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Electrospray Tandem Mass Spectrometry of a Novel Series of Amphipathic Functionalized Ether-linked Di- and Trisaccharides and Cyclic Oligosaccharides†

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Electrospray mass spectrometry (ESMS) has aided the structural characterization of a novel series of amphipathic functionalized ether-linked di- and trisaccharides, composed of units of alkyl derivatives of glucofuranose and either units of glucofuranose or diacetylgalactose. The structural elucidation of a novel eight-membered macrocyclic ether-linked disaccharide and an 11-membered macrocyclic ether-linked trisaccharide was also effected using ESMS. Low-energy collision-induced dissociation MS/MS analysis of the $[M + H]^+$ precursor ions confirmed the characteristic fingerprint patterns obtained in the conventional electrospray spectra and proved to be a specific and very sensitive method for the detection and characterization of these novel amphipathic molecules.

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INTRODUCTION

Amphiphilic carbohydrate molecules are a large class of compounds which have acquired important biotechnological applications and, consequently, notoriety since the mid-1970s. Their physical, chemical and biochemical properties allow them to behave as surfactants,[1] liquid crystals,[2–4] emulsion stabilizers for the *in vivo* use of synthetic oxygen carriers[5] and liposomes[6] and they can also be used as solvents for membrane proteins[7] or as inducers for asymmetric synthesis[8,9] or separation.[10,11]

A carbohydrate amphipathic molecule possesses two moieties, a hydrophilic head which is usually a sterically compressed mono-, di- or oligosaccharide and a hydrophobic (or lipophilic) aliphatic chain which usually adopts a straight chain configuration (*i.e.* a tail). The hydrophilic heads of the carbohydrate amphipathic molecules are usually derived from either naturally occurring oligosaccharides which contain common *O*-glycosidic linkages or from synthetic ether-linked di- or oligosaccharides. The latter type of linkage is characterized by an enhanced chemical stability compared with more conventional glycosidic bonds. Villa and coworkers[12,13] have previously described the synthesis of a novel series of functionalized ether-linked di- and trisaccharide substrates formed without a glycosidic function. The structures of some representative members of this novel series are shown in Fig. 1. It should be emphasized that the ether linkages between the alkyl chain and the carbohydrate part forming these molecules were resistant to severe acid hydrolytic treatment, indicating that these amphipathic molecules were very resilient to harsh acidic conditions. Further studies of the compounds described here are under way to resolve the vesicular and liquid-crystal aspects of these molecules. These novel molecules have also been examined for surface activity and their surface tensions (γ) and critical micellar concentrations (CMC) were measured in water.[14] The macrocyclic ether-linked oligosaccharides **4** and **5** are currently being assessed as possible chelating agents and could be compared with analogues of cyclodextrins.

To our knowledge, there have been no previous studies on the mass spectrometric characterization of these amphipathic ether-linked compounds. Early attempts to obtain structural information on this novel series of compounds **1**–**5** by conventional mass spectrometry using gas chromatography/mass spectrometry (GC/MS) with either electron impact (EI) ionization or chemical ionization (CI), using methane as reagent gas, failed and did not allow molecular mass determination.

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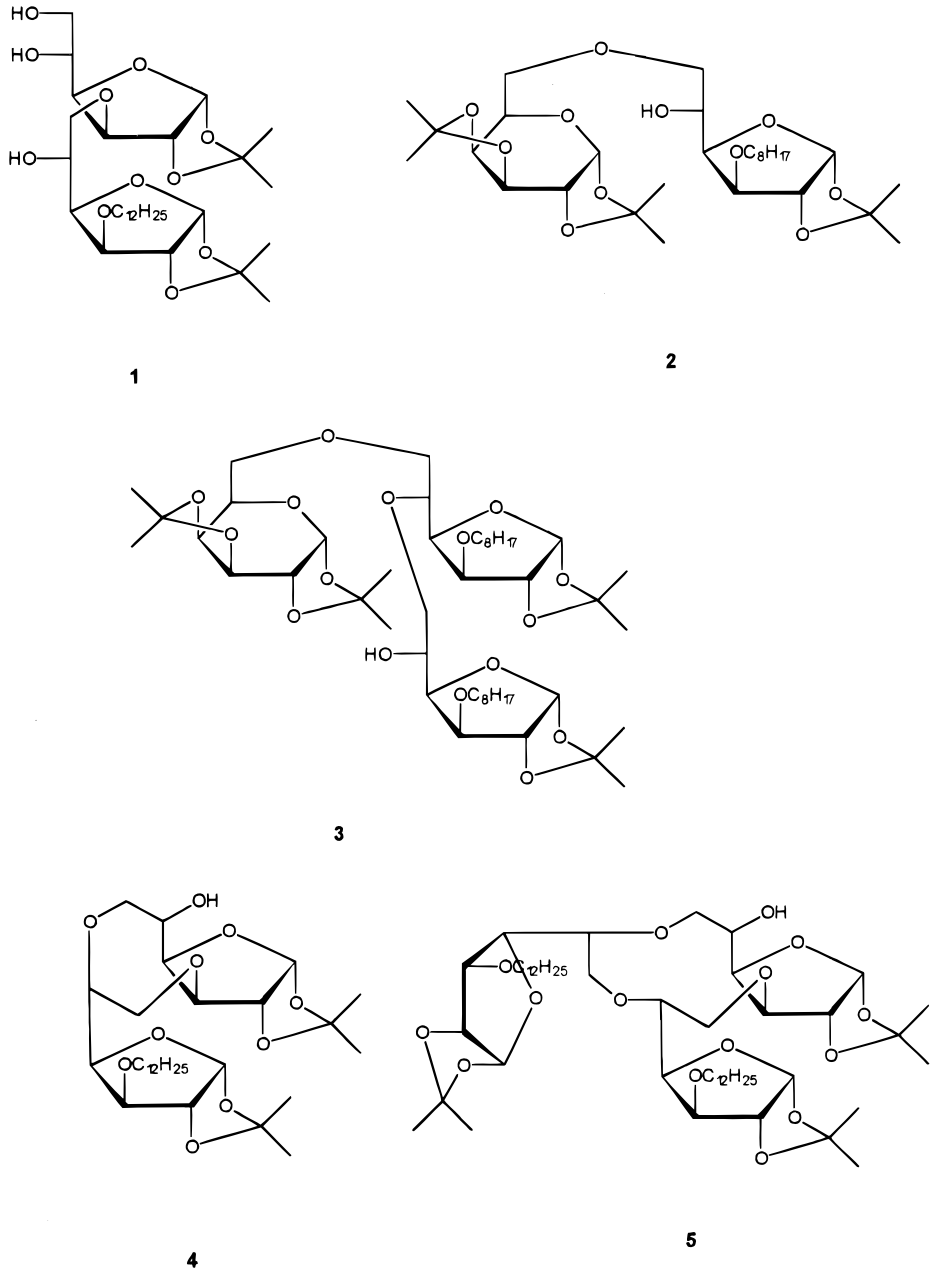

Figure 1. Structures of amphipathic functionalized ether-linked di-and trisaccharides **1**–**5**.

Although the mass spectra obtained contained neither the expected molecular ions nor the protonated molecular ions, some diagnostic fragment ions were observed.

We have opted to use electrospray mass spectrometry (ESMS) since this is, arguably, one of the softest ionization methods, which requires neither derivatization nor excessive manipulation of the analytes. Electrospray ionization is well established as a robust technique for use with combined liquid chromatography/mass spectrometry (LC/MS), which allows the rapid, and sensitive analysis of a wide range of analytes from low molecular mass polar compounds (<200 Da) to biopolymers >100 kDa.[15]

As a continuation of our interest in the MS and MS/MS of bioactive molecules,[16–21] we now report on the structural characterization of the amphipathic ether-linked, non-glycosidic carbohydrates **1**–**5** using ESMS. Structural information was also derived from low energy MS/MS analysis of diagnostic fragments derived from the protonated precursor molecules.

EXPERIMENTAL

The A—O—B disaccharides of the type A(6 → *n*)B, namely 6-*O*-(3-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranos-3-yl)-3-*O*-dodecyl-1,2-*O*-isopropylidene-α-D-glucofuranose (**1**) and 6-*O*-(6-deoxy-1,2,3,4-di-*O*-isopropylidene-α-D-glucofuranos-6-yl)-1,2-*O*-isopropylidene-3-*O*-octyl-α-D-glucofuranose (**2**), obtained by linking the D-glucose derivative (A) with either a D-glucose or D-galactose derivative (B), were synthesized according to the method reported by Villa and co-

workers,[12,13] the key step being the nucleophilic attack of a monosaccharide alkoxyde on the C-6 site of 3-*O*-alkyl-5,6-anhydro-1,2-*O*-isopropylidene-α-D-glucofuranose. Each reaction was performed in toluene–DMSO using KOH as the base. The synthesis of the eight-membered ether-linked macrocyclic disaccharide 5,6-*O*-(3,6-dideoxy-1,2-*O*-isopropylidene-α-D-glucofuranos-3-yl)-3-*O*-dodecyl-1,2-*O*-isopropylidene-α-D-glucofuranose (**4**) and the 11-membered ether-linked macrocyclic trisaccharide cyclo-6-*O*-(3,6-dideoxy-1,2-*O*-isopropylidene-α-D-glucofuranos-3,6-diyl)-5-*O*-(5,6-dideoxy-3-*O*-dodecyl-1,2-*O*-isopropylidene-α-D-glucofuranos-5,6-diyl)-3-*O*-dodecyl-1,2-*O*-isopropylidene-α-D-glucofuranose (**5**) has been described elsewhere.[22]

Electrospray mass spectra were obtained using an API III triple-quadrupole mass spectrometer (SCIEX, Thornhill, Ontario, Canada) equipped with an atmospheric pressure ionization (API) source operated in the ionspray mode. A Macintosh Quadra 950 computer was used for data acquisition and data processing. Samples were admitted to the mass spectrometer by flow injection of 0.25 μl of the analyte solution into a stream of aqueous acetonitrile introduced at a flow rate of 10 μl min^{-1}. The voltage of the ionspray needle was maintained at 5 kV and the orifice voltage was typically 100 V, since this had been predetermined to be the optimum orifice voltage for the production of the protonated molecules. A 0.6 l min^{-1} flow of high purity air was used as a nebulizing gas.

