Stereospecific Synthesis and Characterization of Aminoglycoside Ligands from Diethylenetriamine

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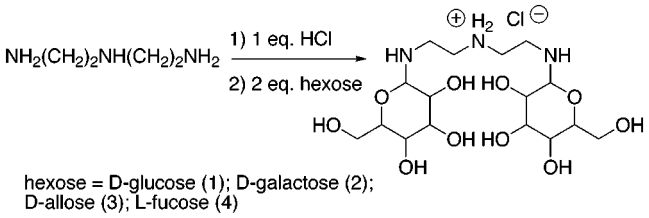
A convenient synthetic route to diglycosylamines derived from diethylenetriamine and D-glucose, D-galactose, D-allose, or L-fucose is presented where the stereochemically pure product is easily isolated and crystallized as a hydrochloride salt. The diglucosyl diethylenetriamine analogue is characterized by X-ray crystallography, and the chloride ion is shown to stabilize the compound in the crystalline state. These ligands provide simple and efficient routes to various diastereomeric metal *N*-glycoside coordination complexes that are currently being studied by mass spectrometry.

Introduction

Oligosaccharides are ubiquitous in biological systems, but much remains to be determined about the structures and functions of specific glycans. Accordingly, many analytical techniques are being developed to characterize these structurally complicated and diverse molecules.[1,2]

Of particular interest is the investigation of metal cationized oligosaccharides by mass spectrometry (MS) and tandem mass spectrometry (MS^n). Previous research indicates that it is possible to obtain linkage, anomeric configuration, and stereochemical information on metal-coordinated oligosaccharides.[3−14] Although mass spectrometry in not a tool traditionally used to distinguish stereoisomers, stereochemistry of individual monosaccharides as well as α versus β configuration of glycosidic bonds in disaccharides can be determined by MS^n.[9−14] In such studies it was found that axial versus equatorial stereochemistry of the C2 and C4 hydroxyl groups could be differentiated by the cross ring cleavage patterns observed in the gas phase. This procedure allowed for relatively rapid analysis of several saccharides with a given metal−ligand system. However, it was limited by the fact that it was much more time consuming to screen the efficacy of different metals for a given saccharide or ligand because each individual metal−ligand complex required synthesis a priori.

Scheme 1



During the course of our studies it was found that cooling a portion of the concentrated reaction mixtures to 4 °C for several days afforded a small amount of white precipitate. Elemental analysis along with ^{1}H NMR and mass spectra indicated the compound was 1,3-*N*,*N*-di-β-D-glucopyranosyldiethylenetriamine (**1**). It was envisioned that the purposeful synthesis of such a ligand could lead to a method for rapidly screening the effect of inserting different metals into **1**, and/or analogues, using MS/MS spectra. We expect a systematic investigation of how different metals may promote different types of dissociation pathways when the complexes are analyzed by MS^n to provide information which could then be used to design other systems which differentiate stereochemistry.

Diglycosylamines derived from ethylenediamine and diaminopropane have been previously reported in the literature.[15−17] However, in only one case was a solution structure reported, and in another, no proof of structure other than elemental analysis was given. We have developed a method for the synthesis of diglycosylamines derived from diethylenetriamine which can be easily isolated and crystallized as a hydrochloride salt. Furthermore, the structure has been fully characterized by ^{13}C NMR as well as X-ray crystallography.

Results and Discussion

Scheme 1 outlines the general procedure for preparation of diglycosylamines derived from diethylenetriamine (dien). This method proved to be a convenient one-pot synthesis in which the product precipitated from solution

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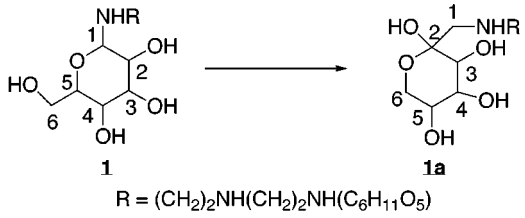

Figure 1. Schematic of the Amadori rearrangement: conversion of **1**, an aminoaldose, to **1a**, an aminoketose.

