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Atmospheric pressure photoionization mass spectrometry of per-O-methylated oligosaccharides related to D-xylans

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ABSTRACT

Three D-xylan type per-O-methylated trisaccharides with various types of linkages between the D-xylopyranose units were examined by atmospheric pressure photoionization (APPI) mass spectrometry in the positive ion mode. The most interesting feature of a thermospray mass spectrum using the APPI source with UV lamp switched off, is the exclusive production of $[M+Na]^+$ adduct ions. $[M+Na]^+$ cationized ions are the most abundant species in the case of APPI mass spectrometry. The second ionization process has no analogy in the case of substances studied using APPI to date. This aspect involves the addition of a water molecule to the molecular ion of a per-O-methylated saccharide, giving rise to $[M+H_2O]^{\cdot+}$ adduct ions. The $[M+H_2O]^{\cdot+}$ species are readily detected at m/z 544, and are clearly visible for all three isomers studied. The MS/MS spectrum of $[M+Na]^+$ ions contains a base peak at m/z 375, produced by a Y-type cleavage of the trisaccharide, along with a hydrogen rearrangement on the terminal interglycosidically linkage glycosidic oxygen atom. The $[M+H_2O]^{\cdot+}$ species fragment largely give rise to ions at m/z 175, 143 and, as a result, the m/z 111 ion is unique to nonreducing terminal units.

1. Introduction

APPI is a relatively recent ionization technique, which had considerable attention in the past few years from the analytical chemistry community. Initially restricted to small hydrophobic molecules, its use was then extended to larger, polar compounds.[1–4] To our knowledge, this technique has not been extended to the study of oligosaccharides.

D-Xylans and D-glucuronoxylans comprise branched polysaccharides with various branching points on the basic linear chain. Mass spectrometric analysis of saccharides has traditionally relied on GC–MS, after a preliminary hydrolysis step.[5] In addition, methylation analysis of *O*-methyl alditol acetates continue to provide basic information concerning the structure of poly- and oligosaccharides.[5] Electron ionization (EI) MS has been used since the early 1970s to analyze methylated mono- and oligosaccharides.[6,7] Chemical ionization (CI) also represents an interesting alternative method.[8,9] Fast atom bombardment mass spectrometry (FAB MS) has also been used at high collision energy.[10] More recently electrospray ionization (ESI) and matrix-assisted laser desorption-ionization (MALDI) combined with tandem mass spectrometry has been shown to provide valuable structural information, when applied under low-energy collision activation conditions.[11–13] Naturally occurring saccharides and saccharide conjugates comprise mixtures of several molecular species, in which the saccharide and aglycon parts of the molecules are different. Reverse-phase high-performance liquid chromatography (RP-HPLC) thus represents a method of choice for reducing the complexity of the samples prior to their analysis by mass spectrometry. The development of APPI, which allows the use of organic solvents (even a non-polar solvent) offers some new possibilities for LC–MS. This method[14–16] has been successfully applied to the analysis of a variety of compounds including polyaromatic hydrocarbons,[17] flavonoids,[18] drugs and other biomolecules,[19–21] such as biological matrices or hydrophobic peptides.[22] The coupling of this method with liquid chromatography has also been shown to be efficient.

The ionization in APPI is based on the photoionization of particular species that have a lower ionization energy (IE) than the photon energy ($h\nu$). A krypton discharge lamp is typically used for this purpose, which largely produces 10 eV photons. Photoionization is achieved in a modified heated nebulizer or other type of liquid spray nozzle. The basic mechanism[23] involves ionization of the analyte (M) according to the scheme below

$$M + h\nu \rightarrow M^{+\cdot} + e^- \quad \text{if IE (M)} < h\nu$$

However, the dominant ion observed by APPI is typically $[M+H]^+$ or $[M+Na]^+$. This is at variance with typical gas phase photoionization experiments, which are performed in diluted media, and in which a the molecular ion $M^{+\cdot}$ is generally detected.[23,24] The direct photoionization of the analyte is not very efficient due to the strong UV absorption by the nebulizing gases and by the

