

Article

Electrospray Mass Spectrometric Characterization of Novel Unsaturated Neutral Carbohydrate Bolaforms Obtained by Catalytic Metathesis

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Espectrometria de massa de eletrospray de íon positivo foi usada na caracterização de novos carboidratos *bolaforms* neutros insaturados 1, 2, 3 e 4 obtidos pela reação catalítica com complexos de tungstênio de cloro-arylóxido. A dissociação ativada por colisão na interface pressão atmosférica/vácuo proporcionou informações estruturais importantes com relação à fragmentação desses novos carboidratos.

Electrospray mass spectrometry in the positive ion mode was used for the structural characterization of novel unsaturated neutral carbohydrate bolaforms 1, 2, 3 and 4 obtained by catalytic metathesis reaction with chloro-aryloxide complexes of tungsten. Collision activated dissociation (CAD) at the atmospheric pressure/vacuum interface provided valuable structural information regarding the fragmentation of these novel unsaturated neutral bolaforms.

Keywords: *electrospray-MS, characterization, unsaturated bolaforms*

Introduction

The metathesis reaction is a well established reaction that promotes the conversional synthesis of new olefins by coupling of two terminal double bonds resulting in the evolution of ethylene^{1,2}. It has been shown that the metathesis reaction of olefins bearing a variety of functional groups can be achieved using tungsten based catalysis³⁻⁵.

We have previously shown in our laboratories that the intermolecular metathesis reaction can be successfully applied for the synthesis of novel unsaturated neutral carbohydrate bolaforms and macrocyclic carbohydrate derivatives⁶⁻⁸. Bolaforms represent a group of compounds which consist of two hydrophilic heads joined together by a long spacer composed of an aliphatic chain. Interest in bolaforms, due to their unusual properties as surfactants, has increased in the past few years. Bolaforms are able to form micelles, vesicles and very thin monolayer lipid membranes⁹, and have been used as liquid crystals.

Among one of the most interesting classes of bolaforms is the neutral form containing carbohydrate units at both ends of the long aliphatic chain spacer. In this rationale, we discuss the mass spectrometric characterization of members of this novel series of unsaturated neutral carbohydrate bolaforms whose structures are shown in Fig. 1. To our knowledge, there has been no previous study of any kind on the mass spectrometric characterization of these types of neutral bolaforms.

We have opted to use electrospray mass spectrometry for the structural characterization of this class of novel compounds because this ionization method, which is, arguably, the softest ionization to date, requires neither special derivatization nor excessive handling of the analytes which is usually associated with the other ionization methods. Electrospray ionization (ESI) is well established as a robust technique for use with combined liquid chromatography/mass spectrometry (LC/MS) which allows rapid, accu-

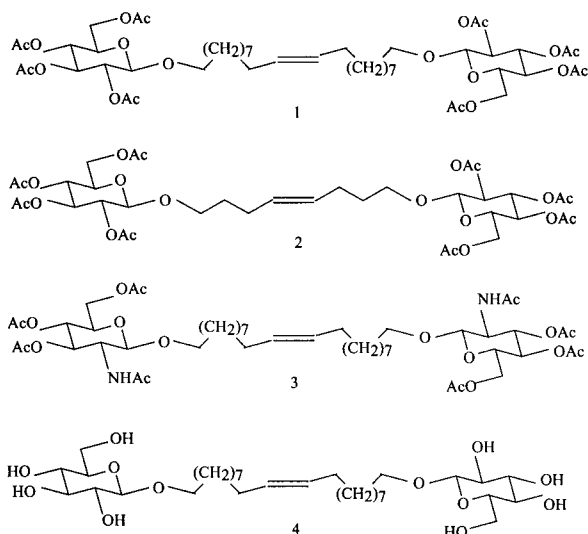


Figure 1. Chemical structures of unsaturated neutral carbohydrate bolaforms 1-4.

rate and sensitive analysis of a wide range of analytes from low molecular weight polar compounds (less than 200 Da) to biopolymers larger than 100 kDa¹⁰. Under appropriate experimental conditions, gas phase fragmentations are minimized and the ions thus formed, which possess low internal energy, are sufficiently stable to pass from the ion source to the detector without dissociation. This is characteristic for ions produced by “very soft” ionization processes.