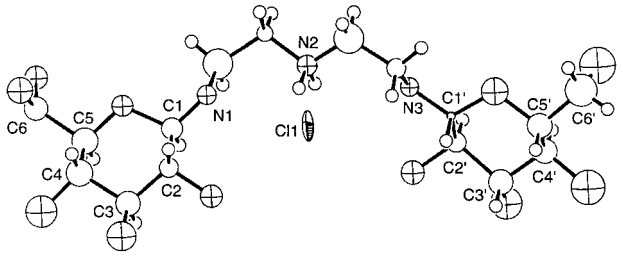

Figure 2. Ortep diagram for the crystal structure of **1**. Disorder at the C6 oxygen arises from favorable hydrogen bonding with the molecule in the unit cell on either side.

in high yield (80−90%). In general, yields were improved by allowing the reaction to stir for 1 h after initial observation of the precipitate and then cooling to 4 °C before filtration.

Although glycosylamines formed from a reducing sugar and an amino acid often undergo an Amadori rearrangement[18,19] (Figure 1), aliphatic *N*-glycoside compounds are less prone to such effects.[20,21] Analysis of **1** by ^{13}C NMR indicated that the Amadori rearrangement had not occurred after formation of the *N*-glycoside bond. DEPT 90 and DEPT 135 experiments indicated that the number of quaternary, methyne, and methylene carbons is 0, 5, and 3 respectively, in contrast to the result expected for the rearranged product **1a** (Figure 1), namely, 1 quaternary, 3 methyne, and 4 methylene carbons.

^{13}C NMR spectra also indicate that all of these compounds have an axis of symmetry. In the ^1H NMR spectra of **1** through **4** the anomeric hydrogen is a doublet with a coupling constant of 8.5−9.5 Hz. Such coupling constants are in the range for those resulting from an axial−axial configuration of H1 and H2 and therefore, for these sugars, a β *N*-glycosidic linkage.[22] The NMR spectra of **1** through **4** are complicated by the fact that the only solvent these compounds are very soluble in is H_2O which leads to partial hydrolysis of the *N*-glycoside bonds within 5 min. Solubility in other polar solvents such as DMSO is negligible. To maximize sample concentration and minimize hydrolysis time, the spectra were obtained in D_2O using very concentrated samples (0.6−0.7 M) on a high-field instrument (500 MHz). Although the presence of α anomeric products in the precipitates may not have been detected due to overlapping resonances with the small "hydrolysis" peaks, they would represent a very small percentage of the overall product composition.

The identity and connectivity deduced from the ^{13}C and ^1H NMR data are consistent with an X-ray crystal structure of compound **1**. The Ortep diagram for **1** is shown in Figure 2.

The preference for formation of the β isomer can be rationalized in terms of the competition between substitution at the sterically least hindered position (β) versus substitution at a position which incurs stabilization from the anomeric effect (α). In this case, where the monosaccharide moieties are in the $^4C_1$ conformation (see Figure 2), the steric factor favors the β anomer while the anomeric factor favors the α anomer. An amino substituent, however, will not be stabilized by the anomeric effect to as great an extent as a more electronegative substituent such as a group 5 halogen or oxygen.[23] Therefore, substitution is driven predominantly by sterics and the reaction is stereoselective for the β configuration.

More importantly, the X-ray crystal structure obtained for **1** gives some insight as to why crystalline products were easily obtained for **1**−**4** while the corresponding reaction with mannose did not yield a precipitate, even though mass spectrometric analysis indicated that the desired dimannosyltriamine had been formed in solution (see Figure 3). As can be seen from the Ortep diagram in Figure 2, the chloride ion imparts some rigidity to the structure. There are hydrogen bonds from the central nitrogen and from the hydroxyl groups on C2/C2′ to the chloride ion. The latter observation is relevant because glucose, galactose, allose, and fucose all possess an equatorial C2 hydroxyl group while mannose has an axial C2 hydroxyl. It is evident upon examination of the Ortep diagram that in order for such H-bonding to occur between chloride and the axial hydroxyl groups on C2/C2′ of the mannose moiety, significant structural contortion would be necessary which might then preclude crystallization. To further test this hypothesis, the reaction was again performed, this time using altrose, which also possesses an axial C2 group. As expected, no solid product was formed under these conditions, although the major product detected by mass spectrometric analysis was the protonated diglycosylamine complex (*m*/*z* 428, data not shown).