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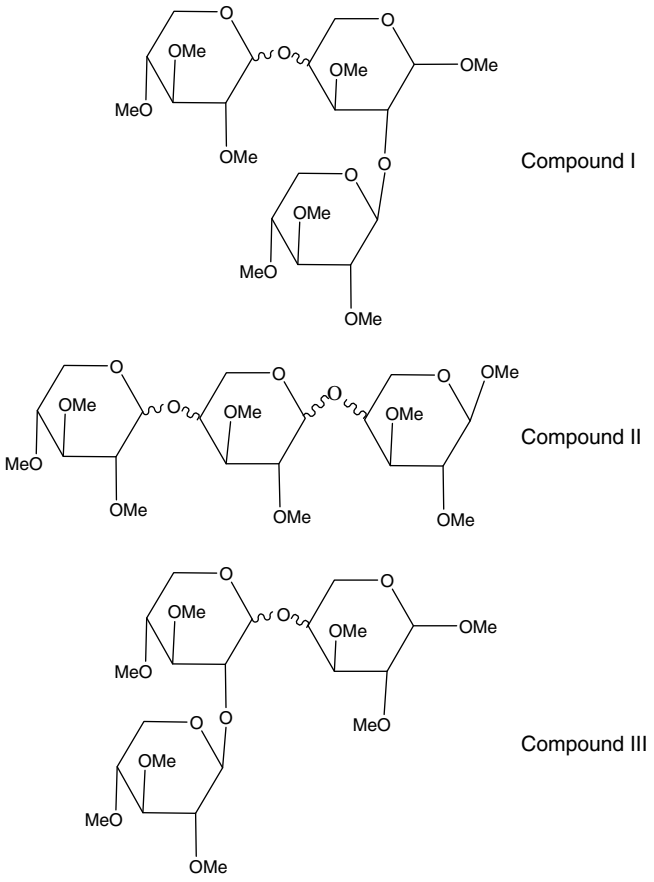


Scheme 1.

solvent. For this reason, APPI sources are frequently operated in conjunction with a third molecule, which is usually referred to as dopant (D), in order to enhance the ionization efficiencies.[10] In this case, ionization is based on a charge transfer to the analytes from dopant molecules (e.g., toluene) that have been ionized using 10 eV photons, which is higher than the ionization potentials (IPs) of typical target molecules, but lower than the IPs of virtually all of the constituents of air as well as most common solvents. Furthermore, because molecules of interest are ionized near their ionization thresholds, there is minimal fragmentation that typically results in a cluttered mass spectrum. These features provide tremendous benefits for analyzing mixtures and samples in complex matrices. The method of electron ionization, even when low energy (12 eV) electrons[6,7] are used, by contrast, imparts energy that greatly exceeds the dissociation thresholds, leading to extensive fragmentation.

APPI was used in this study to analyse some per-O-methylated oligosaccharides of the D-xylan type. The experiments were carried out in the positive ion mode. More detailed studies of the O-methylated oligosaccharides will be the subject of future. The structures of the three studied compounds are depicted in Scheme 1. Figure 1 shows the mass spectrum of compound **I**, obtained using the APPI source with the UV lamp switched off. (e.g., thermospray conditions). The conspicuous feature of this thermospray mass spectrum of isomeric compounds **I–III** is the exclusive production of $[M+Na]^+$ adduct ions detected at *m*/*z* 549 for all three isomers. These ions are also generated under matrix assisted laser desorption/ionization (MALDI) and electrospray (ESI) conditions.[11–13] $[M+Na]^+$ cationized ions remain the most abundant species under APPI conditions. Figures 2–4 show APPI mass spectra of **I–III** recorded in the same conditions as before, but with the photoionization lamp switched on and with H_2O/MeOH (50/50 v/v) as the solvent admitted into the ion source. The second observed ionization of molecules of analyte has no analogy in substances studied to date by APPI MS. It corresponds to the addition of a water molecule to the molecular ion of per-O-methylated saccharides, giving rise to $[M+H_2O]^{\cdot+}$ adduct ionradicals. The $[M+H_2O]^{\cdot+}$ species is detected at *m*/*z* 544, and is clearly visible in all three isomers studied (see Figs. 2–4). As a rule the APPI mass spectra of the studied isomers show two peaks at *m*/*z* 111 and 143. The only difference between