Tandem mass spectrometric experiments were performed using the API III instrument. Fragment ion spectra of mass-selected ions were induced by collisions with argon in the second (RF-only) quadrupole. The resulting fragments were mass analyzed by the third quadrupole. The target thickness was typically 3×10^{14} atoms cm^{-2} and collision energies of ~100 eV (laboratory frame) were used in all MS/MS experiments.

RESULTS AND DISCUSSION

The electrospray mass spectrum (positive ion mode) of the ether-linked disaccharide (**1**), shown in Fig. 2, was characterized by an abundant protonated molecule $[M + H]^+$ at *m/z* 591 and a major series of fragment ions derived from the $[M + H]^+$ ion. Low-energy MS/MS analyses were conducted to rationalize the pathways leading to the various fragmentations observed in the conventional electrospray mass spectrum of **1**. The product ion spectrum arising from the fragmentation, of *m/z* 591 in the RF-only quadrupole collision cell is presented in Fig. 3. The connectivity between fragment ions was established using MS/MS analyses of precursor and fragment ions. The CID tandem mass spectrum of the ion at *m/z* 591, shown in Fig. 3, suggested the formation of a series of product ions whose formation has been tentatively rationalized in Fig. 4.

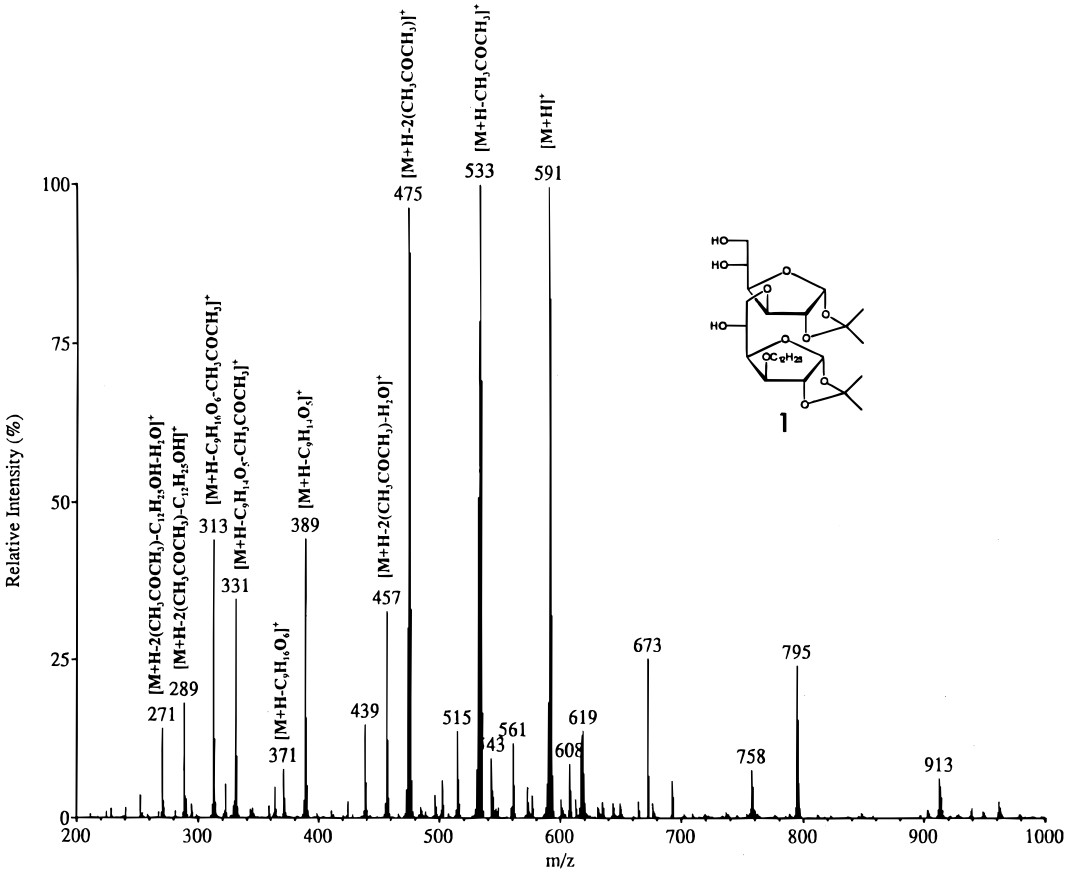

Figure 2. Electrospray mass spectrum (positive ion mode) of the ether-linked disaccharide 6-*O*-(3-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranos-3-yl)-3-*O*-dodecyl-1,2-*O*-isopropylidene-α-D-glucofuranose (**1**).

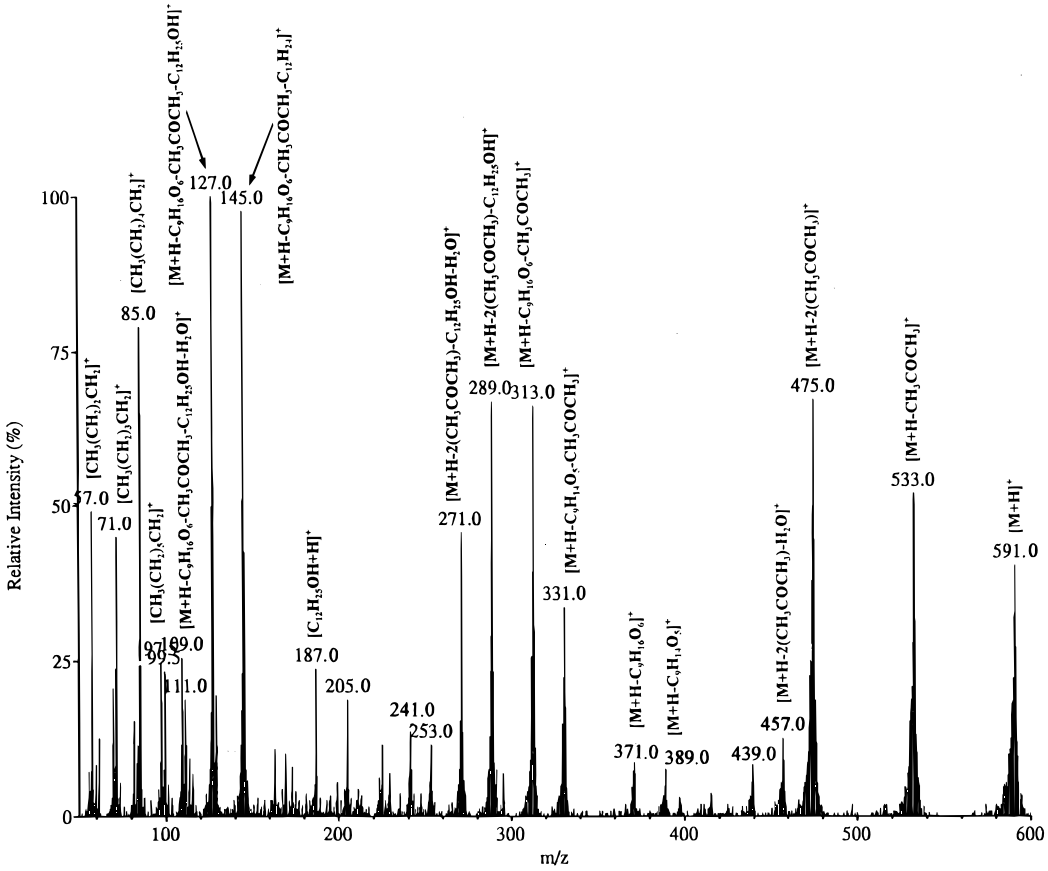

Figure 3. Low-energy CID tandem mass spectrum of the $[M + H]^+$ ion at *m/z* 591 obtained from the ether-linked disaccharide 6-*O*-(3-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranos-3-yl)-3-*O*-dodecyl-1,2-*O*-isopropylidene-α-D-glucofuranose (**1**).

