Gas-Phase Dissociation Mechanisms of Dilithiated Disaccharides: Tandem Mass Spectrometry and Semiempirical Calculations

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Fast atom bombardment ionization followed by tandem mass spectrometry of ^{18}O- and ^{2}H-labeled dilithiated disaccharides is used to differentiate the linkage position of three isomeric disaccharides. The mechanisms of dissociation proposed are based on the mass spectrometry, experimental data, and semiempirical calculations, the latter of which provide information on the stability of the deprotonated dilithiated precursor, $(M + 2Li - H)^+$. Tandem mass spectrometry experiments indicate that, as in the case of monolithiated disaccharides, reducing ring opening occurs followed by two-, three-, and four-carbon chain neutral losses. Semiempirical calculations support the experimental data which suggest that one lithium is tetracoordinate between the two sugar rings, while the second lithium is dicoordinate forming a lithium alkoxide with one of the deprotonated hydroxyl groups of the reducing ring.

Introduction

There are many important biological functions performed by oligosaccharides ranging from glycolipid precursors of protein glycosylation[1] to possible links to biosynthesis of molecules responsible for nitrogen fixation in legumes.[2] Structural elucidation of these carbohydrates thus becomes one of the first steps to understanding how these molecules interact in the living system. In particular, determination of linkage position is one very important aspect. An example of this is seen with the isomeric branched oligosaccharides shown below. Although both inhibit binding of antibodies to blood groups, each is specific to a different blood group.[3]

Galβ1-3GlcNAcβ1-3Galβ1-4Glc and Galβ1-3GlcNAcβ1-3Galβ1-4Glc

1,4	1,2
Fucα	Fucα

Inorganic salts and organometallic compounds are also important to biological systems. Lithium salts have been extensively studied in their uses for psychological disorders, such as manic depression,[4-7] as well as their interactions with ion transport across cell membranes,[5] their antiviral activity,[8] and probably most interesting to us, their effects on carbohydrate metabolism.[6] With these facts in mind, we have chosen to investigate how metals coordinate to oligosaccharides and how we can use specific metals as analytical tools that promote specific dissociation pathways of isomeric oligosaccharides using tandem mass spectrometry.

With the advent of fast atom bombardment mass spectrometry (FABMS), excellent methodology has been proposed for structural elucidation of glycolipids, glycopeptides, peptidoglycans, and branched and linear oligosaccharides using tandem mass spectrometry (MS/MS).[9-19] However, linkage position determination using any mass spectral technique without prior derivitization has not been successful, and in the best of cases the techniques are cumbersome, lengthy, and ambiguous. Recent research using FABMS,[20] plasma desorption time of flight,[21] and laser desorption ionization[22-28] in combination with collision-induced dissociation (CID) in either the positive or negative[29,30] ion mode are thorough and provide some structural details; however, they still do not provide unambiguous information as to linkage position.

In our earlier studies[31,32] we showed how the linkage position of different isomeric di-, tri-, and tetrasaccharides could be distinguished when the monolithiated precursor undergoes CID after FAB ionization. This current study

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focuses on the mechanism of dissociation of the dilithiated isomers. As the carbohydrate gets larger and more complex, more lithium ions (or different metal ions) must coordinate to the oligosaccharide in order to obtain linkage position information. Therefore, we need to compare how CID of the dilithiated precursor differs from that of the monolithiated isomers and determine the dissociation mechanisms associated with the multimetal coordinated species. Labeling experiments using ^{18}O and ^{2}H, in combination with MS/MS, MS/MS/MS, and semiempirical calculations, are used to support proposed dissociation pathways for three dilithiated isomeric disaccharides. Particular attention is given to the 1,3-isomer because of its very different spectra and reaction mechanism. Semiempirical calculations of the 1,3- and 1,2-linked isomers were used to obtain the ΔH_f's for the most stable conformation of the dilithiated species. In this particular case, determination of the dissociation pathway for the 1,3-linked isomer was not possible prior to determining the site of deprotonation provided to us by the theoretical calculations.