Furthermore, dissociations may be induced or activated by collision. In this process, a part of the kinetic energy of the ion is converted to internal energy by colliding with a neutral gas-phase species, usually in the pressurized collision cell of a tandem mass spectrometry (MS/MS) instrument¹¹⁻¹³. Ions that have undergone this collisional excitation process may subsequently fragment. Thus, collisionally activated dissociation (CAD)/MS/MS, and in particular low-energy CAD MS/MS characteristic of quadrupole instruments, is a valuable method for generating structural information if the primary ionization process does not impart enough internal energy for spontaneous fragmentation to occur¹¹⁻¹³. Another way to generate structural information by dissociation of the $[M+H]^+$ ion is by controlled adjustment of the accelerating voltage (0 ± 250 V) applied to the sampling cone (focus voltage) of the electrospray source (known as cone voltage fragmentation). This procedure is also referred to as CAD in the atmospheric pressure/vacuum interface^{14,15}. Unlike CAD experiments performed using tandem mass spectrometers, no mass filtering precedes the latter ion/neutral collisions. The surviving precursor ions, plus the decomposition products generated in the ES source, contribute to the ions observed in such a CAD spectrum.

As a continuation of our interest in the mass spectrometry and tandem MS/MS analysis of bioactive molecules¹⁶⁻²⁰, we now report on the structural characterization of this novel series of unsaturated carbohydrate bolaforms 1-4 using electrospray mass spectrometry. Evidence of the different fragmentation routes was obtained by collisionally activated dissociation in the atmospheric pressure/vacuum interface.

Experimental

Sample preparation

Members of the unsaturated neutral carbohydrate bolaforms, series 1-4, were prepared by the method of Descotes *et al.*^{6,7}

Mass spectrometry conditions

The ES MS spectra (positive-ion mode) were recorded using a Fisons VG-Quattro quadrupole-hexapole-quadrupole mass spectrometer (Fisons VG, Altrincham, UK), equipped with an electrospray ionization source and capable of analyzing ions up to m/z 4000. A personal computer (486, 66 MHz processor) equipped with Fisons MASSLYNX mass spectrometry data system software was used for data acquisition and processing. The temperature of the ES ionization source was maintained at 70 °C. The operating voltage of the ES capillary was 3.50 kV and the high voltage lens was maintained at 0.5 kV throughout. ES mass spectra were recorded with a focus setting varying from 30 to 75 V. Generally, the higher focus voltages induced almost complete fragmentation of the lower molecular weight samples. Conventional ES mass spectra were obtained by scanning in the multi-channel analysis (MCA) mode with a scan time of 1 s per 250 mass numbers. Spectra are an average of 3-4 scans. Conventional ES and MS/MS spectra presented in this rationale have been background-subtracted, smoothed and centered. The mass scale was calibrated in the positive-ion mode using a polyethylene glycol mixture. MS/MS experiments were conducted using the same instrument. Fragment-ion spectra of mass-selected ions were induced by collision with argon in the (RF only) hexapole. Argon collision gas was added to the enclosed chamber of the hexapole to give an indicated pressure of 2×10^{-5} mbar for collisional activation of the sample ions. The resulting fragments were analyzed by the second quadrupole. Collision energies of approximately 50 eV and a focus voltage of 30 V were used in all MS/MS experiments. Precursor-ion scans were obtained by scanning the first quadrupole while selecting a given m/z value with the second quadrupole.

