CHARACTERIZATION BY SOFT IONIZATION MASS SPECTROMETRY METHODS OF MONO- AND OLIGOSACCHARIDES FUNCTIONALIZED AT THE ANOMERIC CENTER

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Abstract
In order to obtain oligosaccharides functionalized with amine groups, aliphatic diamines and polyamines were coupled with maltose by using reductive amination reactions. The synthesized compounds were characterized through electrospray ionization mass spectrometry (ESI-MS), while finer structural details were obtained by using MS^2.

Introduction
In the hands of organic chemists, the reductive amination reaction is a powerful tool for building C-N bonds. Its importance is revealed by the fact that volume 59 of Organic Reactions series *(Ed. L.E. Overman et al. - John Wiley & Sons, 2002*) is completely dedicated to the reductive amination reaction, which uses boranes and their derivatives as reducing agents (2100 reference citations). A multitude of substrates, including aliphatic, aromatic and mixed aldehydes, but also aliphatic and aromatic ketones, can be reductively aminated with primary or secondary amines belonging to the aliphatic or aromatic series, and even by using the ammonium ion.
Dedicated literature abounds with sugar compounds modified with amines, especially in relation with the processes of analytical detection of biological trace samples. The attachment of aromatic amines (some possessing fluorescence) allows the detection of sugars by using high performance liquid chromatography (HPLC) and capillary electrophoresis (CE) techniques.
Keeping in mind our experience regarding the analysis and derivatization of dextrans and maltodextrins [1-3], we set out to synthesize some maltoses aminated with different aliphatic amine components. In this paper, the obtained results regarding the derivatization through reductive amination of maltose, as well as the analysis through electrospray ionization mass spectrometry of the obtained compounds, are presented.

Experimental
The reactions took place in round flasks made of glass, which were equipped with magnetic stirrers, heating sources and straight condensers. The reagents ratios vary depending on the amine, while the working temperature (45-90 °C) depends on the solubility of the participating reacting species. The progress of the reaction was monitored through TLC, visualization of spots being made with 5% ethanolic ninhydrin. The isolated compounds were purified by ion exchange chromatography, using ammonium hydroxide solutions of various concentrations as mobile phases. The products were finally brought in solid state through lyophilization. Before being

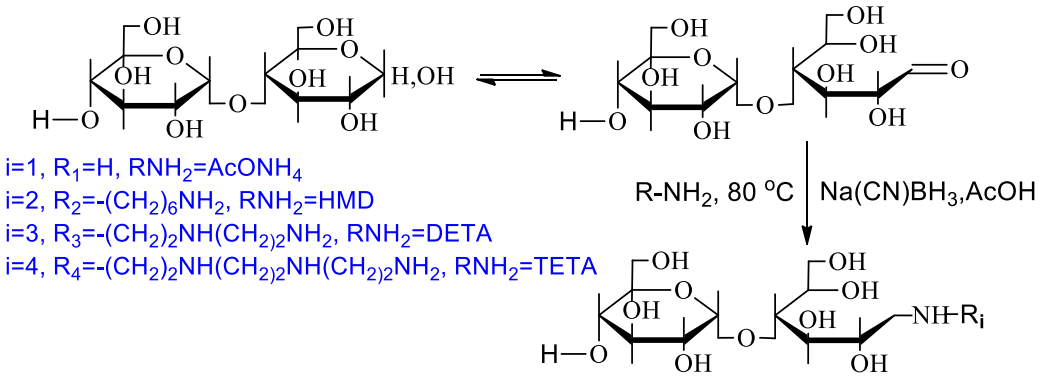
subjected to physical-chemical analysis, the products were investigated regarding their purity through TLC and for identity by reacting them with ninhydrin, all compounds giving positive results during these tests.