Experimental Section

Mass Spectrometry. Mass spectra were obtained using a VG ZAB2-EQ mass spectrometer (VG Analytical, Greater Manchester, U.K.) of BEoQ geometry (o is an rf-only octopole collision cell fabricated in our laboratory and replaces the conventional quadrupole cell in this particular instrument). Details of MS/MS acquisitions and procedures for making ^{18}O- and ^{2}H-labeled compounds are described elsewhere.[32]

MS/MS/MS experiments have also been described previously.[33] Briefly, the precursor ion is transmitted into the MIKES cell and allowed to undergo unimolecular decomposition. A product ion resulting from this decomposition is then transmitted into the rf-only octopole collision cell where it sustains low-energy collisions (100-eV lab-frame-of-reference) with argon ($P = 1 \times 10^{-6}$). The resulting second-generation product ions are analyzed in the second quadrupole (Q) mass analyzer.

Samples. Sophorose (Glu (α)1→2Glu) was purchased from Serva Biochemicals (Westbury, NY). Laminaribiose (Glu (β)-1→3Glu) and lactose (Gal (β)1→4Glu) were both purchased from Sigma Chemical Co. (St. Louis, MO). The disaccharides were used as purchased without additional purification.

Semiempirical Calculations. All MNDO (modified neglect of differential overlap) calculations were performed using a general-purpose semiempirical molecular orbital package (MOPAC) written by James P. Stewart,[34] using a CAChe calculation package developed by Tektronix Corp.[35] Specific details for these calculations are described elsewhere.[32]

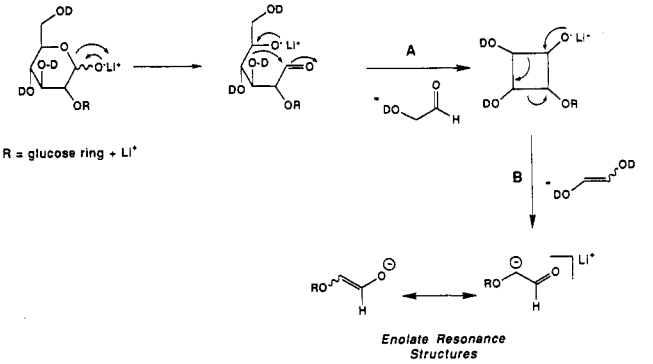
In calculating the heats of formation for the dilithiated species ([M + 2Li - H]+), several different procedures were introduced. Initially, the x, y, and z coordinates for the 1,3-disaccharide, laminaribiose, and the 1,2-disaccharide, sophorose, were transferred from the Cambridge Data Base to the CAChe system. A lithium atom was introduced within coordination distance of different oxygens on the molecule as described earlier[32,36] and the structure continually refined to a point where the change in energy on successive iterations was less than 0.3 kcal/mol. At this point a hydrogen atom from a hydroxyl group of either the reducing or nonreducing ring was substituted with another lithium atom and then the MNDO program was again initiated. This was repeated until each of the hydroxyl hydrogens was, in turn, replaced with lithium. In this way we were able to obtain theoretical data on the most likely sites for coordination of both lithium atoms to the 1,2- and 1,3-disaccharides.

Table I. List of Five Isomeric Disaccharides of Different Linkages with Corresponding Neutral Diol or Aldehyde Losses Observed in the MS/MS Spectra of the Mono- and Dilithiated Precursors

		neutral losses	
compd	linkage	monolithiated	dilithiated
trehalose	1,1[a]	–[b]	–
sophorose	1,2	$C_4H_8O_4$	$C_2H_4O_2$, $C_4H_8O_4$
laminaribiose	1,3	$C_3H_6O_3$	$C_3H_6O_3$, $C_4H_8O_4$
lactose	1,4	$C_2H_4O_2$	$C_2H_4O_2$, $C_4H_8O_4$
gentiobiose	1,6	$C_2H_4O_2$, $C_3H_6O_3$, $C_4H_8O_4$	$C_2H_4O_2$, $C_3H_6O_3$, $C_4H_8O_4$

[a] Both α and β forms of these isomers gave the same neutral losses. [b] No ring cleavage was observed for this isomer since the linkage prevents opening of the hemiacetal reducing ring.