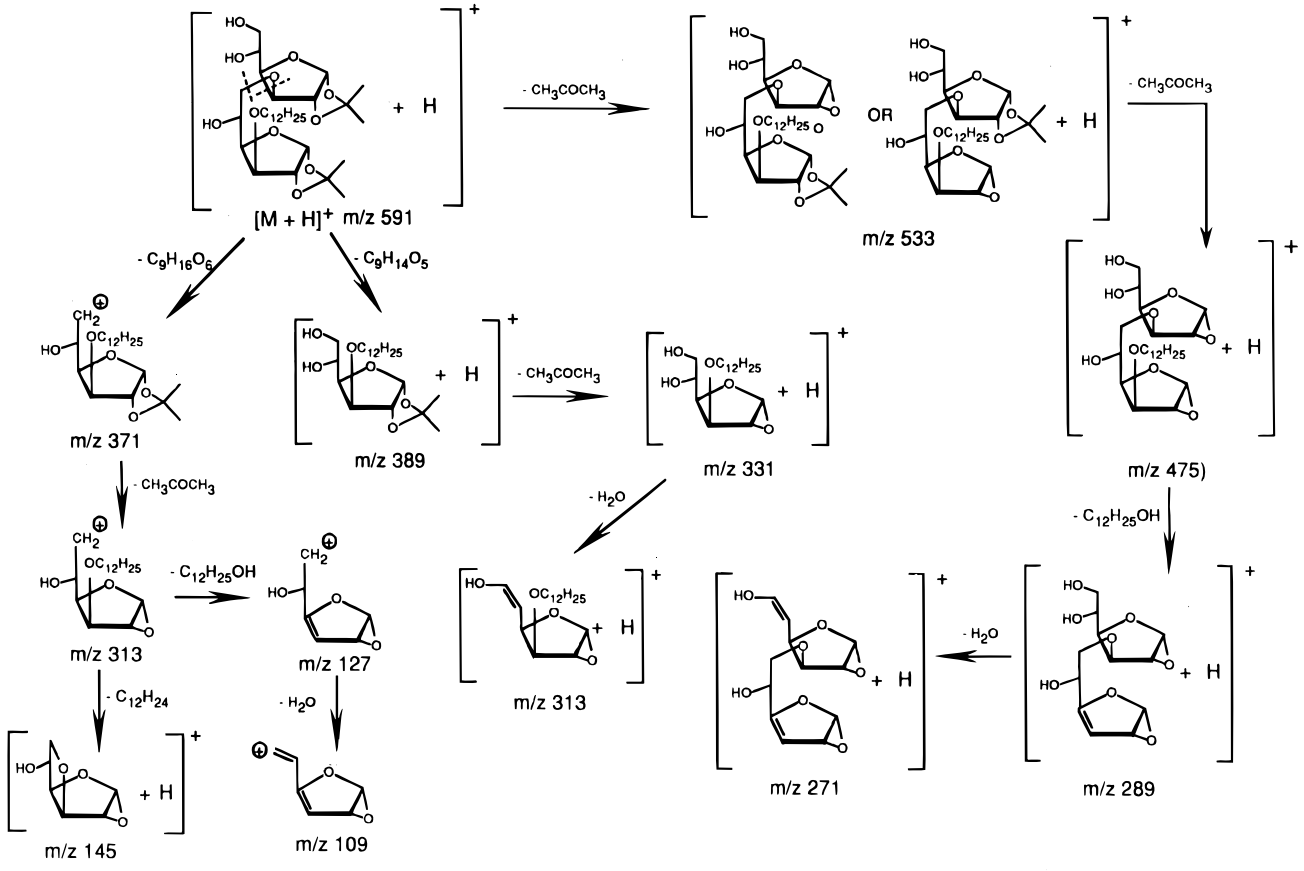

Figure 4. Proposed major fragmentation routes of the $[M + H]^+$ ion at *m/z* 591 from **1** observed during the CID MS/MS experiment.

The formation of this series of fragment ions appears to be governed by three different major routes of fragmentation (Fig. 4). The precursor ion at *m/z* 591 first loses a molecule of acetone, either from the 1,2-*O*- or 1′,2′-*O*-isopropylidene portion of the molecule, to form the epoxide ion at *m/z* 533, which, in turn, loses another molecule of acetone to form the diepoxide ion at *m/z* 475. It should be noted that the presence of the two isopropylidene acetal groups with the attendant possibility of elimination of either one or two molecules of acetone complicates the possible order of their elimination. The elimination of the alkyl chain as a neutral $C_{12}H_{25}OH$ molecule from the ion at *m/z* 475 affords the unsaturated diepoxide ion at *m/z* 289, which, in turn, loses a molecule of water to produce the di(unsaturated epoxide) ion at *m/z* 271 (Fig. 3). It is noteworthy that the formation of this series of fragment ions usually occurs by losses of neutral molecules in either a concerted or a consecutive fashion.

Another fragmentation route, which leads to a different series of fragment ions, is governed by the heterolytic cleavage of the $C_{3'}$—O_6 portion of the $C_{3'}$—O_6—C_6 ether bond, with the transfer of a hydrogen atom to the glucose moiety containing the alkyl chain to form the fragment ion at *m/z* 389. This latter fragment ion loses a molecule of acetone to afford the ion $[M + H - C_9H_{14}O_5 - CH_3COCH_3]^+$ at *m/z* 331, which, in turn, loses a molecule of water to form the ion at *m/z* 313 (Fig. 4). Heterolytic cleavage of the O_6—C_6 portion of the $C_{3'}$—O_6—C_6 ether linkage, with loss of the neutral 1,2-*O*-isopropylidene-α-D-glucofuranose, affords the carbocation $[M + H - C_9H_{16}O_6]^+$ product ion at *m/z* 371. This latter ion loses a molecule of acetone to afford the fragment ion at *m/z* 313. The product ion at *m/z* 313 fragments further by loss of the unsaturated alkyl chain $C_{12}H_{24}$ to afford the 2,6-anhydro-1,2-epoxide ion at *m/z* 145. The same product ion at *m/z* 313 may lose its neutral side chain as a molecule of $C_{12}H_{25}OH$ to afford the derived carbocation at *m/z* 127, which, in turn, loses a molecule of water to afford the fragment ion at *m/z* 109.

A mixture of amphipathic, functionalized, ether-linked disaccharide **2** and trisaccharide **3**, obtained during the synthesis of A—O—B disaccharide of the type A(6 → *n*)B, proved to be difficult to purify by conventional methods. The ESMS of this mixture of **2** and the trisaccharide 6-*O*-[6-*O*-(6-deoxy-1,2,3,4-di-*O*-isopropylidene-α-D-galactopyranos-6-yl)-5-deoxy-1,2-*O*-isopropylidene-3-*O*-octyl-α-D-glucofuranos-5-yl]-1,2-*O*-isopropylidene-3-*O*-octyl-α-D-glucofuranose (**3**) was recorded and is shown in Fig. 5. The ES mass spectrum of **2** and **3** contained the protonated molecules at *m/z* 575 and 890 for the respective disaccharide and trisaccharide components and afforded a series of diagnostic fragment ions originating from these two protonated molecules (Fig. 5). Characterization of these two protonated molecules was effected using low-energy collision-induced dissociation (CID) MS/MS experiments.