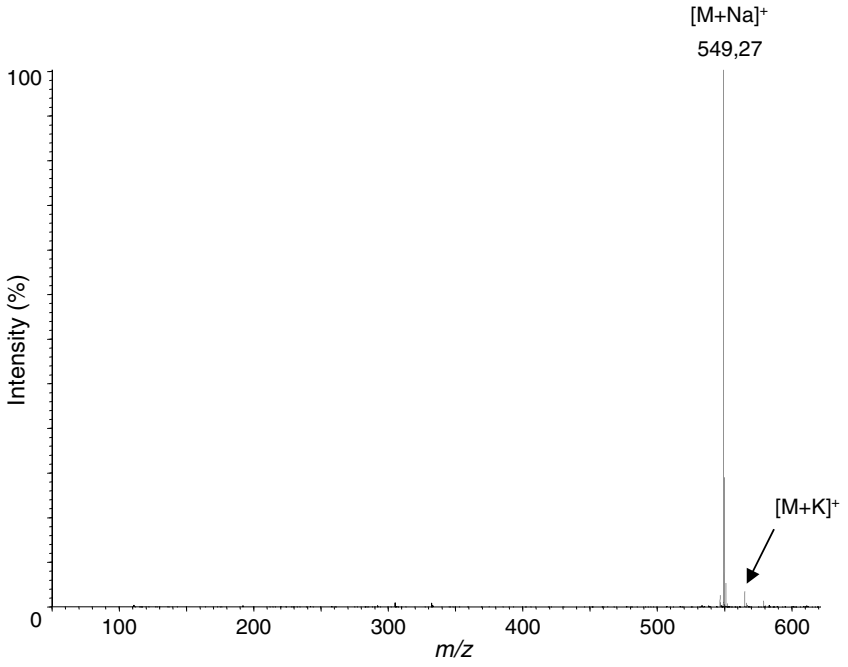


Figure 1. Thermospray mass spectrum of compound **I**.

the isomers is the relative abundance of these peaks. These results differ from data obtained in FAB experiments, in which the fragmentation of cationized saccharides involve the cleavage of the sugar ring.[10,26]

The fragmentation pathways of the two major quasimolecular ions, that is, $[M+Na]^+$ and $[M+H_2O]^{\cdot+}$ species, have been studied by tandem mass spectrometry. No major differences between three isomers studied can be seen. Because of this, only the spectrum of compound **I** is shown. The MS/MS spectrum of $[M+Na]^+$ ions, depicted as an example in Figure 5, contains a base peak at *m*/*z* 375, which is produced by a Y-type cleavage[25] of the trisaccharide, and a hydrogen rearrangement on the terminal interglycosidally linkage glycosidic oxygen atom (Scheme 2). The shortage of numbers of units in oligosaccharides by the Y mode also occurs in the case of FAB, MALDI ToF/ToF and ESI MS/MS fragmentation techniques.[6,9] However, as can be seen in Figure 6, the $[M+H_2O]^{\cdot+}$ species fragment largely generates ions at *m*/*z* 175, 143 and consequently *m*/*z* 111. The latter originates from nonreducing