Scheme I. 1,2-Dilithiated Isomer



Results and Discussion

Collision induced dissociation of dilithiated oligosaccharide precursors ([M + 2Li - H]+) produce ions that are somewhat different from those generated in the MS/MS experiment of the monolithiated precursors ([M + Li]+). Table I lists the neutral losses which are observed in the MS/MS experiments of both the mono- and dilithiated ions for five isomeric disaccharides having different linkages. The neutral losses result from reducing ring cleavage and correspond to two, three, or four-carbon-chain alcohols and/or aldehydes.[32] On the basis of the differences observed in Table I, one might expect that the mechanisms involved in the formation of these ions are also different. In order to probe these mechanisms, ^{18}O and ^{2}H labeling studies, in combination with MS/MS and MS/MS/MS, were carried out on three of the five isomeric dilithiated disaccharides listed in Table I. Semiempirical calculations are also presented on the 1,3- and 1,2-linked isomers which exhibit very different rearrangement and dissociation processes.

Tandem Mass Spectrometry

Sophorose, 1,2-Linked Disaccharide. Collision induced dissociation of the ^{18}O-labeled dilithiated precursor indicated that the label is retained in both ions which resulted from losses of the two- and four carbon chain neutral species, thus indicating that the oxygen at the anomeric carbon is not lost in either of these dissociation pathways (Scheme I). (All ^{18}O, ^{2}H, and MS/MS/MS spectra are available as supplementary material). This is consistent with labeling experiments of the monolithiated isomer which indicated a four-carbon neutral loss which also did not include the anomeric oxygen. The MS/MS spectrum of the perdeuterated dilithiated precursor showed that the first two carbon neutral loss is an aldehyde

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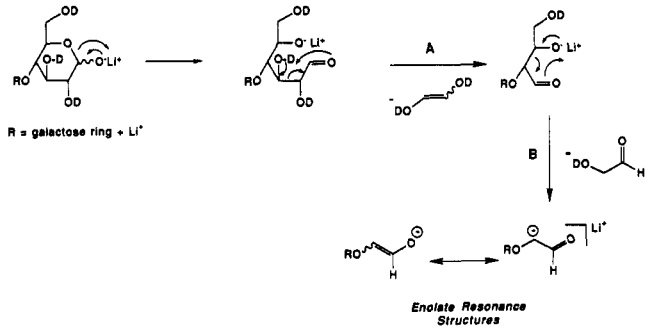
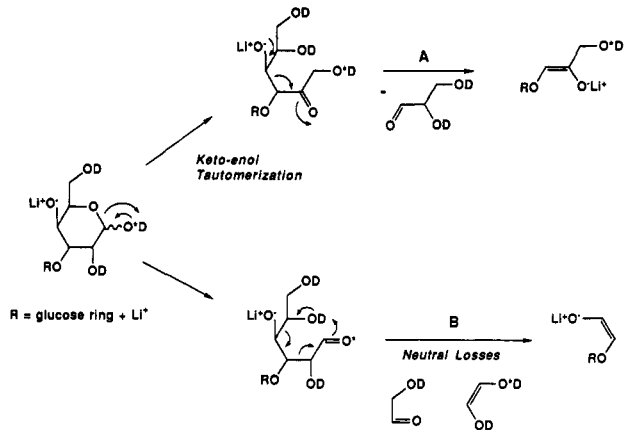
Scheme II. 1,4-Dilithiated Isomer



Scheme III. 1,3-Dilithiated Isomer



since loss of the expected deuterated ethylene diol is 62 AMU instead of the observed 61 AMU. The four-carbon loss of 123 AMU is the same as that seen in the MS/MS spectrum of the monolithiated 1,2-isomer. Since the MS/MS/MS spectrum of the dilithiated precursor (m/z 355) indicated that the four-carbon neutral loss was in fact a second-generation product ion (m/z 355 → 295 → 235), it became clear that two consecutive two-carbon neutral losses were taking place.

It is proposed in Scheme I that for the 1,2-dilithiated isomer the hemiacetal opens up to form the lithium alkoxide with lithium migration from the oxygen on the anomeric carbon to the ring oxygen. The negative charge on the oxygen is then the site from which rearrangement and dissociation proceed to produce a stable cyclobutane ion with resulting loss of the aldehyde. From this ion, the loss of the ethylene diol is then generated which gives rise to the stable enolate resonance structures as shown. Although the cyclobutane ring has approximately 25 kcal of ring strain associated with its formation, the amount of internal energy absorbed using 80 eV collisions (laboratory-frame-of-reference) (E_{cm} = 8 eV) exceeds this small energy requirement. This mechanism is thus supported by all the data generated for this particular isomer.