Results and Discussion

The positive ion electrospray mass spectrum (ES MS) of the unsaturated bolaform (E,Z)-di-Q-(2,3,4,6-tetra-Q-

acetyl- β -D-glucopyranosyloxy)-1,20-eicos-10-ene, $[C_{48}H_{76}O_{20}$, M.Wt.=973] **1**, recorded with a low cone voltage (~ 30 V) showed the sodiated molecular ion $[M+Na]^+$ at m/z 996 in high relative abundance (100%). We also noticed the formation of a characteristic ion at m/z 523 which occurred by fragmentation of the spacer aliphatic chain of the sodiated molecular ion accompanied by a hydrogen atom transfer. The structures of this diagnostic fragment ion is indicated in Fig. 2a. It is noteworthy to mention the presence of the 2,3,4,6-tetra-O-acetyl- β -D-glucopyranosyloxy ion A_1^+ at m/z 331. The relative distribution of this series of charged ions can be changed by varying the controlled adjustment of the accelerating voltage applied to the sampling cone of the electrospray source. Indeed, the collisionally activated dissociation at the atmospheric pressure/vacuum interface with a voltage of ~ 40 V showed an increase in abundance of the fragment ion at m/z 523, the A_1^+ fragment ion at m/z 331 and in the appearance of its derived $[A_1-AcOH]^+$ ion at m/z 271 (see Fig. 2b).

The positive ion ES MS of the second member of this novel series was that of the unsaturated bolaform (E,Z)-di-Q-(2,3,4,6-tetra-O-acetyl- β -D-glucopyranosyloxy)-1,8-oct-4-ene, $[C_{36}H_{52}O_{12}$, M.Wt. = 804] **2**, recorded with a low cone voltage (~ 30 V) showed a very simple ES MS composed of the protonated molecular ion $[M+H]^+$ at m/z 805 and the sodiated molecular ion $[M+Na]^+$ at m/z 827 (see

Fig. 3a). The CAD at the atmospheric pressure/vacuum interface, with a cone voltage of ~ 40 V, resulted in the production of the characteristic A_1^+ ion at m/z 331 and its $[A_1-AcOH]^+$ derived fragment ion at m/z 271 (see Fig. 3b).

The positive ion ES MS of the third member of this novel series which contained the N-acetylglucosamine residues, namely (E,Z)-di-Q-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- β -D-glucopyranosyloxy)-1,20-eicos-10-ene, $[C_{48}H_{78}O_{18}N_2$, M.Wt = 971] **3**, was recorded with a cone voltage of 30 V and is shown in Fig. 4a. This ES MS shows the protonated molecular ion $[M+H]^+$ at m/z 971 (100% relative abundance), the sodiated molecular ion $[M+Na]^+$ at m/z 993, a diagnostic fragment ion at m/z 642 whose structure is indicated in Fig. 4a and minute amounts of the 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- β -D-glucopyranosyloxy ion A_1^+ at m/z 330. The CAD atmospheric pressure/vacuum interface with a cone voltage of 40 V produced an ES MS shown in Fig. 4b which indicates a decrease in the $[M+H]^+$ ion at m/z 971 and the production of the fragment ion at m/z 951 assigned to $[M+Na-CH_2CO]^+$. An increase in the A_1^+ fragment ion at m/z 330 was also noticed.

Finally, the ES MS of one of the deprotected members of these unsaturated bolaforms, (E,Z)-di-Q-(β -D-glucopyranosyl)-1,20-eicos-10-ene, $[C_{33}H_{60}O_{12}$, M.Wt. = 636] **4**, is shown in Fig. 5. We noticed the presence of the