No precipitate was observed after several hours when the synthesis of **1** was repeated, omitting the addition of HCl. When 1 equiv of HCl in ether was added to the reaction mixture, however, **1** precipitated from solution. This result supports the conclusion that under these conditions the chloride ion is playing an important role in the crystallization process. The fact that attempts to induce crystallization of **1** with bulkier counterions such as HSO_4^-, NO_3^-, and $CH_3C(O)O^-$ were unsuccessful may be because these counterions are not suited for the space provided by the two monosaccharide and "bridging" dien moieties.

As mentioned earlier, ligands such as **1** provide simple and efficient routes to various metal *N*-glycoside complexes which are currently being explored in the solid state[24,25] and the gas phase.[9−14] For example, the mass spectrum of the products from reaction A (Figure 4)

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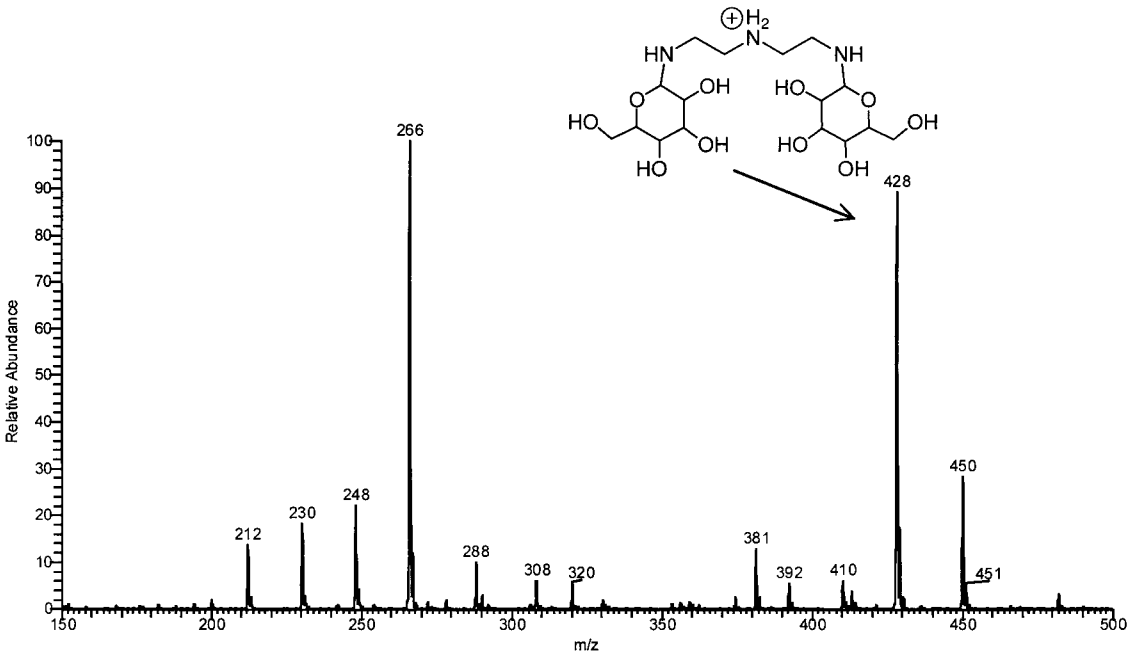

Figure 3. Mass spectrum of a reaction mixture (as in Scheme 1) where mannose has been used as the hexose. The ion at *m*/*z* 428 is the protonated ligand $[M + H]^+$ and *m*/*z* 450 is the sodium adduct $[M + Na]^+$ made in solution.

consists predominantly of singly and doubly charged metal *N*-glycoside complexes. Reaction B, on the other hand, yields only a moderate to low abundance of these ions of interest, along with many other species which are presumably side reactions and unreacted starting material. However, tandem mass spectra of these ions prepared by methods A and B are identical, indicating that A is the method of choice for MS studies. In addition, method A allows for the comparison of binding affinities of several metals to these types of carbohydrate ligands, a subject of future studies.