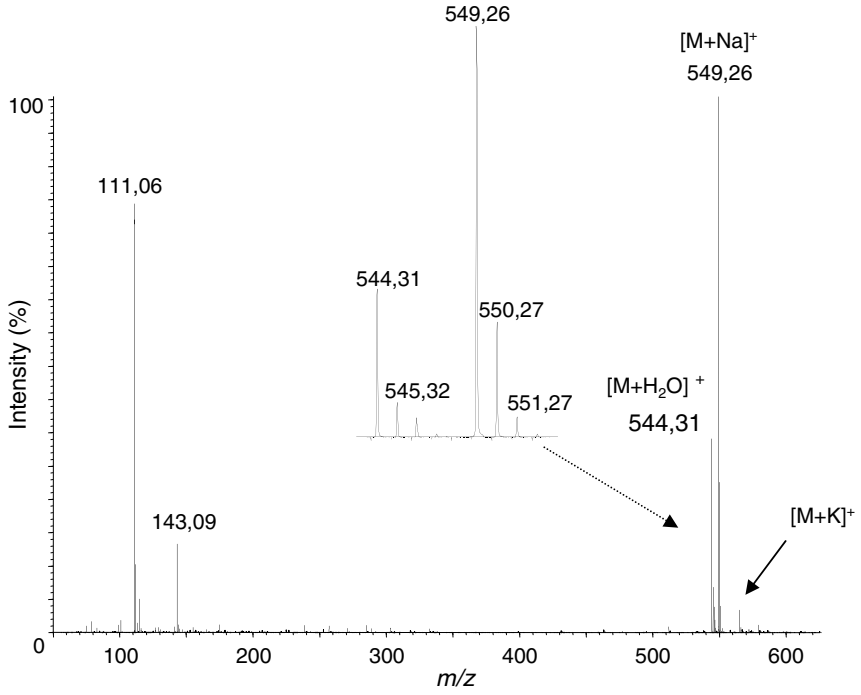


Figure 2. APPI mass spectrum of compound **I** recorded in H_2O/MeOH 50/50.

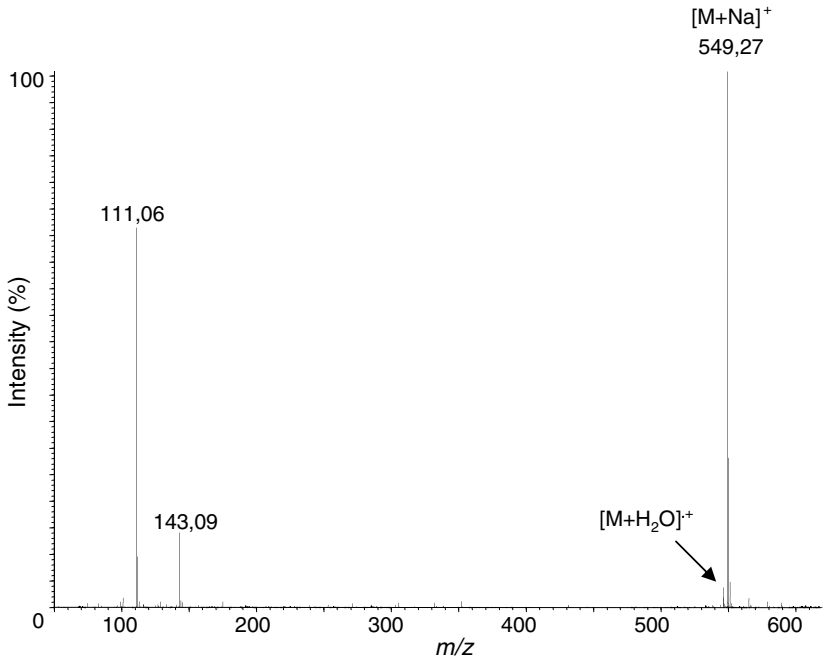


Figure 3. APPI mass spectrum of compound **II** recorded in H_2O/MeOH 50/50.

terminal units, as described in Scheme 3. From the CID mass spectrum, a less intensive glycosidic cleavage, namely the C_1-type in two forms $[C_1 + H]^+$ and $[C_1 + H_2O]^+$ can be observed at *m*/*z* 207 and 225, respectively. The tentative mechanism of APPI fragmentation of compounds **I–III** is introduced on the example in Schemes 2 and 3.