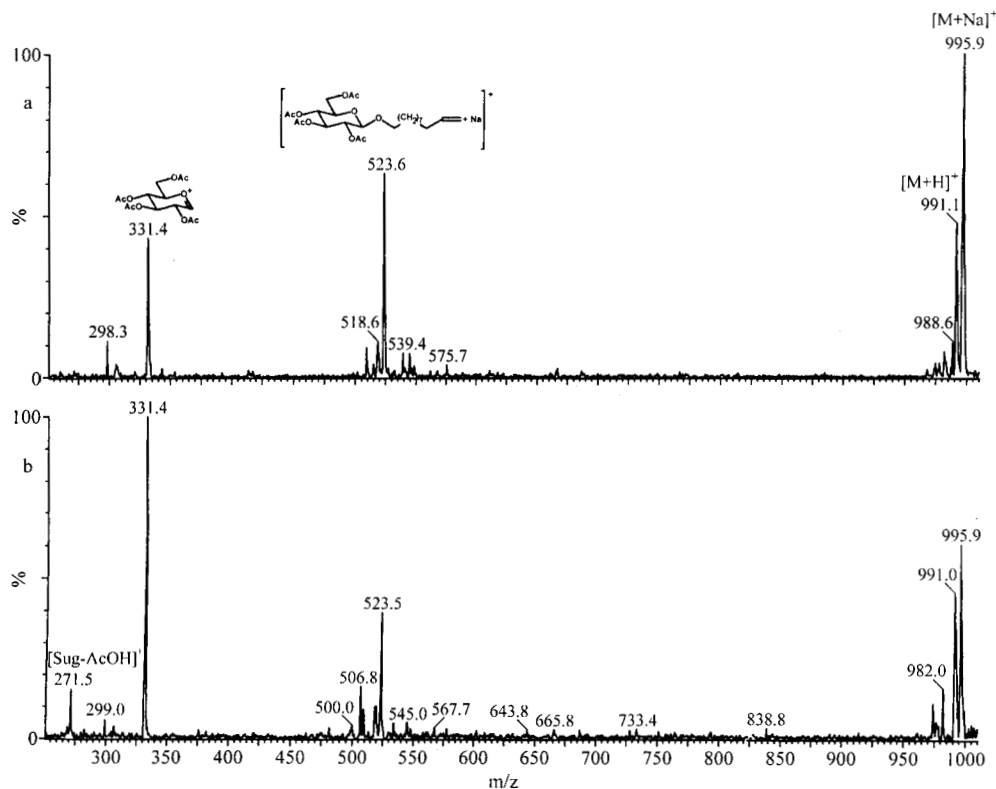


Figure 2. Electrospray mass spectra of the unsaturated bolaform (E,Z)-di-Q-(2,3,4,6-tetra-O-acetyl- β -D-glucopyranosyloxy)-1,20-eicos-10-ene **1** recorded with a cone voltage of a) 30 V and b) 40 V.