Experimental Section

General. ^{1}H NMR and [^{1}H] ^{13}C NMR spectra were recorded on a 500 MHz instrument in D_2O. MS analyses were carried out in the positive ion mode using electrospray ionization on an ion trap instrument as described elsewhere.[9] D-Glucose, diethylenetriamine, and 1 M HCl in ether were purchased from Aldrich (Milwaukee, WI). D-Allose was purchased from ICN Biomedicals, Inc. (Aurora, OH). D-Galactose and L-fucose were purchased from Sigma (St. Louis, MO). All reagents were used as received.

1,3-*N*,*N*-Di-*β*-D-glucopyranosyldiethylenetriamine (1). Diethylenetriamine (1.56 mL, 14.4 mmol) was dissolved in methanol (15 mL) and cooled to 0 °C. Hydrochloric acid (1 M solution in ether, 14.4 mL, 14.4 mmol) was added dropwise with stirring. The flask was warmed to room temperature, and methanol (50 mL) was added to redissolve the white precipitate which had appeared. Glucose (5.19 g, 28.8 mmol) which had been dissolved in 6 mL of hot water was added to the reaction flask followed by 6 mL more methanol. The resulting solution remained clear for 20 min, after which time a white precipitate appeared. The reaction mixture was allowed to stir for 1.25 h, and the precipitate was collected and dried under vacuum to give 5.38 g of **1** (80%).

Recrystallization of **1** was achieved by dissolving 661 mg of the precipitate in 5 mL of water in a large test tube. A 45 mL portion of 1:1 ethanol:methanol was carefully added to the tube and allowed to slowly infuse into the water layer with gentle swirling as necessary. Once crystals had begun to form, the solution was left at room temperature for 30 min more and then kept at 0 °C overnight before filtering to obtain 413 mg of white crystalline product. ^{1}H NMR: δ 2.40−3.10 (10H, m), δ 3.15−3.35 (6H, m), δ 3.52 (2H, dd, J = 5.5, 12.0 Hz), δ 3.72 (2H, dd, J = 1.5, 11.0 Hz), δ 3.86 (2H, d, J = 8.5 Hz). ^{13}C NMR: δ 41.25, 47.39(−CH2−CH2-), δ 60.78 (C6/C6′), δ 69.75, 72.86, 76.49, 76.53 (C2−C5/C2′−C5′), δ 89.52 (C1/C1′). MS: $[M + H]^+$ *m*/*z* 428.

Anal. Calcd for $C_{16}H_{34}ClN_3O_{10}$: C, 41.42; H, 7.39; N, 9.06. Found: C, 41.12; H, 7.63; N, 8.90.

1,3-*N*,*N*-Di-*β*-D-galactopyranosyldiethylenetriamine (2). Diethylenetriamine (0.52 mL, 4.8 mmol) was dissolved in methanol (15 mL). Hydrochloric acid (1 M in ether, 4.8 mL, 4.8 mmol) was added dropwise with stirring at room temperature. Galactose (1.73 g, 9.6 mmol) which had been dissolved in 2 mL hot water was added to the reaction flask along with 10 mL more of methanol. The resulting solution remained clear for 15 min after which time a white precipitate appeared. The reaction mixture was allowed to stir 1 h and then kept at 4 °C overnight. The product was collected and dried for 2.5 h under vacuum to give 1.90 g of white solid **2** (85%). A 600 mg portion of crude material was recrystallized as for **1** using 2 mL water and 10 mL ethanol/methanol to yield 336 mg of product: ^{1}H NMR: δ 2.99−3.10 (7H, m), δ 3.34 (2H, dd, J = 8.5, 9.5 Hz), δ 3.50−3.64 (9H, m), δ 3.81 (2H, dd, J = 1.0, 3.5 Hz), δ 3.86 (2H, d, J = 9.0 Hz). ^{13}C NMR: δ 41.35, 47.52 (−CH2−CH2-), δ 61.18 (C6/C6′), δ 68.90, 70.59, 73.43, 75.70 (C2−C5/C2′−C5′), δ 90.04 (C1/C1′). MS: $[M + H]^+$ *m*/*z* 428.

