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Electrospray ionization mass spectrometry: a key analytical tool for the characterization of regioselectively derivatized maltooligosaccharides obtained starting from natural β-cyclodextrin

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The development of natural cyclodextrins (CDs) for various industrial applications (agroalimentary, cosmetic or pharmaceutical) constitutes a continuous challenge. For the integration of these agricultural plant products in the creation of super-absorbent biodegradable and hypoallergenic materials (water-retaining agents, cosmetic hydrating and texturing, pharmaceutical and horticultural products) to replace synthetic polymers, we have developed chemical methods to access regioselectively C-6-derivatized maltooligosaccharides starting from CDs. These compounds are highly suitable for further chemical modifications and are expected to give access to a new class of polymeric materials with potential applications such as water-retaining agents in the disposable nappies industry. For the structural analysis of carbohydrates, electrospray ionization mass spectrometry (ESI-MS) offers precise results, analytical versatility and very high sensitivity. We report herein the rapid and convenient follow-up of chemical reactions, the purity evaluation of intermediates and final products, and the structural characterization of derivatized maltooligosaccharides, obtained by acidic cleavage (acetolysis) of halogenated and esterified CDs, using ESI-MS in combination with the high-resolution (HRMS) and tandem mass spectrometry (MS/MS) capabilities of a quadrupole orthogonal time-of-flight (Q-TOF) mass spectrometer. Copyright © 2006 John Wiley & Sons, Ltd.

Since natural and modified oligosaccharides have become the focus of increasing interest in food and agriculture,[1] cosmetic[2,3]and pharmaceutical[3,4] applications, there have been greater demands for the need to access modified homologous oligosaccharides by more convenient and efficient routes. In spite of this, there has been relatively little published work to date to reflect such demands due to the difficulties in achieving good regioselectivity by chemical modification of linear oligosaccharides and in accessing pure homologous linear oligosaccharides on a large scale. Nevertheless, it is known that maltooligosaccharides (linear oligosaccharides constituted of glucose monomer molecules $\alpha(1\rightarrow4)$ linked) with a degree of polymerization (DP) from 6 to 8 can be obtained readily by acetolysis of esterified α-, β- and γ-cyclomaltooligosaccharides (cyclodextrins, CDs) under H_2SO_4 catalysis (Kuzuhara's method[5,6]) or under $HClO_4$ catalysis (Vasella's method[7]).We have recently adapted these procedures[8] to synthesize regioselectively C-6-derivatized maltoheptaoses (DP7) **4** to **10** in high purity and yield, starting from natural β-CD (Scheme 1). These compounds are highly suitable for further chemical modifications and are expected to give access to anionic, cationic, zwitterionic or amphiphilic oligosaccharides.

One important aspect in the design of synthetic routes to modified maltoheptaoses involves rapid chemical process control and structural identification of reaction components using appropriate analytical tools. Due to the complexity of the compounds involved and the need to accurately and rapidly follow the progress of such reactions, simple nuclear magnetic resonance (NMR) experiments do not readily provide the required quick unambiguous structural elucidation and purity evaluation of intermediates and final products. In contrast, the rapidity and structural analytical power of mass spectrometry (MS) are now well established for the characterization of native or modified oligosaccharides using either electrospray (ESI) or matrix-assisted laser desorption (MALDI) ionization methods.[9,10]

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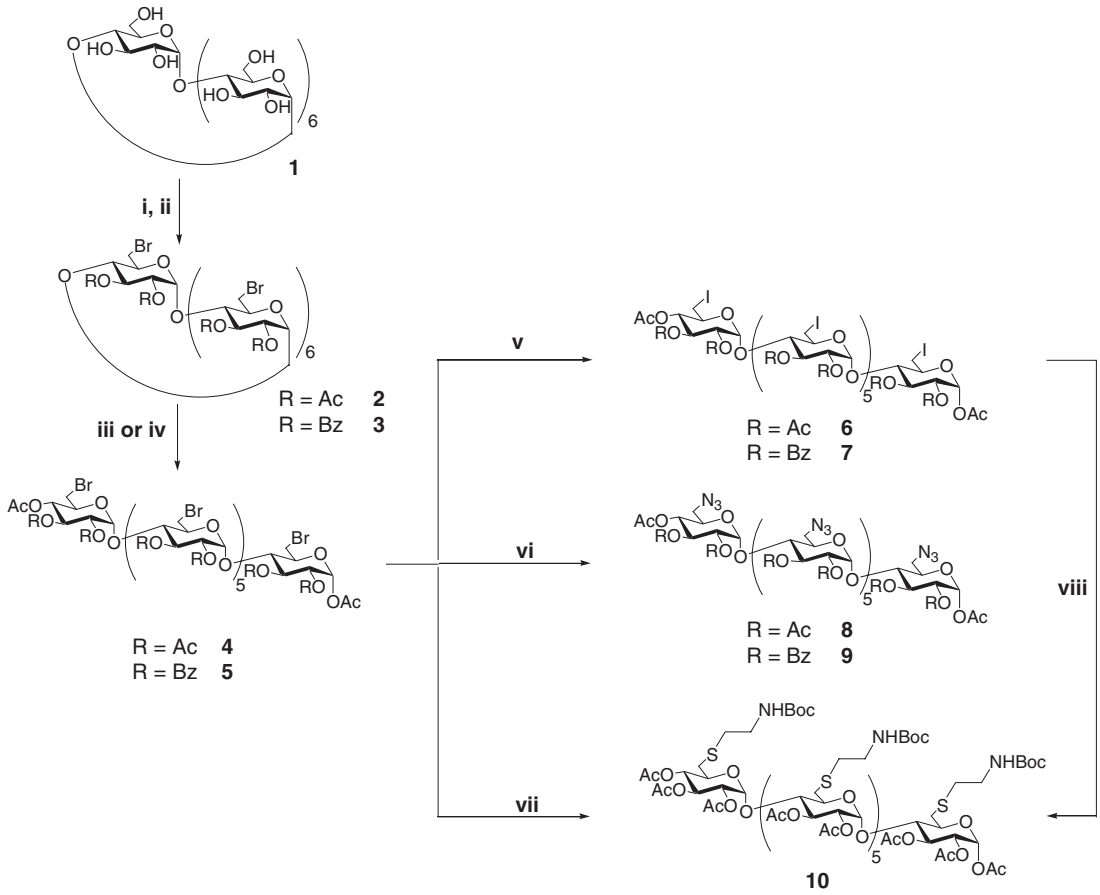