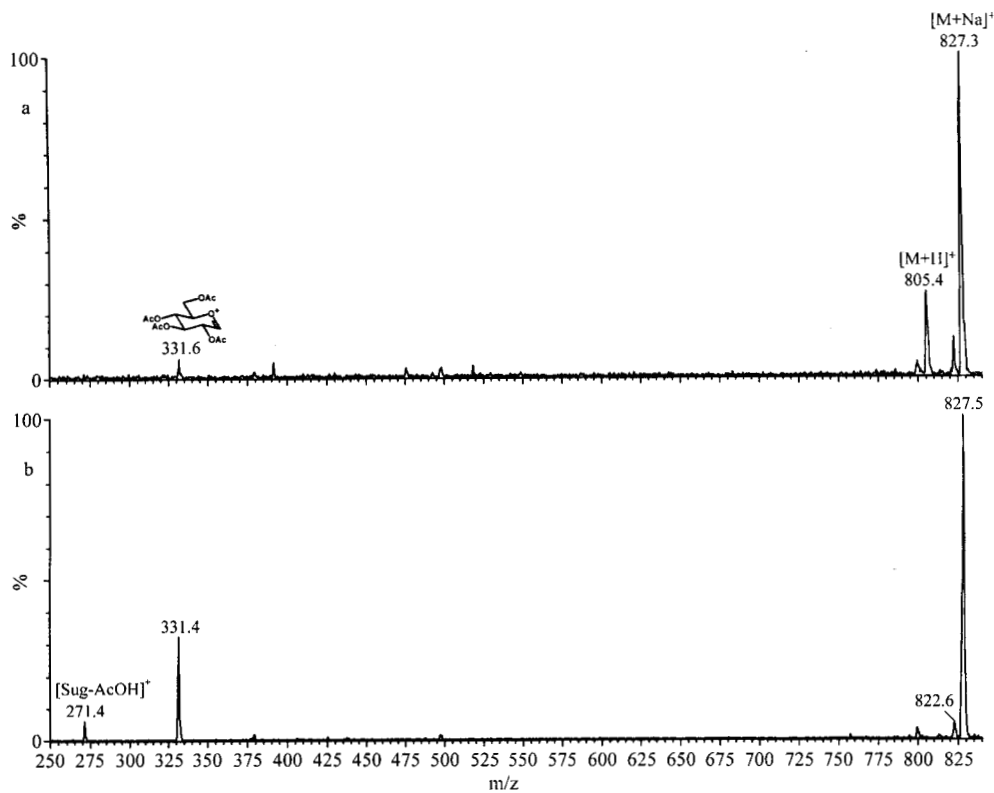


Figure 3. Electrospray mass spectra of the unsaturated bolaform (E,Z)-di-Q-(2,3,4,6-tetra-Q-acetyl-β-D-glucopyranosyloxy)-1,8-oct-4-ene 2 recorded with a cone voltage of a) 30 V and b) 40 V.

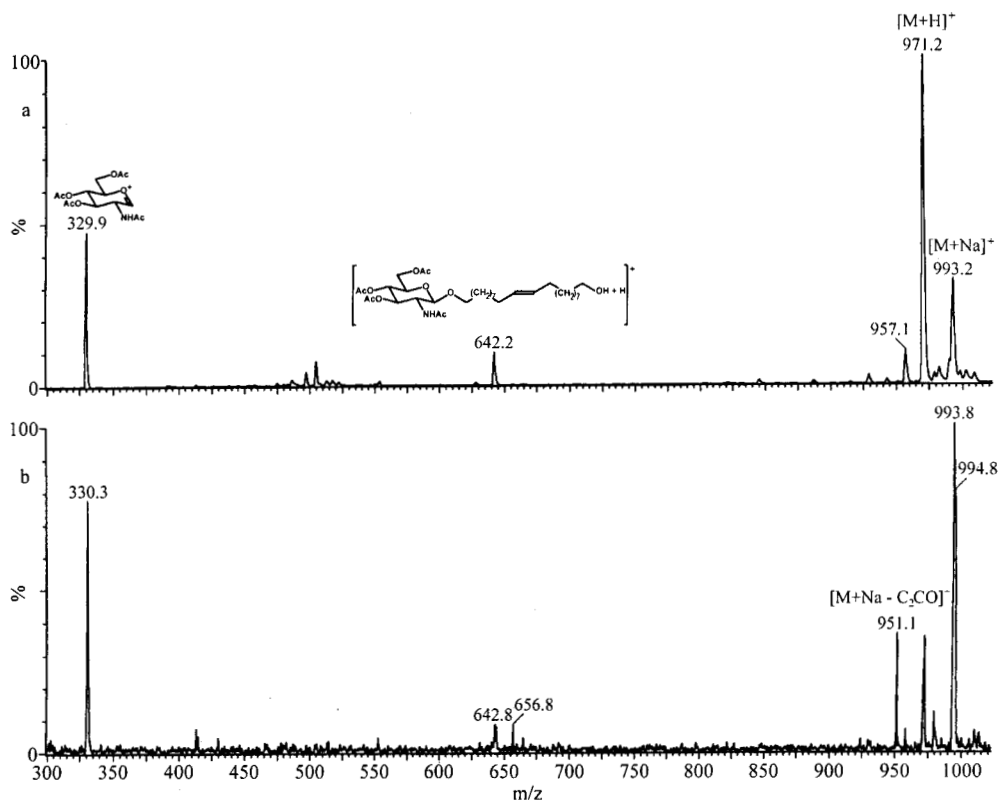


Figure 4. Electrospray mass spectra of the unsaturated bolaform (E,Z)-di-Q-(2-acetamido-3,4,6-tri-Q-acetyl-2-deoxy-β-D-glucopyranosyloxy)-1,20-cis-10-ene 3 recorded with a cone voltage of a) 30 V and b) 40 V.