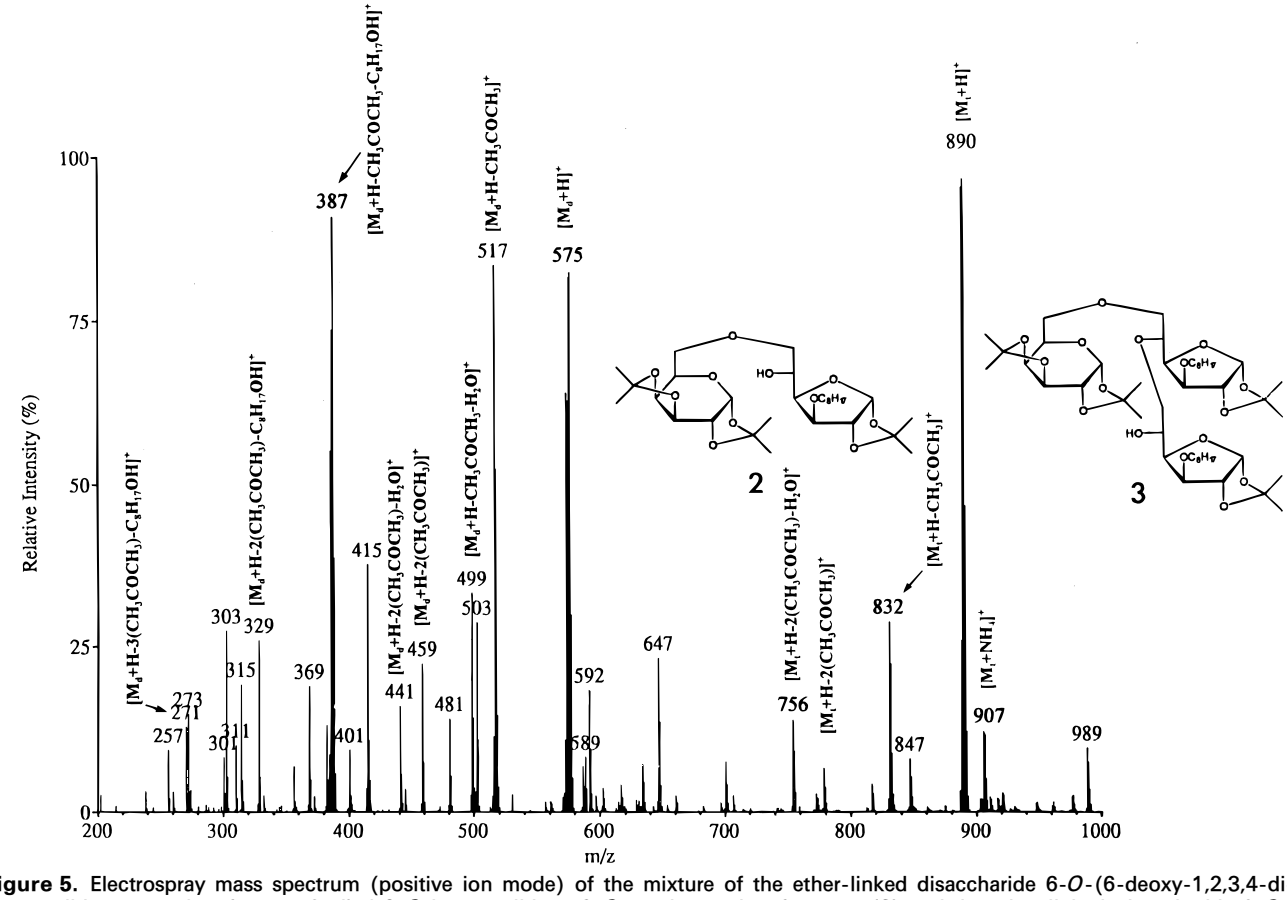

Figure 5. Electrospray mass spectrum (positive ion mode) of the mixture of the ether-linked disaccharide 6-*O*-(6-deoxy-1,2,3,4-di-*O*-isopropylidene-α-D-glucofuranos-6-yl)-1,2-*O*-isopropylidene-3-*O*-octyl-α-D-glucofuranose (**2**) and the ether-linked trisaccharide 6-*O*-[6-*O*-(6-deoxy-1,2,3,4-di-*O*-isopropylidene-α-D-galactopyranos-6-yl)-5-deoxy-1,2-*O*-isopropylidene-3-*O*-octyl-α-D-glucofuranose-5-yl]-1,2-*O*-isopropylidene-3-*O*-octyl-α-D-glucofuranose **3**.

The CID tandem mass spectrum of the precursor ion $[M + H]^+$ at *m/z* 575 is shown in Fig. 6. The proposed fragmentation route of the protonated molecule obtained from disaccharide **2** by the CID MS/MS experiment has been rationalized and the assignment of fragment ion structures is presented in Fig. 7. The fragmentation of the disaccharide protonated molecular ion $[M + H]^+$ at *m/z* 575 produces the fragment ion $[M + H - CH_3COCH_3]^+$ at *m/z* 517 by the loss of an acetone molecule. The latter product ion loses the alkyl chain as a neutral molecule of $C_8H_{17}OH$ to afford the fragment ion at *m/z* 387. Similarly, the ion at *m/z* 517 can lose a neutral molecule of 1,2,3,4-di-*O*-isopropylidene-α-D-galactopyranose to afford the carbocation fragment at *m/z* 257, which fragments further through loss of H_2O and the alkyl chain (Fig. 7). The precursor ion at *m/z* 575 loses, concertedly, two molecules of acetone to afford the fragment ion at *m/z* 459, which dissociates further via losses of $C_8H_{17}OH$, acetone and water to yield the fragment ions at *m/z* 329, 271 and 253, respectively. The heterolytic cleavage of the $C_{6'}$—O_6 portion of the $C_{6'}$—O_6—C_6 bond of the precursor ion at *m/z* 575 affords the fragment ion at *m/z* 261 corresponding to the 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose ion, which fragments further by loss of a molecule of acetone, followed by a molecule of water.

The $[M + H]^+$ ion at *m/z* 890, obtained by ESMS of the mixture of **2** and **3** (Fig. 5), was selected for the CID MS/MS experiment. The corresponding product ion spectrum is again dominated by characteristic losses of molecules of H_2O, alkyl chain and acetone and is shown in Fig. 8. The fragmentation routes of the protonated molecules obtained from trisaccharide **3** by CID MS/MS of the protonated molecule $[M + H]^+$ at *m/z* 890 is tentatively depicted in Fig. 9. Although the type of fragmentation indicated is similar to those observed in the previous two CID MS/MS of **1** and **2**, the fragmentation route of **3** appears to be governed by different mechanisms. The precursor ion $[M + H]^+$ first loses two molecules of acetone and a molecule of water in a concerted fashion to afford the product ion at *m/z* 756. In a separate pathway, the precursor ion at *m/z* 890 dissociates via a heterolytic cleavage of the $C_{6'}$—O_6—C_6 ether linkage at the O_6—C_6 bond followed by elimination of a neutral molecule of 1,2,3,4-di-*O*-isopropylidene-α-D-galactofuranose and two neutral molecules of $C_8H_{17}OH$ to afford the disaccharide carbocation containing two double bonds at *m/z* 370. The latter fragment ion dissociates further, as indicated in Fig. 9. Finally, a double heterolytic cleavage of the $C_{6''}$—O_6—C_6 and $C_{6'}$—O_5—C_5 ether linkage bonds with a transfer of a hydrogen atom affords the major fragment ion at *m/z* 315, which undergoes subsequent cleavages to yield a series of second generation fragment ions as shown in Fig. 9.

The ESMS of another novel member of this series, the ether-linked eight-membered macrocyclic disaccharide **4**, was recorded and is shown in Fig. 10. As expected, this ES mass spectrum contained the proto-

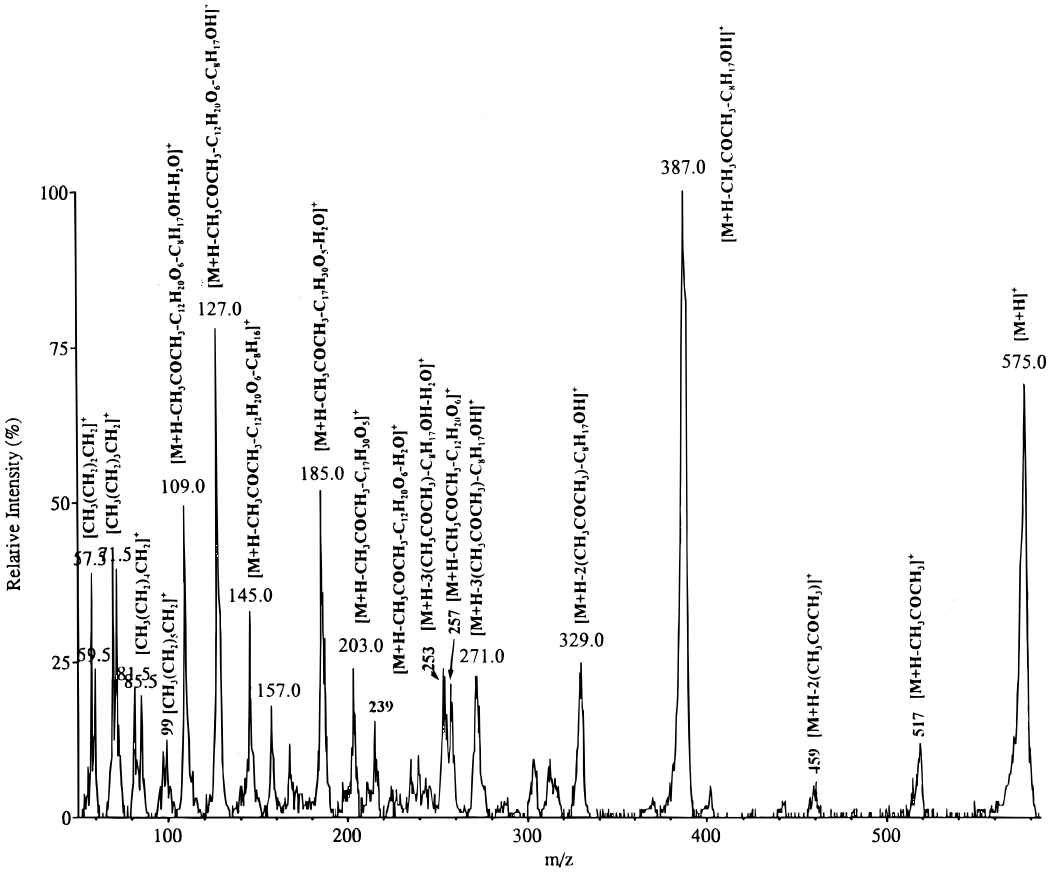

Figure 6. Low-energy CID tandem mass spectrum of the $[M + H]^+$ ion at *m/z* 575 obtained from the ether-linked disaccharide 6-*O*-(6-deoxy-1,2,3,4-di-*O*-isopropylidene-α-D-glucofuranos-6-yl)-1,2-*O*-isopropylidene-3-*O*-octyl-α-D-glucofuranose (**2**).