In conclusion, we have successfully extended the application of APPI to per-O-methylated trisaccharides related to D-xylan. This is the first report of both linear (II) and branched (I and III) oligosaccharides examined under UV radiation at a dense medium and under atmospheric pressure. The predominant pathway involved the production of glycosidic fragments without cross ring fragments.[26] Tandem CID fragmentation of $[M+Na]^+$ ions result in fragment ions. The advantage of this technique is, that the spectra show no internal residue loss.[27] These results differ from those obtained by ESI and MALDI, in which cross ring cleavages are the dominant feature of spectra of per-O-methylated oligosaccharides. This finding demonstrates the potential value of the APPI

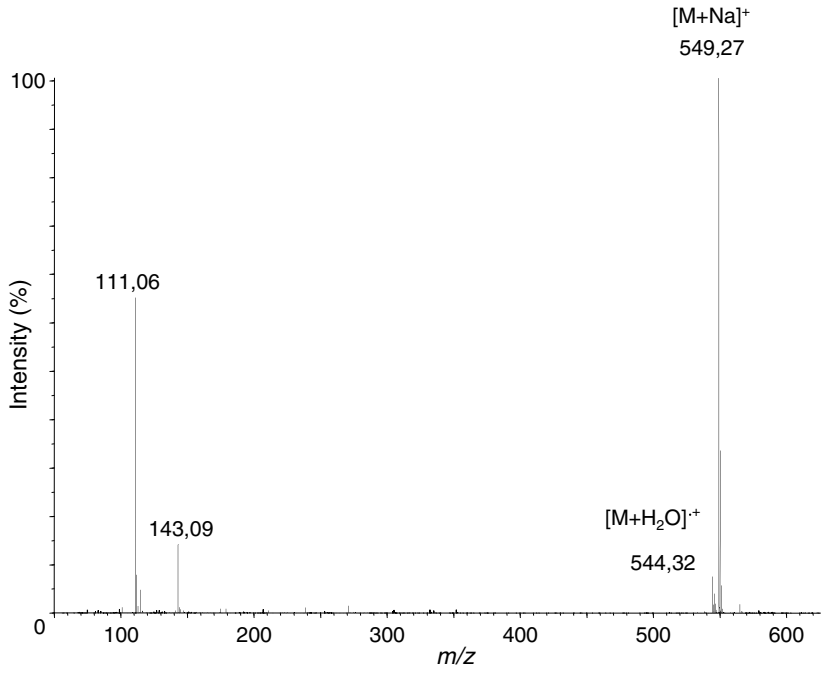


Figure 4. APPI mass spectrum of compound **III** recorded in H_2O/MeOH 50/50.

