) was attributed to the loss of a neutral *dap* ligand from the precursor ion. The *m/z* 277 (neutral loss of 90 Da) and *m/z* 247 (neutral loss of 120 Da) product ions are assigned to losses of $C_3H_6O_3$ and $C_4H_8O_4$, respectively, resulting from cross-ring cleavages in the monosaccharide ring. Such cross-ring cleavages are commonly observed as dissociation pathways for monosaccharides [24–27] and have been observed previously from metal–*N*-glycoside complexes [14–17]. The *m/z* 203 product ion (neutral loss of 164 Da) results from the combined loss of a neutral *dap* ligand and a $C_3H_6O_3$ cross-ring cleavage fragment. The product ion spectra in Fig. 2 are similar to those obtained from other related five-coordinate, two-ligand *N*-glycoside systems [17].

Isotopic labeling studies were performed using a variety of labeled D-glucose complexes in order to identify the carbon centers lost from the precursor ion following the dissociation of the *m/z* 367 ion. $^2H_2$-labeled or singly ^{13}C-labeled D-glucose was used in the synthesis in place of unlabeled D-glucose. Ions at *m/z* 368 and 369 were observed in the low-resolution mass spectra of the reaction mixtures containing the ^{13}C and $^2H_2$-labeled D-glucose, respectively, indicating the incorporation of the labeled monosaccharides into the Ni–*N*-glycoside complexes. Product ion spectra were obtained following collision induced dissociation (CID) of the labeled precursor ions, with the

Table 2
Summary of product ion spectra of isotopically labeled precursor ions

	Three-carbon neutral loss	*dap* + three-carbon neutral loss	Four-carbon neutral loss
D-glucose (unlabeled)	$C_3H_6O_3$	$C_3H_6O_3$	$C_4H_8O_4$
D-glucose-6-$^2H_2$	$C_3{}^2H_2H_4O_3$	$C_3{}^2H_2H_4O_3$	$C_4{}^2H_2H_6O_4$
D-glucose-4-^{13}C	$^{13}CC_2H_6O_3$	$^{13}CC_2H_6O_3$	$^{13}CC_3H_8O_4$
D-glucose-3-^{13}C	$C_3H_6O_3$	$C_3H_6O_3$	$^{13}CC_3H_8O_4$

results being summarized in Table 2. Both C-4 and C-6, but not C-3, were present in $C_3H_6O_3$ losses. The third carbon center that is lost can be assigned to C-5. Neutral losses possessing C-6 and C-4, but not C-5, would require a considerable rearrangement in the ion structure during the dissociation. Such extensive rearrangements are likely to make the generation of product ions unfavorable. The isotopic labeling studies also demonstrate that C-3, C-4, and C-6 are absent from the precursor ion following loss of $C_4H_8O_4$. Using similar reasoning as above, it can be concluded that the fourth carbon center lost is C-5.

As was shown in an earlier publication [17], five-coordinate, two-ligand systems often do not display dissociation pathways that are characteristic of a single stereochemical feature (i.e. the presence or absence of product ions cannot be used to identify stereochemical features of the precursor ions). However, as can be seen from Fig. 2, the product ion spectra are unique for each of the four diastereomeric precursor ions based on ion intensities. It may then be possible to utilize the *m/z* 367 precursor ions to differentiate the four diastereomeric monosaccharides in Table 1.

Although the product ion spectra in Fig. 2 demonstrate that the *m/z* 367 precursor ion may potentially be capable of stereochemical differentiation of monosaccharides, the methodology depends upon the reliable measurement of relative ion intensities. Examples of the variability of relative ion intensities obtained from MS^2 experiments on an LCQ quadrupole ion trap are not available. Thus efforts were made to demonstrate whether the differences in the relative ion intensities were reproducible and large enough to reliably differentiate the monosaccharide complexes. In this study, ten product ion spectra generated from two signal-averaged scans were obtained for each of the four diastereomeric *m/z* 367 precursor ions. The results are summarized in Table 3. The standard deviations associated with the relative ion intensities are always less than 2% of the most intense product ion for a given product ion spectrum. (The standard deviations did not decrease significantly when the product ion spectra were generated by signal-averaging ten scans instead of two scans.) Such a variation is smaller than the differences in relative product ion intensities for all complexes. It is concluded that the product ion spectra of the *m/z* 367 precursor ions are sufficiently reproducible such that a diastereomeric monosaccharide complex is unlikely to be incorrectly identified for another, unlike other