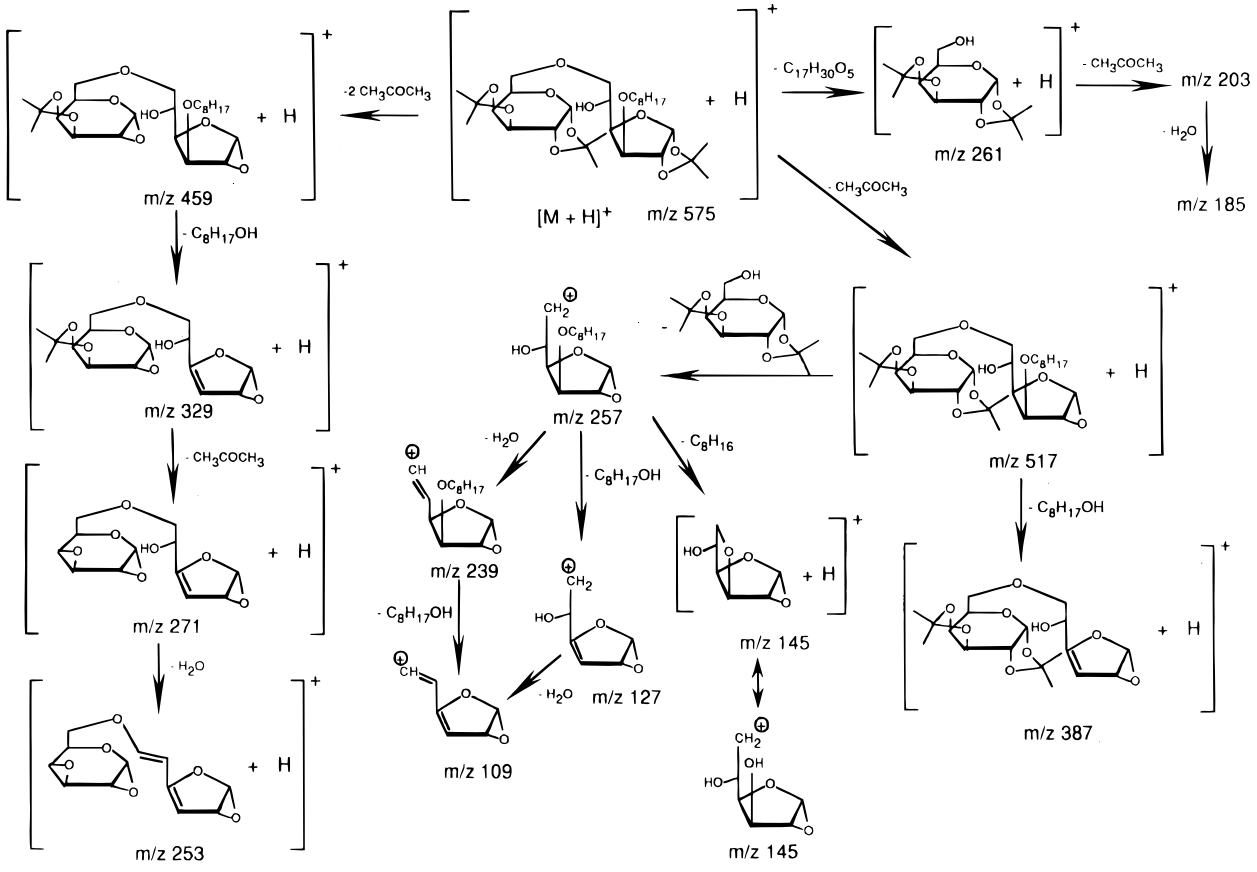

Figure 7. Proposed major fragmentation routes of the $[M + H]^+$ ion at *m/z* 575 from **2** observed during the CID MS/MS experiment.

nated molecule $[M + H]^+$ at *m/z* 573 and afforded a series of diagnostic fragment ions arising from the protonated molecule. The CID tandem mass spectrum of the protonated molecule $[M + H]^+$ at *m/z* 573 (Fig. 11) afforded a series of fragment ions, and the rationalization of the observed fragmentation is tentatively described in Fig. 12. Although a wealth of structural information is obtained from this CID tandem mass spectrum, it is very difficult to establish the genealogy of the product ions produced. In particular, the spectrum is complicated by ions arising from a series of multiple fragmentations which yield intermediate ions of very low abundances. Indeed, the precursor ion $[M + H]^+$ at *m/z* 573, under CID MS/MS conditions, can form different series of diagnostic product ions by four different pathways. For example, the precursor ion at *m/z* 573 can lose one molecule of water to form the product ion at *m/z* 555 which, in turn, loses a molecule of acetone to afford the fragment ion at *m/z* 597, which fragments further by loss of another molecule of acetone to afford the fragment ion at *m/z* 439. It is noteworthy that the precursor ion at *m/z* 573 can lose, by a concerted mechanism, two molecules of acetone and a molecule of water (not necessarily in that order) to afford the ion at *m/z* 439 (Fig. 12). An additional pathway of fragmentation occurs by loss of two acetone molecules from the precursor ion to afford the ion at *m/z* 457, which subsequently proceeds via double cleavage of the eight-membered macrocyclic ring to afford either a carbocation or a protonated epoxide fragment ion at *m/z* 313. The fourth type of fragmentation pathway occurs by concerted losses of two molecules of acetone and a neutral molecule of $C_{12}H_{25}OH$ originating from the alkyl chain of the precursor ion to afford the ion at *m/z* 271 (Fig. 12).

The intramolecular cyclization of the ether-linked trisaccharide 3-*O*-dodecyl monoacetone glucose (6 → 5')-3'-*O*-dodecyl monoacetoneglucose (6' → 3')-5',6'-anhydro monoacetoneglucose, in the presence of traces of KOH in DMSO–toluene afforded a low yield of a mixture of two compounds which could not be separated by chromatographic methods.[21] This mixture was introduced into the mass spectrometer and electrosprayed and the resulting ES mass spectrum is shown in Fig. 13. The peak at *m/z* 944 corresponds to the protonated molecule of the expected ether-linked 11-membered macrocyclic trisaccharide **5**. The peak at *m/z* 673 is a reaction by product of the synthetic reaction, and further investigation will be required to establish the structure of this fragment ion. The low-energy CID tandem mass spectrum of the protonated molecule $[M + H]^+$ at *m/z* 944 of the macrocyclic trisaccharide is shown in Fig. 14. The structures of the product ions observed in Fig. 14 are tentatively assigned in Fig. 15. The low-energy collision MS/MS of the precursor ion of **5** shows straightforward and simple rearrangement pathways similar to those of **1-4**. Therefore, in the CID tandem mass spectrum of the protonated molecule $[M + H]^+$ at *m/z* 944, we could only decipher three different fragmentation routes. Indeed, the protonated

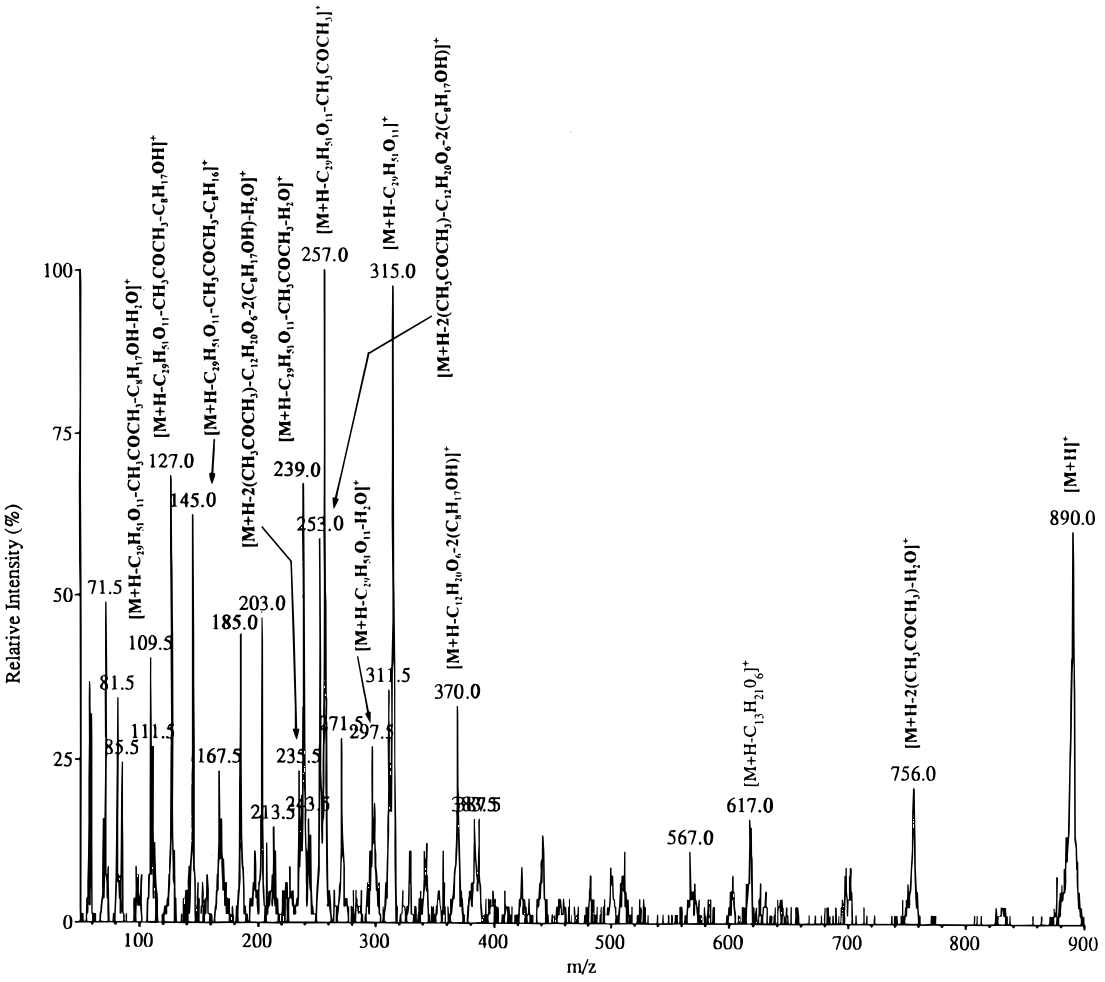

Figure 8. Low energy CID tandem mass spectrum of the $[M + H]^+$ ion at *m/z* 890 obtained from the ether-linked trisaccharide 6-*O*-[6-*O*-(6-deoxy-1,2,3,4-di-*O*-isopropylidene-α-D-galactopyranos-6-yl)-5-deoxy-1,2-*O*-isopropylidene-3-*O*-octyl-α-D-glucofuranose-5-yl]-1,2-*O*-isopropylidene-3-*O*-octyl-α-D-glucofuranose (**3**).

molecule at *m/z* 944 may lose either one, two or three molecules of acetone to afford the product ions at *m/z* 886, 828 and 770. The major fragmentation route of this precursor ion seems to be governed by the concerted losses of the three molecules of acetone to afford, perhaps, the unstable product ion at *m/z* 770 (not seen in the CID tandem mass spectrum), which again either eliminates a neutral molecule of the $C_{12}H_{25}OH$ to afford the fragment ion at *m/z* 398, or breaks down to the fragment ion at *m/z* 313 (Fig. 15). The fragment ion at *m/z* 398 eliminates a neutral molecule of $C_6H_8O_4$ to afford the abundant fragment ion at *m/z* 254. Similarly, the abundant fragment ion at *m/z* 313 degrades further to afford the fragment ions at *m/z* 295, 145, 127 and 109 (Fig. 15).