Lactose, 1,4-Linked Disaccharide. For the 1,4-isomer, the CID spectrum of the anomeric ^{18}O labeled compound showed a loss of the label upon dissociation (Scheme II). This is in contrast to the 1,2-isomer and clearly shows that the first two-carbon loss must include the anomeric carbon. The spectrum arising from CID of the perdeuterio compound also showed losses opposite those for the 1,2-linked isomer; i.e., the first loss is that of the ethylene diol. Once again the MS/MS/MS spectrum indicated two consecutive two-carbon-chain losses, i.e., 355 → 295 → 235. Therefore, for this isomer the ethylene diol loss is followed by loss of the aldehyde.

On the basis of this data, a mechanism for dissociation is outlined in Scheme II. In this particular case the sequential losses are reversed with those for the 1,2-linked isomer, but both isomers give rise to two consecutive two-carbon-chain neutral losses with subsequent generation of stable enolate resonance structures. It is interesting to note that in pathway A the retro-aldol reaction proceeds from the hydroxide while in pathway B it proceeds from the lithium alkoxide. Because there is no way for the lithium alkoxide to initiate the retro-aldol reaction in pathway A (i.e., there is no carbonyl function β to the alkoxide) this may simply be a case where the only pathway available for dissociation is through the hydroxide. Interestingly, this lithium alkoxide initiated retro-aldol is observed as the first step in the 1,3-linked isomer which is discussed below.

Laminaribiose, 1,3-Linked Disaccharide. The 1,3-linked isomer exhibits three very different features which are absent in both the 1,2- and 1,4-linked isomers. The first, and most obvious, difference is that there is no two-carbon loss observed for the 1,3-isomer (Table I). Secondly, our previous results[32] indicated that ring opening followed by keto–enol tautomerization is required in order to obtain the three-carbon loss observed for all 1,3-isomers. CID spectra of the perdeuterated and ^{18}O-labeled monolithiated laminaribiose (β) or nigerose (α) precursor showed that the neutral loss composition was $C_3H_4D_2O_3$ (^{18}O label not lost). In order to obtain this composition after opening of the reducing ring hemiacetal, the carbonyl on C-1 would have to undergo keto–enol tautomerization thereby transposing the carbonyl to C-2. In this configuration the hydroxyl group on C-4 is now β to the carbonyl thus allowing the retro-aldol reaction to proceed. Lastly, we will present theoretical and experimental data here which suggests that the lithium ion resides on the deprotonated hydroxyl group of the fourth carbon on the reducing ring as opposed to coordinating on the oxygen of the anomeric carbon.

The CID spectra of the ^{18}O-labeled dilithiated 1,3-isomer showed that the ^{18}O label is retained in the ion resulting from the three carbon neutral loss (Scheme III). However, the ion which results from the loss of the four-carbon neutral species does not contain the ^{18}O label. The MS/MS spectrum of the perdeuterio compound showed that the three-carbon loss corresponds to $C_3D_2H_4O_3$. Both the ^{18}O and ^{2}H labeling experiments are consistent with the three-carbon neutral loss data obtained for the monolithiated precursor. This strongly suggests that the dissociation pathway for the three carbon neutral loss from the dilithiated precursor also proceeds via retro-aldol rearrangement of the alkoxide after keto–enol tautomerization from C-1 to C-2.

The four-carbon loss is much more difficult to determine since the linkage prevents the loss of a contiguous four-carbon chain. Therefore, this loss must either come from the nonreducing end or two two-carbon fragments (carbons 5 and 6 and carbons 1 and 2) must be lost simultaneously. There is no way to envision this four-carbon neutral loss when the C-1 hydroxyl group was deprotonated, and the MS/MS/MS experiment ruled out the possibility of a loss of formaldehyde as a consecutive loss (355 → 265 ↛ 235). Loss of a four-carbon neutral from the nonreducing end is also ruled out, since the anomeric ^{18}O is lost during this dissociation process.