The spectral characterization through HCIT-MS analysis of maltose derivatives, obtained following the reductive amination reaction with different amine components, was done on a High Capacity Ion Trap (HCIT) Ultra PTM instrument (Bruker Daltonik, Bremen, Germany), belonging to the Institute of Chemistry Timișoara of Romanian Academy and courtesy of dr. Gh. Ilia. In all cases, the samples were injected in the spectrometer with the help of a microsyringe, attached to a push-syringe, with a speed of 250 μL h^{-1}. Nitrogen was used as a nebulizer gas, with a flowrate of 5 L min^{-1} and at 7 p.s.i., and at a 300 °C desolvation temperature. The instrument was programmed to operate in positive ion mode, at an ESI potential of 3.0 kV.

For the analysis, the samples were dissolved in MeOH/H_2O (1:1 v/v) to a concentration of 5-7 pmol μL^{-1} (stock solutions), which were diluted as needed.

Results and discussion

The reactions that occur during the derivatization of maltose through reductive amination are presented in **Scheme 1**, while the reaction conditions, which depend on the amine being used, are presented in **Table 1**.



Scheme 1. The reductive amination reactions of maltose with different amine components

Table 1. The reaction conditions for derivatization of maltose with various amine components

No.	Aliphatic amine (mol)	Substrate (mol)	Reducing agent (mol)	Acid (mol)	Time (h)	Control MS	Chemical control
1	**$AcONH_4$**	**maltose**	$Na(CN)BH_3$	AcOH	25	$[M+H]^+$	$Ny^{\#}$
	$15 \cdot 10^{-3}$	$1.5 \cdot 10^{-3}$	$20 \cdot 10^{-3}$	$21 \cdot 10^{-3}$		344.00	+
2	**HMD**	**maltose**	$Na(CN)BH_3$	AcOH	24	$[M+H]^+$	
	$92 \cdot 10^{-3}$	$9 \cdot 10^{-3}$	$18 \cdot 10^{-3}$	$50 \cdot 10^{-3}$		443.00	+
3	**DETA**	**maltose**	$Na(CN)BH_3$	AcOH	120	$[M+H]^+$	
	$90 \cdot 10^{-3}$	$3 \cdot 10^{-3}$	$9 \cdot 10^{-3}$	$30 \cdot 10^{-3}$		430.00	+
4	**TETA**	**maltose**	$Na(CN)BH_3$	AcOH	74	$[M+H]^+$	
	$210 \cdot 10^{-3}$	$9 \cdot 10^{-3}$	$20 \cdot 10^{-3}$	$120 \cdot 10^{-3}$		473.00	+

$^{\#}$Ny - ninhydrin (ethanolic solution of ninhydrin)

In all cases, the $[M+H]^+$ pseudomolecular ion's peak was revealed (see **Table 1**). This was isolated and fragmented through CID, obtaining the MS^2 spectrum (and also, whenever possible, the MS^3 and even the MS^4 spectrum), which gave the minimal number of structural data (characteristic fragmentations for the sugar and, respectively, the amine component). To give an example, the MS^2 and, respectively, MS^3 spectra of the product obtained after amination of maltose with ammonium acetate are shown in **Figs. 1** and **2**. To facilitate the assignments, the main fragmentations of the analyzed product are presented in **Scheme 2**. The MS^3 spectrum of the P fragment (see **Scheme 2**), which is also the base peak, gives all structural details that confirm the structure of the synthesized product.