From the examples presented in this rationale, the potential of ESMS/MS for the structural analysis of this novel series of amphipathic ether-linked carbohydrate molecules becomes evident. Also, the presence of isopropylidene acetal groups on these amphipathic carbohydrate molecules seems to direct the ESMS/MS fragmentations. Product ions resulting from losses of acetone initiate some of the principal fragmentation routes. The conventional ESMS fragmentation routes of this series of compounds show some similarities with the isobutane CI fragmentations reported for simple monosaccharides such as 1,2:4,5-di-*O*-isopropylidene-β-D-fructofuranose.[23]

In the MS/MS analysis described, we have not attempted to rationalize the different fragmentation routes of this series of novel compounds by obtaining precursor ion spectra of the various intermediate ions. Assignment of ion structures was based mainly on the interpretation of product ion spectra of selected precursor ions. The elimination sequences of the neutral molecules from the various fragment ions are numerous and their verification would require the synthesis of suitable molecules containing stable ^{13}C and ^{18}O isotopes, and would represent a mammoth task. The aim of this study was to establish the fragmentation routes of this series of novel amphipathic carbohydrate molecules by ESMS. Finally, it is imperative to mention that although the purity of this novel series of synthetic ether-linked carbohydrates **1**, **4** and **5** was established by high-resolution NMR and elemental combustion analysis (i.e. gave correct elemental analysis and NMR spectra consistent with the proposed structures),[22] their respective ES mass spectra showed some low-abundance ions originating from unidentified polymeric reaction by-products. This may well indicate that by the

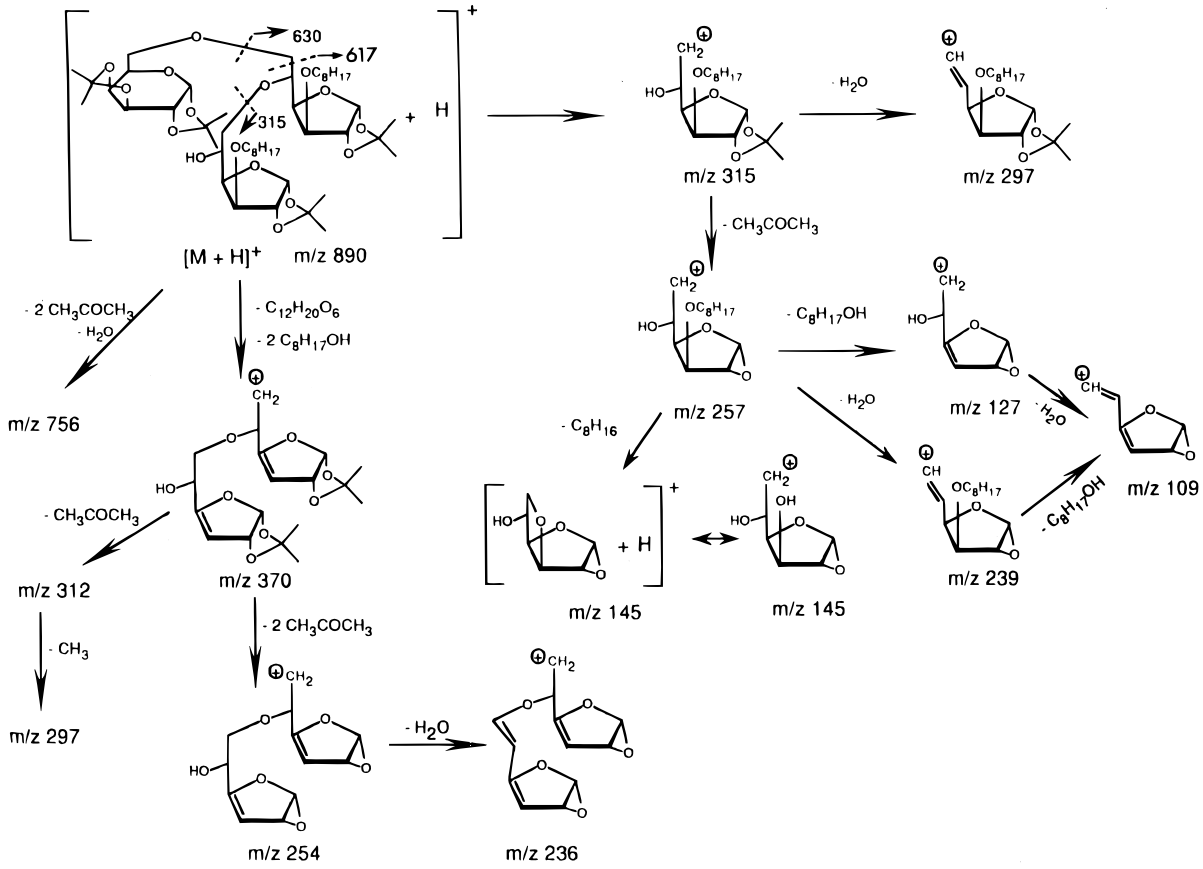

Figure 9. Proposed major fragmentation routes of the $[M + H]^+$ ion at *m/z* 890 from **3** observed during the CID MS/MS experiment.

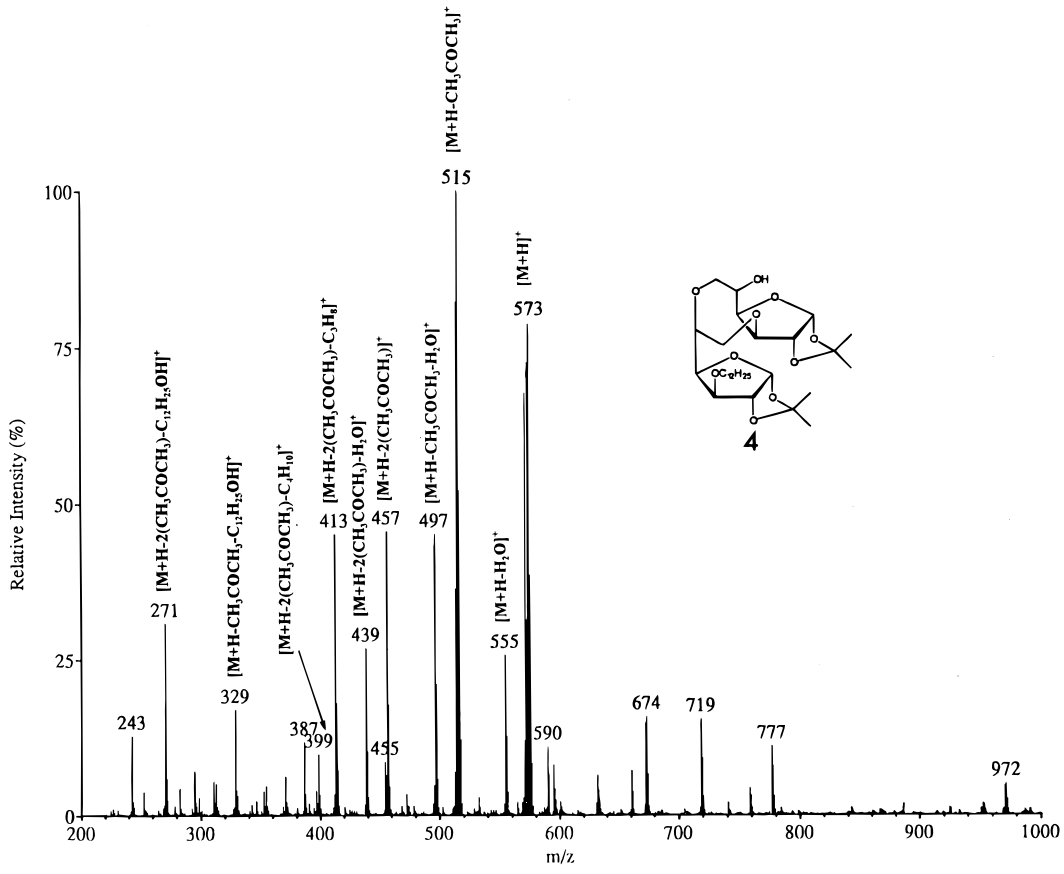

Figure 10. Electrospray mass spectrum (positive-ion mode) of the ether-linked macrocyclic disaccharide 5,6-*O*-(3,6-dideoxy-1,2-*O*-isopropylidene-α-D-glucofuranso-3-yl)-3-*O*-dodecyl-1,2-*O*-isopropylidene-α-D-glucofuranose (**4**).