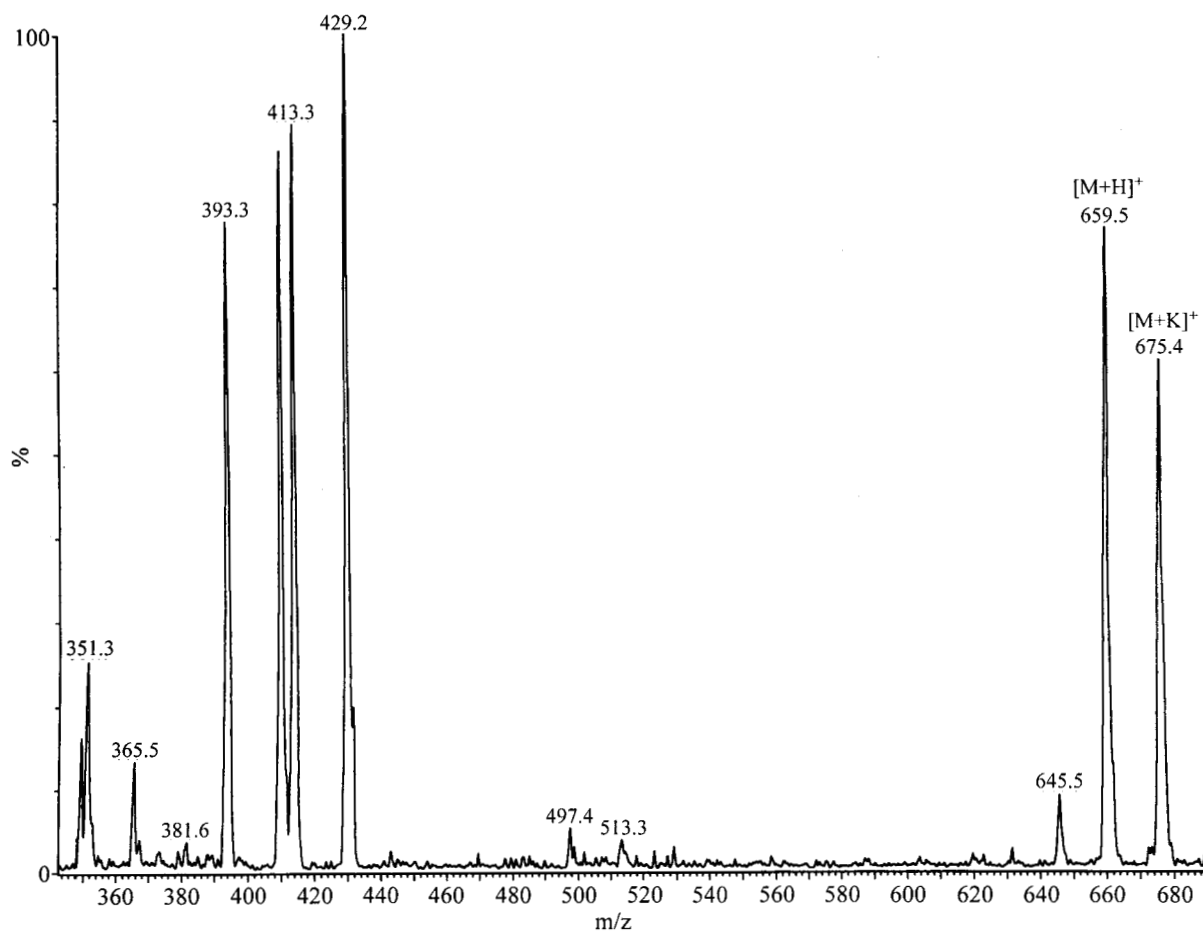


Figure 5. Electrospray mass spectrum of the unsaturated bolaform (E,Z)-di-Q-(β -D-glucopyranosyl)-1,20-icos-10-ene 4.