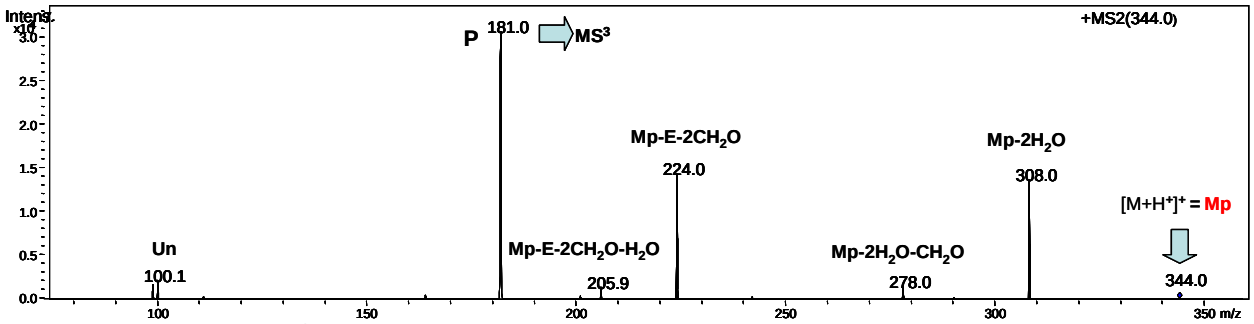


Figure 1. The MS^2 spectrum obtained after (+)-ESI-HCIT-MS analysis of the aminated derivative of maltose, following ammonium acetate reductive amination

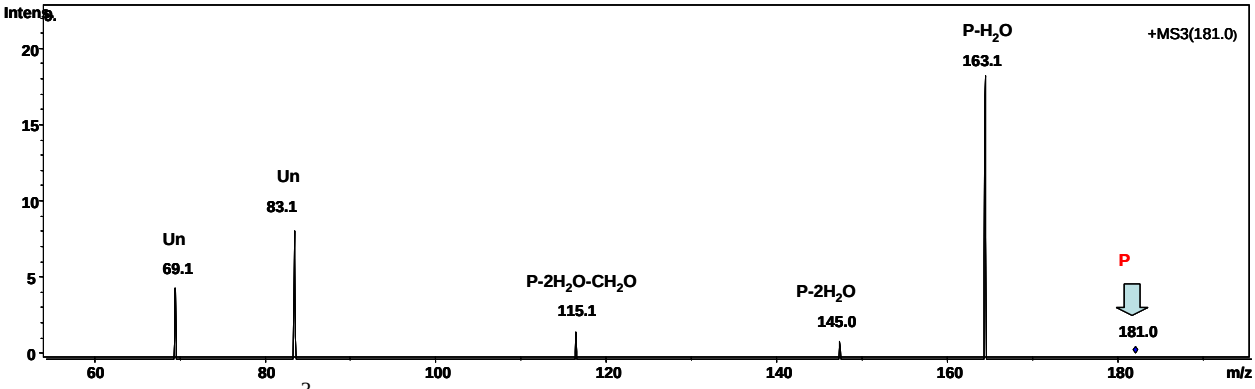
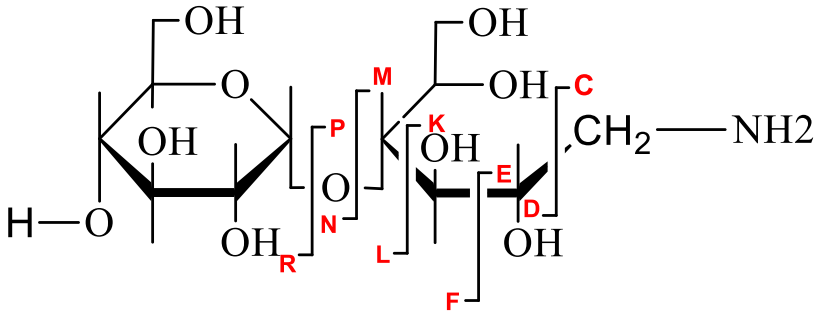


Figure 2. The MS^3 spectrum obtained after (+)-ESI-HCIT-MS analysis of the aminated derivative of maltose, following ammonium acetate reductive amination



Scheme 2. Fragmentation possibilities for the aminated derivative of maltose, obtained through the reductive amination with ammonium acetate

Conclusions

The derivatization of the anomeric site through attachment of aliphatic amines is done in mild conditions with satisfying yields and with procedures that were established by this research. The determination of the mass spectrometry conditions for ESI-HCIT-MS analysis of the aminated derivatives of maltose gives clear, sharp and reproducible spectra. Owing to the reactivity of the free amine groups, the thus obtained aminated derivatives can be used in many complexation reactions with hydrophilic agents.

Acknowledgements

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