Anal. Calcd for $C_{16}H_{34}ClN_3O_{10}$: C, 41.42; H, 7.39; N, 9.06. Found: C, 41.05; H, 7.69; N, 8.82.

1,3-*N*,*N*-Di-*β*-D-allopyranosyldiethylenetriamine (3). The reaction was carried out as for **2** using 0.31 mL (2.9 mmol) of diethylenetriamine, 4.8 mL (2.8 mmol) of 1 M HCl in ether, and 1.0 g (5.6 mmol) of allose instead of galactose; 1.2 g of a white solid **3** was obtained (90%). A 241 mg portion of crude material was recrystallized as for **1** using 0.75 mL water and 12 mL of ethanol/methanol to yield 179 mg of product. ^{1}H NMR: δ 2.94−3.06 (8H, m), δ 3.29 (2H, dd, J = 2.8, 9.6 Hz), δ 3.45 (2H, dd, J = 2.5, 9.5 Hz), δ 3.5−3.6 (4H, m), δ 3.74 (2H, dd, J = 1.5, 11.5 Hz), δ 4.02 (2H, dd), δ 4.12 (2H, d, J = 9.5 Hz). ^{13}C NMR: δ 41.35, 47.47 (−CH2−CH2-), δ 61.26 (C6/C6′), δ 67.00, 70.08, 70.90, 73.71 (C2−C5/C2′−C5′), δ 86.22 (C1/C1′). MS: $[M + H]^+$ *m*/*z* 428.

Anal. Calcd for $C_{16}H_{34}ClN_3O_{10}$: C, 41.42; H, 7.39; N, 9.06. Found: C, 41.59; H, 7.64; N, 8.96.

1,3-*N*,*N*-Di-*β*-D-fucopyranosyldiethylenetriamine (4). The reaction was carried out as for **2** using fucose (1.58 g, 9.6