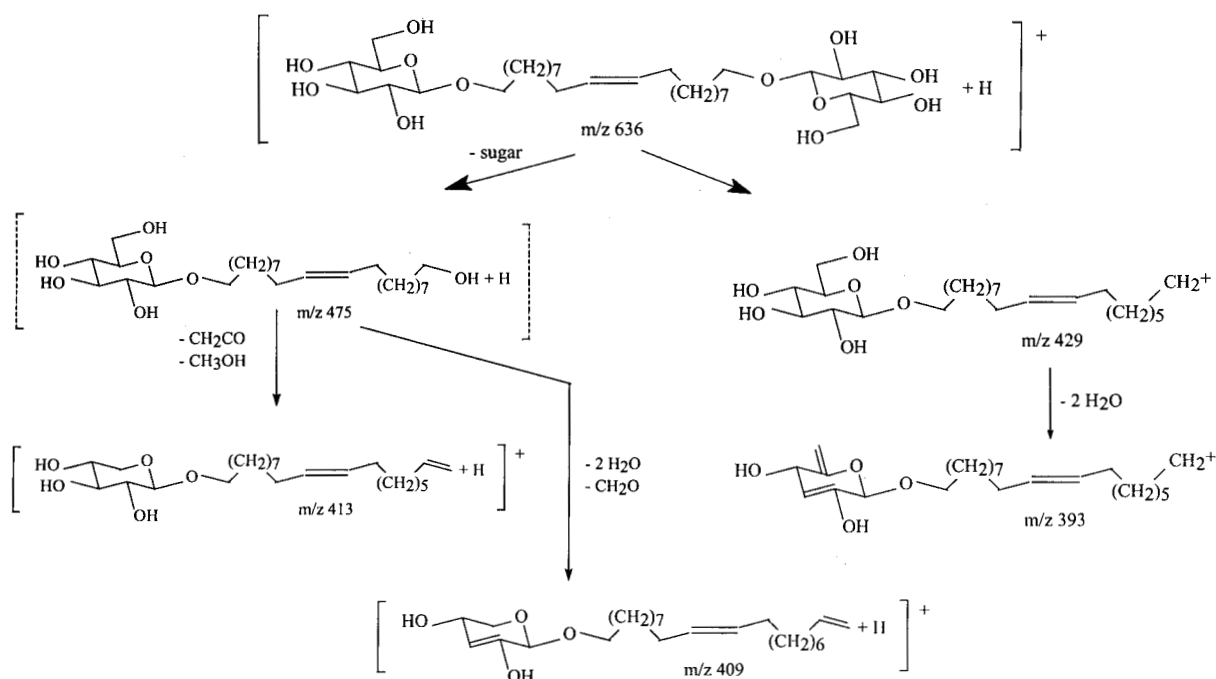


Figure 6. Proposed fragmentation routes of the $[M+H]^+$ ion of 5.

sodiated ion $[M+Na]^+$ at m/z 639 and the potatiated ion $[M+K]^+$ at m/z 675 and a series of derived fragment ions whose respective chemical structures are tentatively assigned in Fig. 6.

It should be noted that the sodiated adducts $[M+Na]^+$ of the unsaturated bolaforms **1-4** were selected for the recording of product ions formed either by the unimolecular or collisionally activated MS/MS dissociations. Unfortunately, CAD MS/MS of the precursor $[M+Na]^+$ ions did not fragment to the expected product ions. This could be due to the resistance of these precursor ions to fragmentation which, in turn, could be attributed to the low energy imparted to these very stable unsaturated amphiphilic molecules. Indeed, CAD MS/MS are usually characterized by low collision energy being imparted to the precursor ions which is fundamental to MS/MS behaviour in triple quadrupole experiments.

In conclusion, ES MS obtained for this novel series of unsaturated neutral carbohydrate bolaforms **1-4** provided considerable information and permitted the characterization of the expected molecular structures. In addition, ES MS recorded with different cone voltages allowed the rationalization of the fragmentation patterns.

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