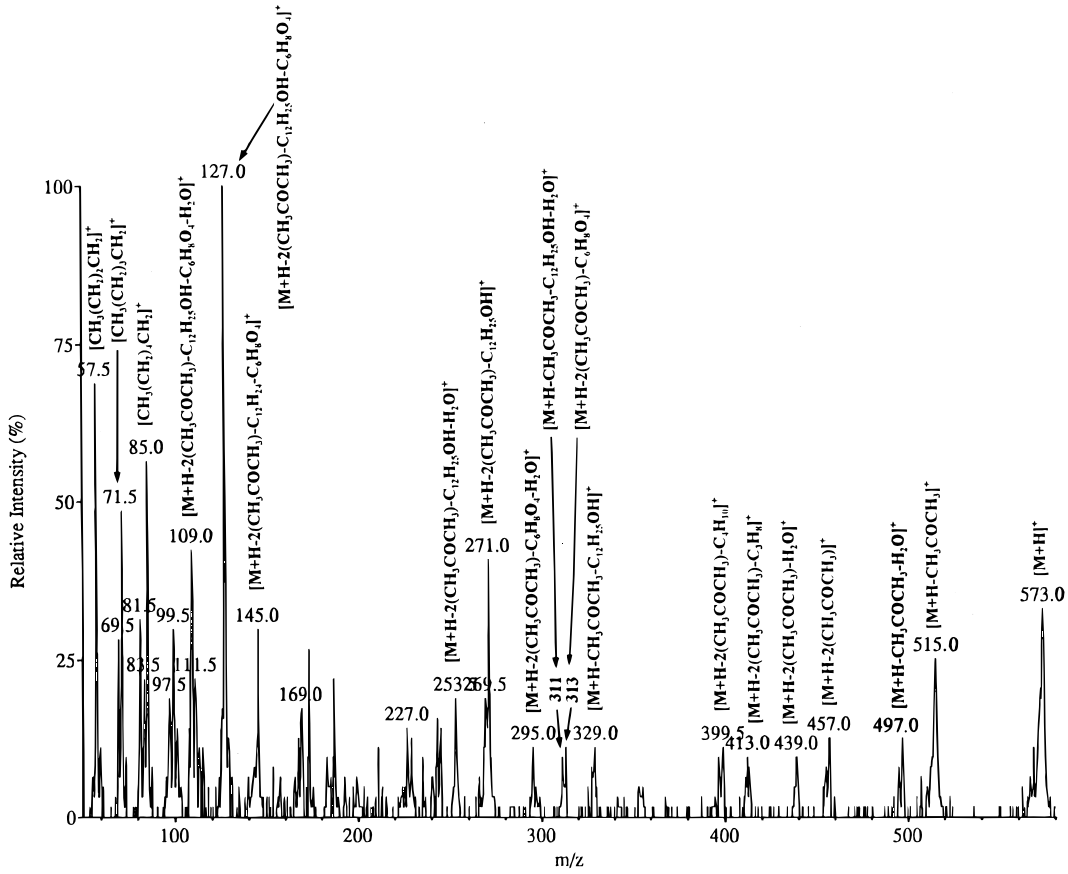

Figure 11. Low-energy CID tandem mass spectrum of the $[M + H]^+$ ion at *m/z* 573 obtained from the ether-linked macrocyclic disaccharide 5,6-*O*-(3,6-dideoxy-1,2-*O*-isopropylidene-α-D-glucofuranos-3-yl)-3-*O*-dodecyl-1,2-*O*-isopropylidene-α-D-glucofuranose, (**4**).

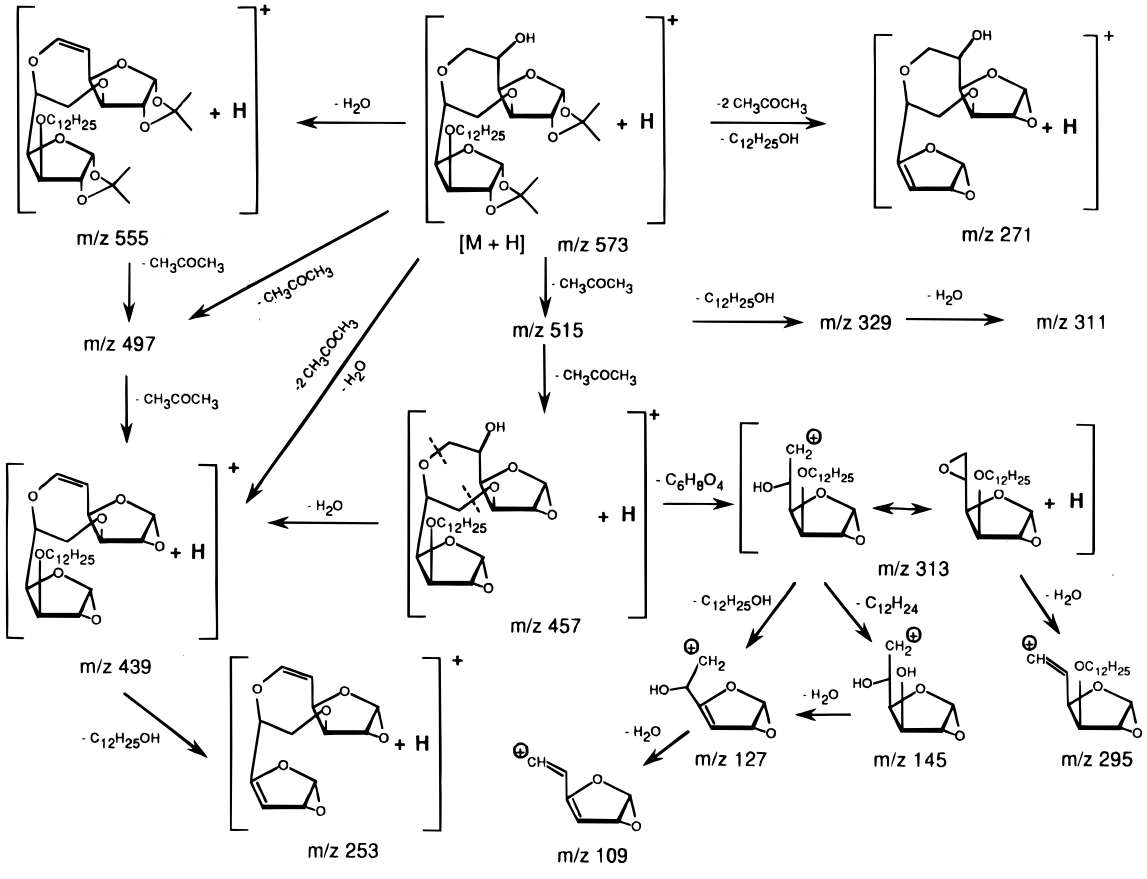

Figure 12. Proposed major fragmentation routes of the $[M + H]^+$ ion at *m/z* 573 from **4** observed during the CID MS/MS experiment.

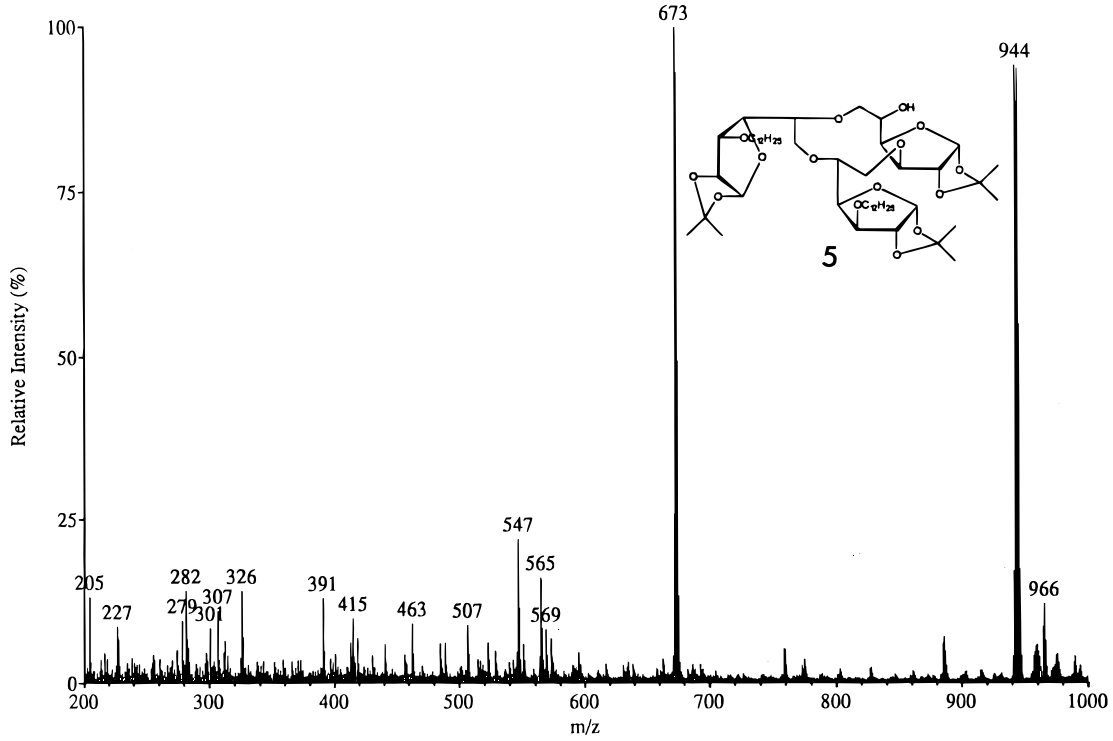

Figure 13. Electrospray mass spectrum (positive-ion mode) of the ether-linked macrocyclic trisaccharide cyclo-6-*O*-(3,6-dideoxy-1,2-*O*-isopropylidene-α-D-glucofuranos-3,6-diyl)-5-*O*-(5,6-dideoxy-3-*O*-dodecyl-1,2-*O*-isopropylidene-α-D-glucofuranos-5,6-diyl)-3-*O*-dodecyl-1,2-*O*-isopropylidene-α-D-glucofuranose (**5**).