After many unsuccessful attempts to propose a dissociation mechanism to support our data, we focused our attention on the MNDO calculations. It was anticipated that the semiempirical calculations would provide important information on the most stable geometry of the

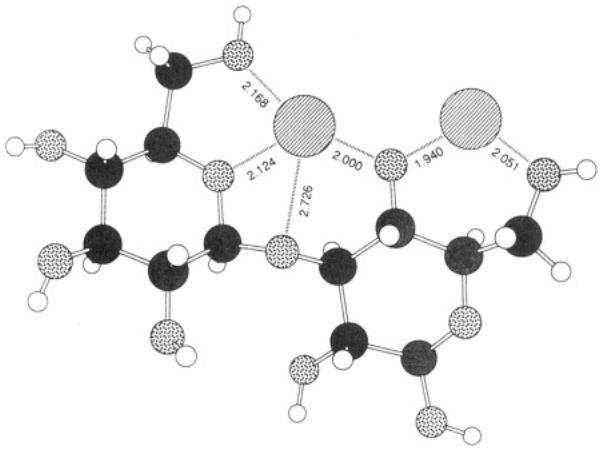


Figure 1.

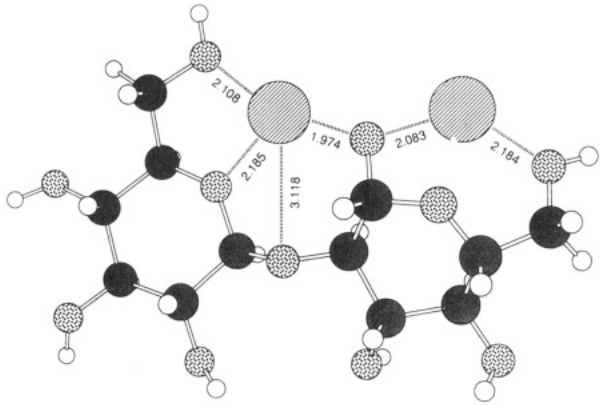


Figure 2.

dilithiated precursor. In fact, the calculations minimize to a conformation in which the hydroxyl group of C-4, not C-1, of the reducing ring is deprotonated (detailed MNDO results discussed below).

On the basis of the collective data, the only way in which $C_4D_3H_5O_4$ can be eliminated from the precursor ion is via the mechanism shown in Scheme III, pathway B. Pathway B involves intramolecular hydrogen transfer from an eight-membered transition state and may explain why the ion produced from this four carbon loss is so much smaller in abundance than that from the three carbon loss. Although pathway A involves an isomerization process, the subsequent retro-aldol reaction appears to be favored over a rearrangement proceeding through an eight-membered transition state.

Semiempirical Calculations

Scheme III indicates that for the 1,3-isomer the second lithium forms an alkoxide at the C-4 position of the reducing ring. However, Scheme I shows the lithium alkoxide residing at the anomeric carbon for the 1,2-isomer. Because of the different proposed mechanisms of dissociation for both these isomers, we decided to investigate them in detail by exhaustive iterations of the MNDO calculations to determine the site of coordination of the second lithium.

As with the 1,6-isomer,[32] the calculations for the monolithiated 1,3- and 1,2-disaccharides minimized to a structure in which the lithium was located near the glycosidic linkage between the two sugar rings where it could be coordinated to the greatest number of oxygens. However, the positioning of the second lithium ion appeared to be crucial to understanding the dissociation pathways. Since the hydroxyl group of the anomeric carbon seemed to be the most likely site for deprotonation, we began all our calculations with the second lithium atom coordinated at this position.

Table II. ΔH_f of Dilithiated Laminaribiose Structures with Different Coordination and Site of Deprotonation Derived by MNDO Calculations

no.	dilithiated structure	ΔH_f (kcal/mol)
1	Li_1 [6,O,4$_r$[a]]$_{nr}$[b] Li_2 [6,4[c]]$_r$	−430
2	Li_1 [6,O,2$_r$]$_{nr}$ Li_2 [2,1]$_r$	−420
3	Li_1 [6,O,2$_r$]$_{nr}$ Li_2 [6,4]$_r$	−409
4	Li_1 [6,O,4$_r$]$_{nr}$ Li_2 [6,O,1]$_r$	−406
5	Li_1 [6,O,4$_r$]$_{nr}$ Li_2 [6,4]$_r$	−403
6	Li_1 [6,O,2$_r$]$_{nr}$ Li_2 [2$_{nr}$,4]$_r$	−401
7	Li_1 [6,O,2$_r$]$_{nr}$ Li_2 [1]$_r$	−400
8	Li_1 [6,O,4$_r$]$_{nr}$ Li_2 [6,O,1]$_r$	−392
9	Li_1 [6,O,2$_r$]$_{nr}$ Li_2 [6]$_r$	−391[d]

[a] r denotes that the coordination to lithium is to the hydroxy group of the indicated carbon of the reducing ring. [b] nr denotes that the coordination to lithium is to the hydroxy group of the indicated carbon of the nonreducing ring. [c] Underlined number indicates that the hydroxyl group of the numbered carbon has been deprotonated. [d] Minimization of this structure forced the second lithium to coordinate only to the C6 oxygen in a zwitterion configuration.