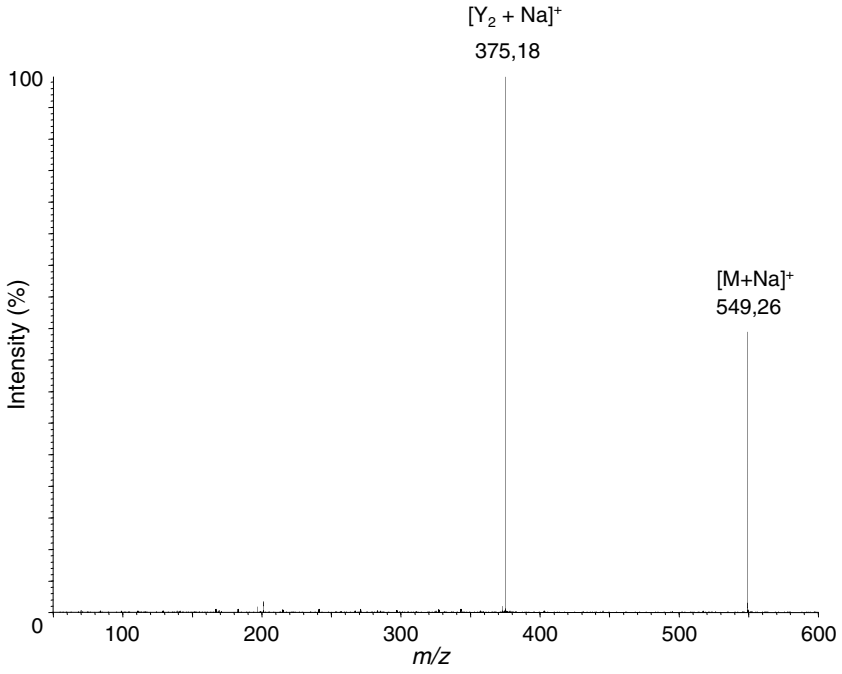
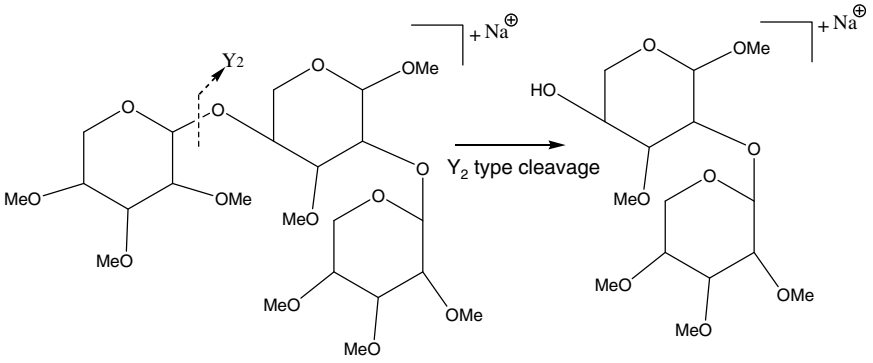


Figure 5. CID mass spectrum of the *m*/*z* 549 ion precursor.



Scheme 2.

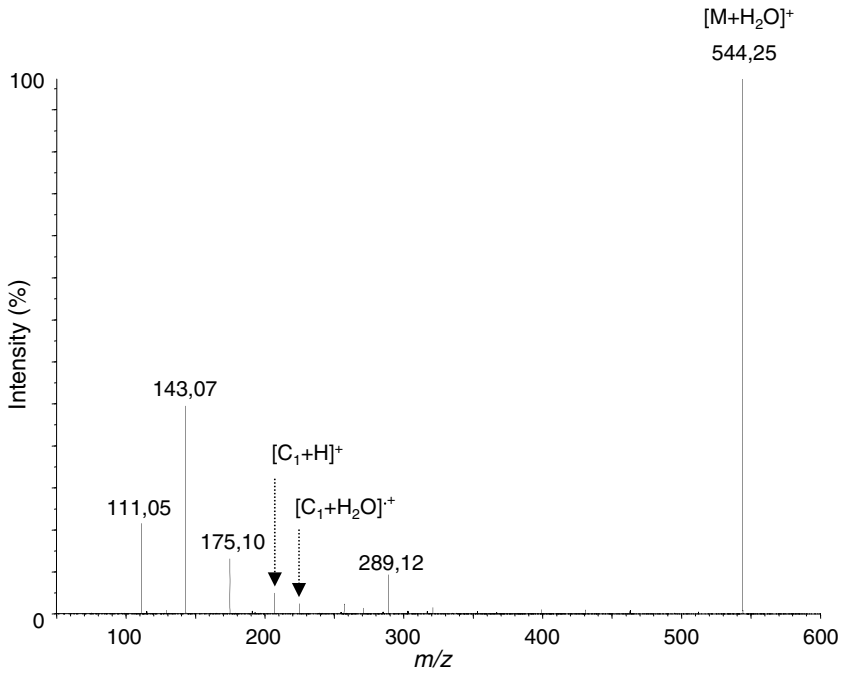
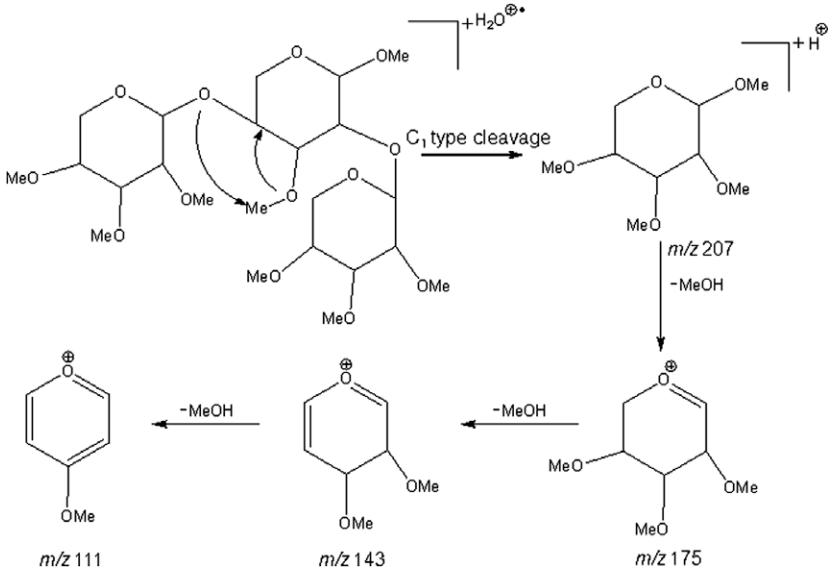