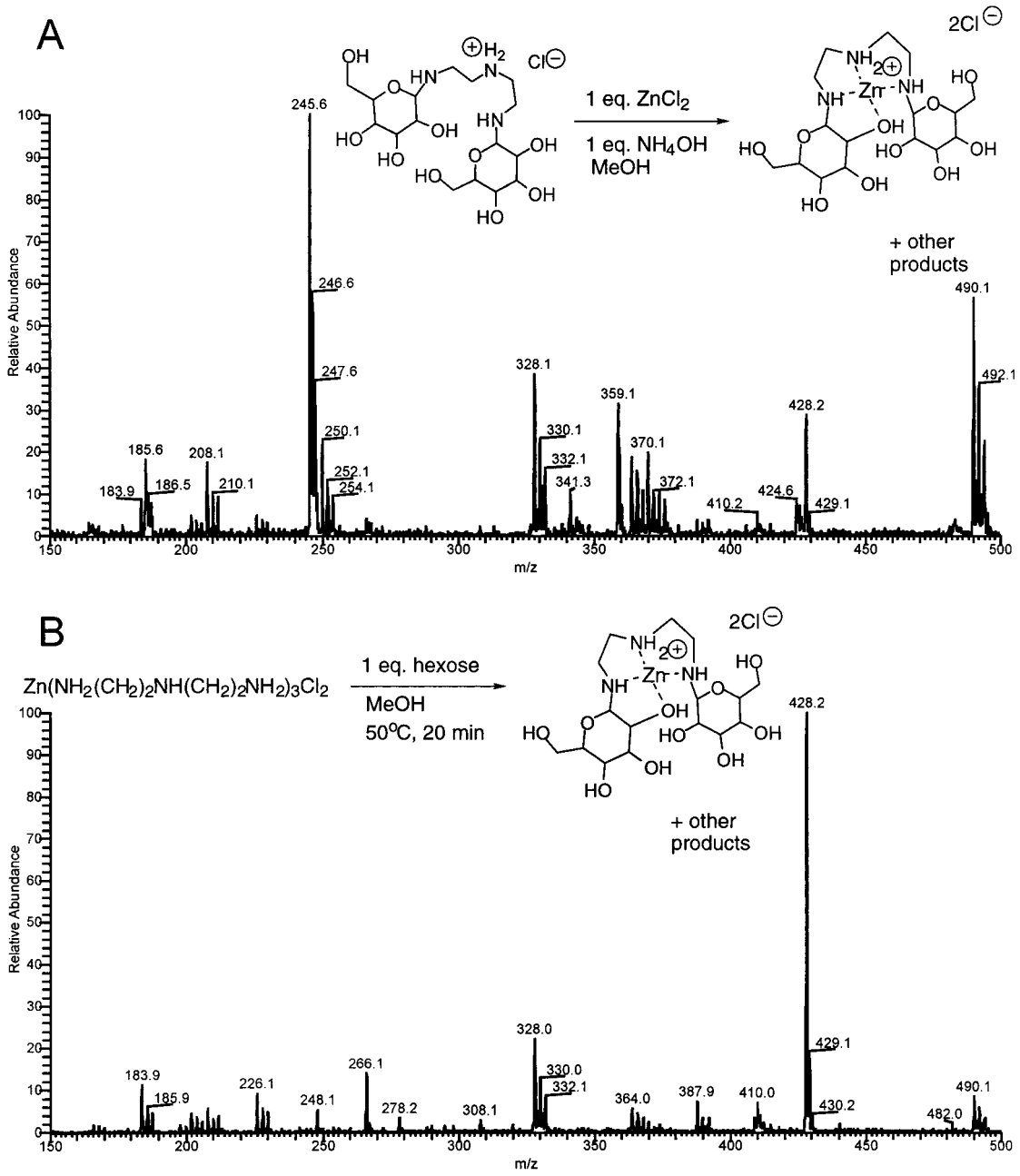

Figure 4. (A) Reaction scheme for sample preparation using the synthesized ligand and the resulting mass spectrum of the reaction mixture. (B) The corresponding scheme and spectrum using the original method of sample preparation.

mmol) instead of galactose. The white solid product was dried 6 h under vacuum to yield 1.74 g of **4** (84%). A 425 mg portion of material was recrystallized as for **1** using 1 mL of water and ~12 mL of ethanol/methanol to yield 213 mg of product. ^{1}H NMR: δ 1.05 (6H, d, J = 6 Hz), δ 2.7−3.0 (8H, m), δ 3.2−3.3 (2H, m), δ 3.47 (2H, dd, J = 3.5, 10.0 Hz), δ 3.6−3.7 (4H, m), δ 3.78 (2H, d, J = 9.0 Hz). ^{13}C NMR: δ 15.74 (C6/C6′), δ 41.18, 47.35 (−CH2−CH2-), δ 70.34, 71.37, 71.57, 73.55 (C2−C5/C2′−C5′), δ 89.76 (C1/C1′). MS: $[M + H]^+$ m/z 428.

Anal. Calcd for $C_{16}H_{34}ClN_3O_{10}$: C, 44.50; H, 7.88; N, 9.73. Found: C, 44.42; H, 8.10; N, 9.54.

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Supporting Information Available: Proton NMR spectra, ^{13}C NMR spectra including DEPT 90 and DEPT 135 data, and experimental procedures and data for the X-ray analysis of compound **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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