Table III. ΔH_f of Dilithiated Sophorose Structures with Different Coordination and Site of Deprotonation Derived by MNDO Calculations

no.	dilithiated structure	ΔH_f (kcal/mol)
1	Li_1 [6,O,1$_r$[a]]$_{nr}$[b] Li_2 [1[c],O,6]$_r$	−429
2	Li_1 [6,O,1$_r$]$_{nr}$ Li_2 [6,4]$_r$	−414
3	Li_1 [6,O,1$_r$]$_{nr}$ Li_2 [6,4]$_{nr}$	−412
4	Li_1 [6,O,1$_r$]$_{nr}$ Li_2 [2$_{nr}$,3]$_r$	−408
5	Li_1 [6,O,1$_r$]$_{nr}$ Li_2 [2$_{nr}$,3]$_r$	−405
6	Li_1 [6,O,1$_r$]$_{nr}$ Li_2 [6,4]$_{nr}$	−404
7	Li_1 [6,O,1$_r$]$_{nr}$ Li_2 [6,4]$_r$	−403
8	Li_1 [6,O,1$_r$]$_{nr}$ Li_2 [1,O,6]$_r$	−402
9	Li_1 [6,O,1$_r$]$_{nr}$ Li_2 [2$_{nr}$, 3]$_r$	−402
10	Li_1 [6,O,1$_r$]$_{nr}$ Li_2 [2$_{nr}$, 3]$_r$	−389

[a] r denotes that the coordination to lithium is to the hydroxy group of the indicated carbon of the reducing ring. [b] nr denotes that the coordination to lithium is to the hydroxy group of the indicated carbon of the nonreducing ring. [c] Underlined number indicates that the hydroxyl group of the numbered carbon has been deprotonated.

Tables II and III list the range of ΔH_f's obtained for the 1,3- and 1,2-isomers, respectively. The nomenclature used is explained and can be followed from Figures 1 and 2 which correspond to conformer 1 in Table II and conformer 1 in Table III, respectively. Briefly, for Figure 1, the first lithium is coordinated to the hydroxyl group at C-6 and the ring oxygen of the nonreducing ring as well as to the hydroxyl of C-4 of the reducing ring. The second lithium is coordinated to the hydroxyl groups of C-6 and C-4 of the reducing ring such that the hydroxyl at C-4 is deprotonated. As can be seen from both Figures 1 and 2, the glycosidic oxygen appears in the same "pocket" with the other oxygens which are coordinated to lithium. However, the distance from the lithium atom to the glycosidic oxygen is always longer, e.g., 3.1 Å for the 1,2-isomer and 2.7 Å for the 1,3-isomer. This is true for all the calculations thus far collected and it would therefore appear that the glycosidic oxygen is merely included in the pocket, but does not really have any significant interaction with the lithium atom (see supplementary material for all x,y,z coordinates and bond distances for all calculations).

Of particular interest is the fact that even though all the calculations were initiated with the second lithium at the deprotonated anomeric position, the two isomers mini-

mized to different structures with the lithiums coordinated to different oxygens. Clearly, the site of deprotonation is particularly important in providing the most energetically stable structure. For example, conformers 1 and 5 in Table II differ by 27 kcal, yet the basic difference between these two is the site of deprotonation; i.e., the sites of lithium coordination are the same.