Table 3
Summary of relative ion intensities and standard deviations of product ions from *m/z* 367 precursor ions; ion intensities are expressed relative to base product ion intensity[a]

	m/z 203	*m/z* 247	*m/z* 277	*m/z* 293
D-glucose	1.60 (0.13)	100	8.97 (0.30)	10.63 (0.17)
D-mannose	12.96 (0.88)	39.21 (0.93)	38.48 (1.67)	100
D-galactose	4.58 (0.07)	100	15.94 (0.30)	33.44 (0.20)
D-talose	30.27 (0.42)	100	64.03 (1.38)	75.75 (1.29)

[a] Parentheses represent the standard deviation.

Table 4
Molar ratios of monosaccharide to metal or amine ligand in various Ni–*N*-glycoside complexes

	Monosaccharide/metal molar ratio	Monosaccharide/*dap* molar ratio
m/z 293 [Ni (*dap*/monosaccharide)–H]$^+$	1:1	1:1
m/z 367 [Ni (*dap*)(*dap*/monosaccharide)–H]$^+$	1:1	1:2
m/z 529 [Ni (*dap*/monosaccharide)$_2$–H]$^+$	2:1	1:1

previously investigated five-coordinate metal–*N*-glycoside complexes [17].

Use of a complex such as [Ni (*dap*)(*dap*/monosaccharide)–H]$^+$ in the assay of monosaccharides may provide benefits that are absent from that of Ni–*N*-glycoside complexes. The ability to synthesize an *N*-glycoside complex over a wide range of monosaccharide quantities is an attractive characteristic of any analysis. The three commonly observed Ni–*N*-glycoside complexes possess different ratios of metal, monosaccharide and amine components as shown in Table 4. It is expected that as the molar ratio of the monosaccharides added to the reaction mixture decreases with respect to that of the metal amine reactant [Ni (*dap*)$_3$ · 2Cl], complexes will be preferentially formed possessing lower molar ratios of monosaccharides compared to other components of the complex. Of these complexes, the *m/z* 367 precursor ion possesses both the lowest monosaccharide/metal and monosaccharide/amine molar ratios. Thus as the quantity of monosaccharides decreases, it is reasonable to expect that the monosaccharides will be preferentially partitioned in the form of the *m/z* 367 complex. Fig. 3(A) displays a representative low-resolution mass spectrum of a reaction mixture synthesized using a 1:10 molar ratio of monosaccharide/Ni (*dap*)$_3$ · 2Cl reactants. (This reaction mixture was prepared in an identical manner to that described in Sec. 2 with the exception that a 4.75 mM monosaccharide stock solution was substituted for the original 47.5 mM monosaccharide stock solution.) It is apparent when comparing Figs. 1 and 3(A) that both *m/z* 293 and 529 ions are absent when lower molar ratios of a monosaccharide are used (i.e. lower than 1:1).

The reverse of this situation (increasing the monosaccharide/Ni (*dap*)$_3$ · 2Cl molar ratio above 1:1) was thought to be troublesome for the formation of the *m/z* 367 precursor ion because one might predict that in the presence of excess monosaccharide, complexes such as the *m/z* 293 and 529 ions would be formed preferentially. Although this prediction is evidenced in Fig. 3(B), where the reaction mixture was generated using a monosaccharide/Ni (*dap*)$_3$ · 2Cl molar ratio of 3:1 via the previously published procedure [15], the intensity of the *m/z* 367 ion is still