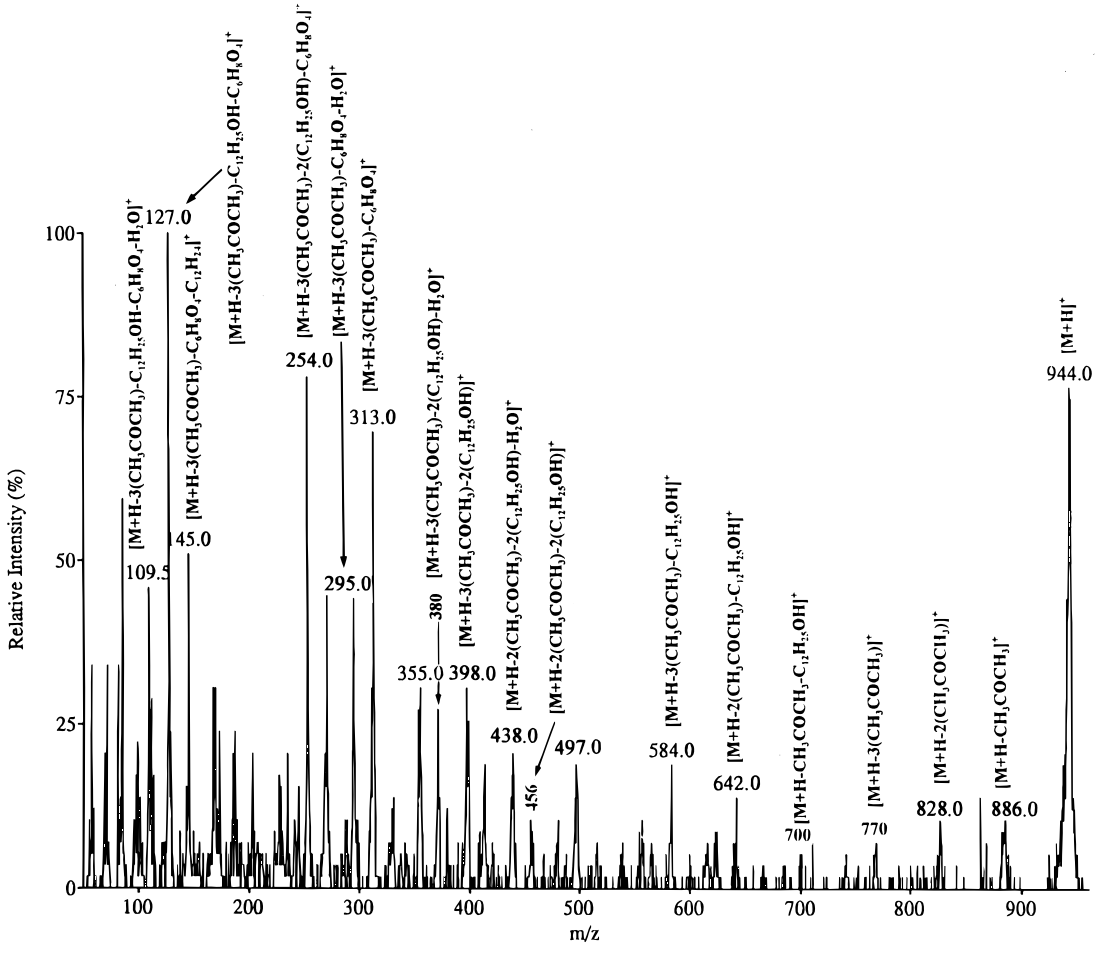

Figure 14. Low-energy CID tandem mass spectrum of the $[M + H]^+$ ion at *m/z* 944 obtained from the ether-linked macrocyclic trisaccharide cyclo-6-*O*-(3,6-dideoxy-1,2-*O*-isopropylidene-α-D-glucofuranos-3,6-diyl)-5-*O*-(5,6-dideoxy-3-*O*-dodecyl-1,2-*O*-isopropylidene-α-D-glucofuranos-5,6-diyl)-3-*O*-dodecyl-1,2-O-isopropylidene-α-D-glucofuranose trisaccharide (**5**).

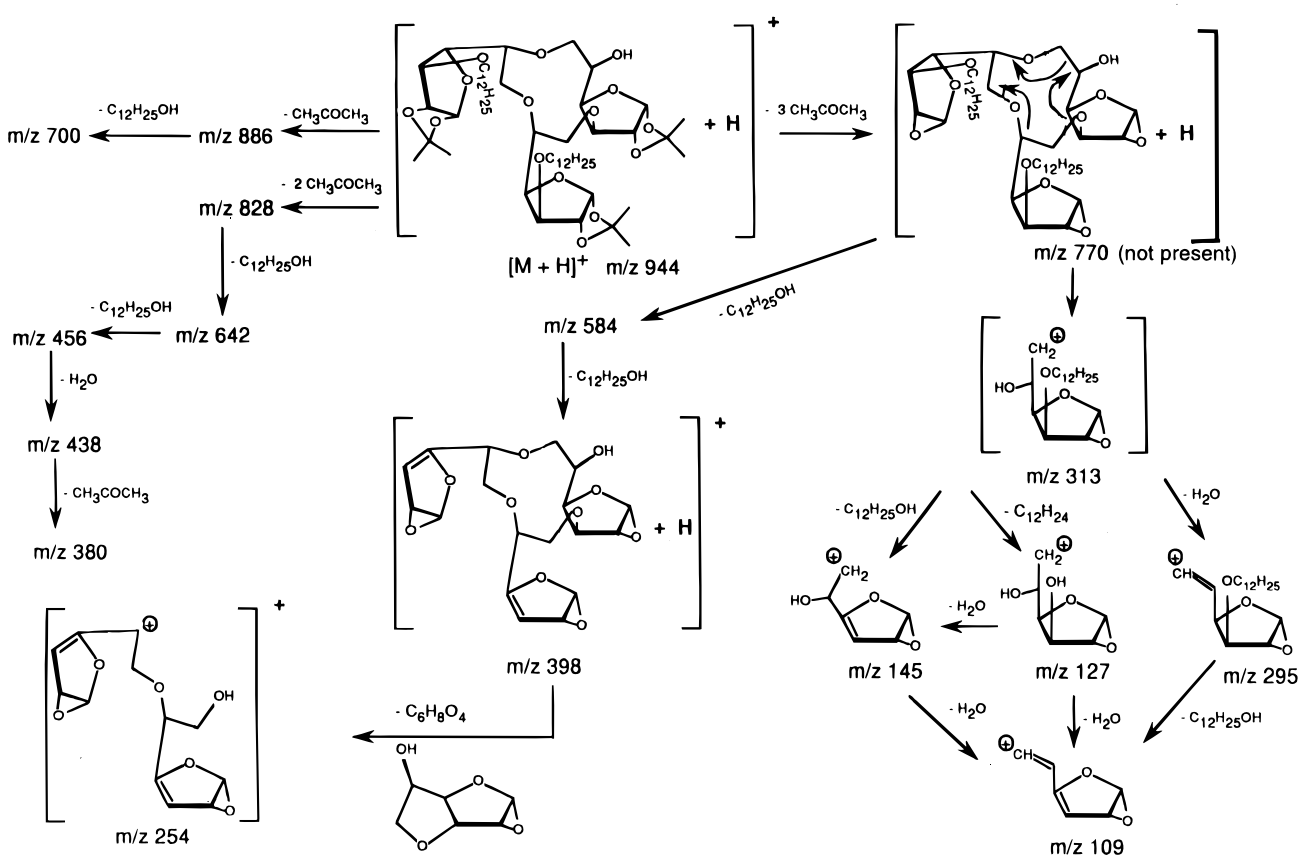

Figure 15. Proposed major fragmentation routes of the $[M + H]^+$ ion at *m/z* 944 from **5** observed during the CID MS/MS experiment.

end of this century, the well accepted paradigm of the 'purity' of synthetic carbohydrates, which is based on NMR in conjunction with elemental combustion analysis, may need to be revised.

CONCLUSION

Preliminary investigation by positive-ion ESMS/MS, using a triple-quadrupole instrument, of this novel series of amphipathic carbohydrate functionalized ether-linked di-and trisaccharide and cyclic oligosaccharides, **1**–**5**, demonstrated that this technique facilitated the characterization of the proposed structures. In addition, MS/MS using low-energy collisional activation of singly protonated precursor ions provided characteristic fingerprints, observed in the conventional electrospray mass spectra of the corresponding compounds, which allowed the proper identification of individual amphipathic carbohydrates in mixtures.

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