Given all the labeling experiments for the 1,3-isomer and the MS/MS/MS studies, we were not initially able to postulate a mechanism for the four-carbon neutral loss with the alkoxide residing at the anomeric position. In this particular case we have used the MNDO calculations as a model for predicting the possible dissociation pathways by first determining the most likely site of metal coordination. Of course, caution is suggested when using this approach. In general, we acquire, analyze, and present both the experimental results and the theoretical calculations when proposing reaction mechanisms and sites of metal coordination.[32,36]

The positioning of the deprotonated oxygen between two lithiums as shown for both the 1,2- and 1,3-isomers is quite interesting. Indeed, many earlier studies have shown this conformation to be quite common among lithium enolates and tetrameric aggregates of lithium aldolate from pinacolone and pivaldehyde.[37,38] The Li–O coordination distances obtained in our MNDO calculations are also consistent with previously reported results for tetra- and dimeric coordination complexes.[38,39]

Although a plethora of crystallographic data exists on Lithium-ligand complexes,[37-39] very little is known about the gas-phase conformations.[40] We anticipate that further MNDO calculations of other metal-coordinated biomolecules will assist us in our future endeavors to determine both the site of metal coordination and MS/MS dissociation pathways of these complexes. (Studies are currently underway to determine the sites of lithium ion coordination for the dilithiated 1,4- and 1,6-isomers.)

Conclusions

In summary, ^{2}H- and ^{18}O-labeling studies of isomeric dilithiated disaccharides and subsequent MS/MS experiments indicate that reducing ring fragmentation occurs, and this fragmentation appears to be directly dependent on both the linkage position and the site of deprotonation. Analytically, one cannot discern the difference between the 1,2- and 1,4-linked oligomers using the dilithiated precursor. However, CID of both the mono- and dilithiated precursors provides unambiguous linkage position information for the 1,2-, 1,3-, 1,4-, and 1,6-linked isomers (the technique does not appear to discriminate between α and β conformations of the linkages).

Mechanisms of dissociation have been proposed after careful consideration of both the experimental and theoretical data, the latter being essential in determining the most likely site of deprotonation for the gas phase $(M + 2Li - H)^+$ species. Coordination of one lithium ion appears to "bridge" the two monomeric saccharides in a tetrameric configuration, while the second lithium is dicoordinate between two oxygens, one of which is deprotonated. In the gas phase, this lithium alkoxide appears to initiate rearrangement and dissociation.

Supplementary Material Available: Cartesian coordinates and Z-matrices of all conformations studied for 1,2 and 1,3 isomers, CID product ion spectra of $[M - H + 2Li]^+$ from anomeric ^{18}O-labeled sophorose, $[M - D + 2Li]^+$ from deuterated sophorose, MS/MS/MS spectrum of $[M - H + 2Li]^+$ from sophorose, CID product ion spectra of $[M - H + 2Li]^+$ from anomeric ^{18}O-labeled lactose, $[M - D + 2Li]^+$ from deuterated lactose, MS/MS/MS spectrum of $[M - H + 2Li]^+$ from lactose, CID product ion spectra of $[M - H + 2Li]^+$ from anomeric ^{18}O-labeled laminaribiose, $[M - D + 2Li]^+$ from deuterated laminaribiose, and MS/MS/MS spectrum of $[M - H + 2Li]^+$ from laminaribiose (124 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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Lanthanide–Chiral Resolving Agent Mixtures as Chiral NMR Shift Reagents

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Mixtures of lanthanide complexes with soluble analogs of chiral liquid chromatographic stationary phases are shown to be useful NMR shift reagents for determining enantiomeric excess. The chiral resolution agents used in this work exhibit different association constants with enantiomeric substrates and associate weakly, if at all, with lanthanide ions. If the lanthanide associates with the substrate, the resolution observed in the spectrum of the substrate is enhanced. Enhancement occurs because the enantiomer concentrated in the bulk solution spends more time bonded to the lanthanide ion than the enantiomer with a higher association constant with the chiral resolving agent. Since the mechanism of interaction of many chiral liquid chromatographic phases is understood, or offers the potential to be understood, it should be possible to assign absolute configurations to the resolved NMR spectra. The method is applicable with donor–acceptor chiral resolving agents such *N*-(3,5-dinitrobenzoyl)-L-leucine and chiral hosts such as the cyclodextrins.

Introduction

Nuclear magnetic resonance spectroscopy is one of the simplest and most common methods of determining enantiomeric excess. One procedure is to synthesize a pair of diastereomers using an optically pure derivatizing reagent. The NMR spectra of the diastereomers often exhibit different chemical shifts for one or more sets of corresponding resonances. An alternative procedure is to

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