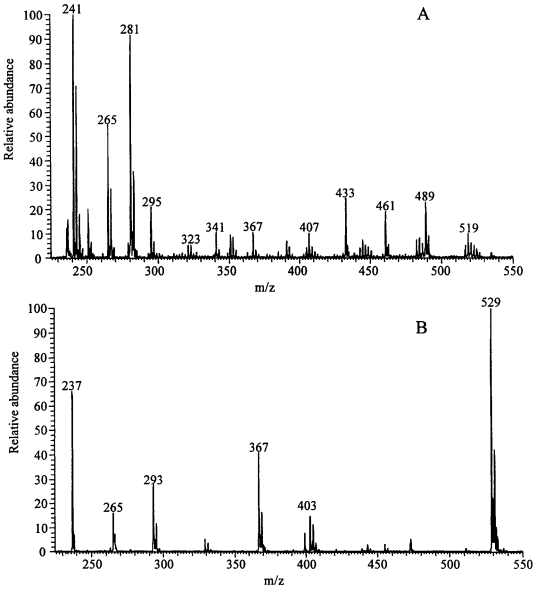


Fig. 3. Low-resolution mass spectrum of Ni (*dap*)$_3$ · 2Cl/D-mannose reaction mixture: (A) 10:1 Ni/D-mannose molar ratio. Total ion current = 7.35×10^5 (arbitrary units); (B) 1:3 Ni/D-mannose molar ratio. Total ion current = 4.64×10^6 (arbitrary units).

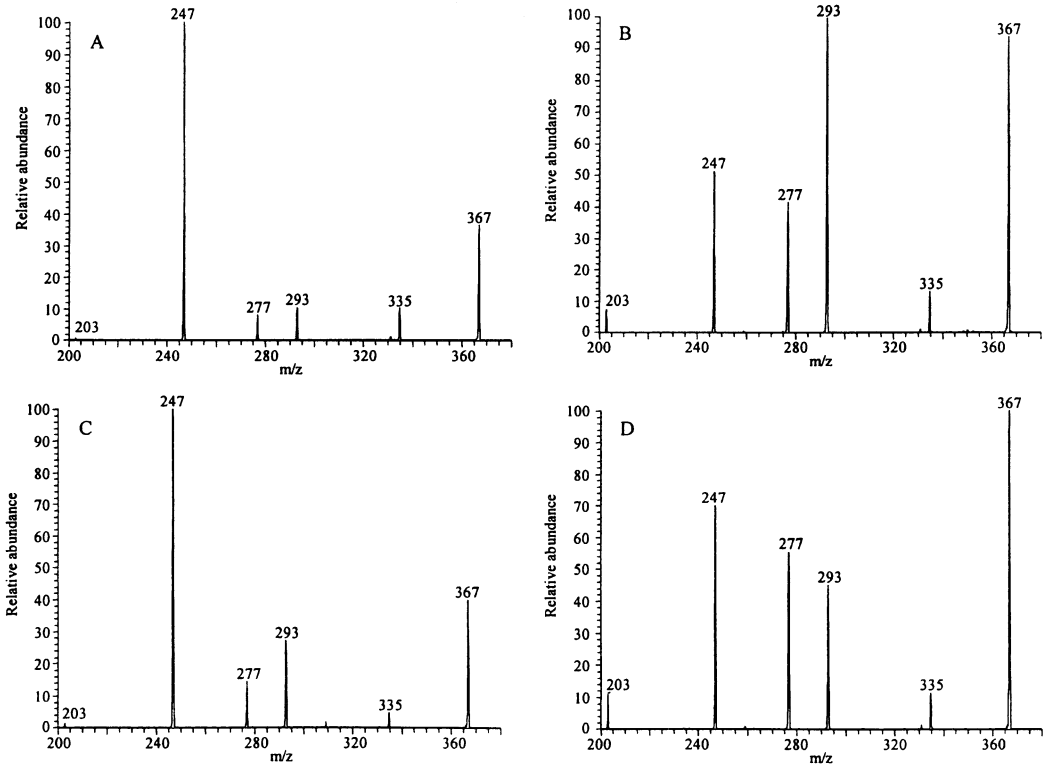


Fig. 4. Product ion spectra (MS^2) of *m/z* 367 [Ni (*dap*)(*dap*/monosaccharide)–H]$^+$ precursor ions generated using 21 μg of monosaccharide: (A) D-glucose complex; (B) D-mannose complex; (C) D-galactose complex; and (D) D-talose complex.

more than sufficiently strong to perform MS^2 experiments. Therefore, a useful yield of the *m/z* 367 precursor ion is generated over a greater range of reactant quantities than that which is observed for other Ni–*N*-glycoside complexes in this reaction mixture. This behavior is very advantageous in the analysis of samples from biological sources where the amount of extracted monosaccharides could vary greatly.