Figure 6. CID mass spectrum of the *m*/*z* 544 ion precursor.



Scheme 3.

technique in the detection of per-O-methylated saccharides, even in trace analysis of complicated mixtures.

2. Experimental

2.1. Chemicals and compounds

All solvents were HPLC grade. methanol was purchased from Prolabo (Fontenay-sous- Bois, France). Water was double distilled and then filtered through a Millipore cartridge (18 MΩ). The oligosaccharides were solubilized in a mixture of H_2O/CH_3OH 50/50. The synthesis of the investigated D-xylan trisaccharides **I–III** (Scheme 1) has been described earlier.[28,29]

2.2. Mass spectrometry

Photoionization experiments were carried out by using the Photospray™ source (Applied Biosystems, Forster City, CA, USA). This source was fitted with a Cathodeon PKS 106 (Cathodeon, Cambridge, UK) Krypton lamp that generates a continuous flow of mainly 10 eV photons with a minor contribution of 10.6 eV photons. Mass spectra were recorded using a hybrid quadrupole-time-of-flight Qstar Pulsar i mass spectrometer (Applied Biosystems). Stock solutions of compounds were prepared in a mixture of H_2O/CH_3OH 50/50 solution at a concentration of 2×10^{-4} mol L^{-1}.

The samples were injected by the flow-injection analysis (FIA) method: 10 μL of the sample solutions were loaded into an injection loop and next eluted with a mixture of H_2O/CH_3OH 50/50. The solvents was introduced into the photospray ionization source using a HPLC pump Agilent 1100 series (Agilent Technologies, Palo Alto, CA, USA) at a flow rate of 200 μL/min. The nebulizer gas was dry, clean air.

Mass spectrometric instrumental parameters were adjusted in order to obtain the best signal-to-noise ratio and to minimize possible in source collision induced dissociation (CID), which have been previously shown to be minor. Operating parameters for the experiments were ISV (ion source voltage) = 1500 V, DP_1 (declustering potential 1) = 10 V, FP (focusing potential) = 50 V, DP_2 (declustering potential 2) = 15 V. The gas flow which protects the lamp was fixed to 2 L/min. Data were acquired using the ANALYST QS software (Applied Biosystems). The temperature of the experiments was 400 °C.

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