Product ion spectra of the *m/z* 367 complexes were also obtained from reaction mixtures possessing 1:10 monosaccharide/Ni $(dap)_3 \cdot 2Cl$ molar ratios to ensure that useful product ion spectra could be obtained from precursor ions with such low intensities. The features of the product ion spectra of the *m/z* 367 precursor ions using 1:10 molar ratios (Fig. 4) were very similar to those observed when using 1:1 molar ratios (Fig. 2). It should be noted that all product ion spectra in Fig. 4 were obtained from reaction mixtures containing only 21 μg of monosaccharide. One noticeable difference is the appearance of an additional product ion at *m/z* 335 (neutral loss of 32 Da). The additional product ion is observed with increasing relative intensity as the monosaccharide/Ni $(dap)_3 \cdot 2Cl$ molar ratio decreases. It is postulated that the *m/z* 335 product ion results from the dissociation of a low intensity background ion that is isobaric with the *m/z* 367 Ni–*N*-glycoside complex. Indeed, the low-resolution mass spectrum of a 235 pmol/μL (9:1 MeOH/H_2O) solution of only Ni $(dap)_3 \cdot 2Cl$ did reveal a low intensity ion at *m/z* 367. Product ion spectra of the suspected isobaric interference obtained under identical excitation conditions as those used in Figs. 2 and 4 revealed the formation of a single product ion at *m/z* 335. Fortunately, although both the isobaric *m/z* 367 ion and its *m/z* 335 product ion are a nuisance, neither significantly affect the results of this assay because no interfering contributions are made to the intensities of the important *m/z* 293, 277, 247, or 203 product ions.

4. Conclusions

In this work, five-coordinate Ni–*N*-glycoside complexes were synthesized for a series of biologically relevant diastereomeric monosaccharides. These complexes consisted of a single tridentate *N*-glycoside ligand and a bidentate *dap* ligand coordinated to a central Ni(II) ion. Following excitation of these precursor ions in a quadrupole ion trap, reproducibly distinct product ion spectra were obtained for each of the diastereomeric complexes. Neutral losses resulted from cross-ring cleavages in the monosaccharide ring, loss of a bidentate *dap* ligand or a combination of the two. Isotopic labeling studies confirmed which carbon centers were lost from the precursor ion as a result of the cross-ring cleavages. Postulation of a source of the stereochemical differentiation achieved when utilizing the five-coordinate complexes is difficult at this time and is beyond the scope of this work. However, it is likely that the axial or equatorial orientation of the C-2 and C-4 monosaccharide substituents affect somewhat the dissociation mechanisms of the *m/z* 367 precursor ions. Significant stereochemical effects upon the dissociation of Ni(II)–*N*-glycoside complexes have been observed previously [12,13,15].

The formation of the *m/z* 367 precursor ion was shown to be practical over a wide range of reactant ratios [3:1 down to 1:10 monosaccharide/Ni $(dap)_3 \cdot 2Cl$ molar ratio]. Also, as little as 21 μg of monosaccharide was used in the synthesis of the Ni–*N*-glycoside complex. Although other analytical techniques are available, which are capable of identifying smaller amounts of diastereomeric monosaccharides [28], use of such low sample quantities has not been previously utilized in the production and MS analysis of diastereomeric metal *N*-glycoside complexes [12–17]. At the lowest sample levels, additional product ions were observed, resulting from the dissociation of low intensity isobaric ions at *m/z* 367. Such artifact product ions did not interfere with the measurement of the product ion intensities of the *m/z* 367 Ni–*N*-glycoside complexes.

Acknowledgement

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