Scheme 1. Synthetic pathway of the target 6-deoxymaltoheptaose derivatives **4–10**.[8]

This communication describes stepwise control of the chemical reactions and structural analysis of the final products using ESI-MS in the positive ion mode in combination with the high-resolution (HRMS) and tandem mass spectrometry (MS/MS) capabilities of a quadrupole orthogonal time-of-flight (Q-TOF) mass spectrometer.[11,12]

EXPERIMENTAL

Sample preparation

Samples of maltooligosaccharides were dissolved ($0.01\,mg\cdot mL^{-1}$) in methanol for acetylated derivatives and in acetonitrile for benzoylated derivatives and the solutions directly introduced ($5\,\mu L\cdot min^{-1}$) through an integrated syringe pump into the ESI source.

Mass spectrometry

High-resolution ESI mass spectra in the positive ion mode were obtained on a Q-TOF *Ultima Global* hybrid quadrupole time-of-flight instrument (Waters-Micromass, Manchester, UK), equipped with a pneumatically assisted electrospray (Z-spray) ionization source and an additional sprayer (Lock Spray) for the reference compound.

The source and desolvation temperatures were 80 and 150°C, respectively. Nitrogen was used as the drying and nebulizing gas at flow rates of 350 and 50 L/h, respectively. The capillary voltage was 3.5 kV, the cone voltage 100 V, and the RF lens1 energy was optimized for each sample (50–200 V). For collision-induced dissociation (CID) experiments, argon was used as collision gas at an indicated analyzer pressure of 5×10^{-5} Torr and the collision energy was optimized for each precursor ion (50–110 V). Lock mass correction, using appropriate cluster ions of sodium iodide, $(NaI)nNa^+$, was applied for accurate mass measurements. The mass range was typically *m/z* 50–4550 and spectra were recorded at 4 s/scan in the profile mode at a resolution of 10 000 (FWHM). Data acquisition and processing were performed with MassLynx 4.0 software.

RESULTS AND DISCUSSION

Heptakis(2,3-di-*O*-acetyl-6-bromo-6-deoxy)-β-CD (2) and heptakis(2,3-di-*O*-benzoyl-6-bromo-6-deoxy)-β-CD (3): synthetic pathway and structure control

Starting from native β-CD, halogenated and esterified β-CDs **2** and **3** were selected for the acetolysis procedures since the subsequent linear products **4** and **5** are suitably substituted for convenient extended chemical modifications. Compounds **2** and **3** were each synthesized in two steps (Scheme 1). The primary hydroxyl groups of the β-CD were first brominated, then the remaining secondary hydroxyl groups were esterified in the presence of acetic anhydride and benzoyl bromide, respectively, to afford the previously described heptakis(2,3-di-*O*-acetyl-6-bromo-6-deoxy)-β-CD[13] **2** ($[M+Na]^+$ *m/z* 2187, found 2186.9050

for $C_{70}H_{91}{}^{79}Br_3{}^{81}Br_4O_{42}Na$ requires 2186.9084), and the unknown heptakis(2,3-di-*O*-benzoyl-6-bromo-6-deoxy)-β-CD **3** ($[M+Na]^+$ *m/z* 3055, found 3055.1339 for $C_{140}H_{119}{}^{79}Br_3{}^{81}Br_4O_{42}Na$ requires 3055.1275), each in 90% overall yield and with a high level of purity as illustrated by their ESI mass spectra shown in Fig. 1. For these two compounds the measured ion represents the ion of the highest signal intensity in the isotope cluster of their $[M+Na]^+$ ions (as for compounds **4** and **5** in Table 1). Their structures were confirmed by MS/MS experiments. The spectrum obtained from CID of the $[M+Na]^+$ ion (*m/z* 3055) of the unknown compound **3** is presented in Fig. 2. The insert presents the high-mass region (*m/z* 2450–3055) with successive losses of HBr (80 Da) and $C_6H_5CO_2H$ (122 Da) which are characteristic of the bromide and benzoyl groups. In the low-mass region (*m/z* 50–450) abundant ions at *m/z* 311/313, 293/295, 189/191 (corresponding to the fragmentation of one modified hexose unit, as illustrated in Scheme 2), and 105 ($C_6H_5CO^+$) are observed.

$1^{I},2^{I–VII},3^{I–VII},4^{VII}$-Hexadeca-*O*-acetyl-$6^{I–VII}$-heptabromo-$6^{I–VII}$-heptadeoxy-$\alpha$-maltoheptaose (4) and $1^{I},4^{VII}$-di-*O*-acetyl-$2^{I–VII}$, $3^{I–VII}$-tetradeca-*O*-benzoyl-$6^{I–VII}$-heptabromo-$6^{I–VII}$-heptadeoxy-α-maltoheptaose (5): acetolysis reaction optimization and halogenated isotopic pattern control

The optimum reaction conditions found for the acetolysis of **2** and **3** used the Kuzuhara procedure (**iii**, Scheme 1) and gave the maltooligosaccharides **4** and **5** in 16 and 32% yields,

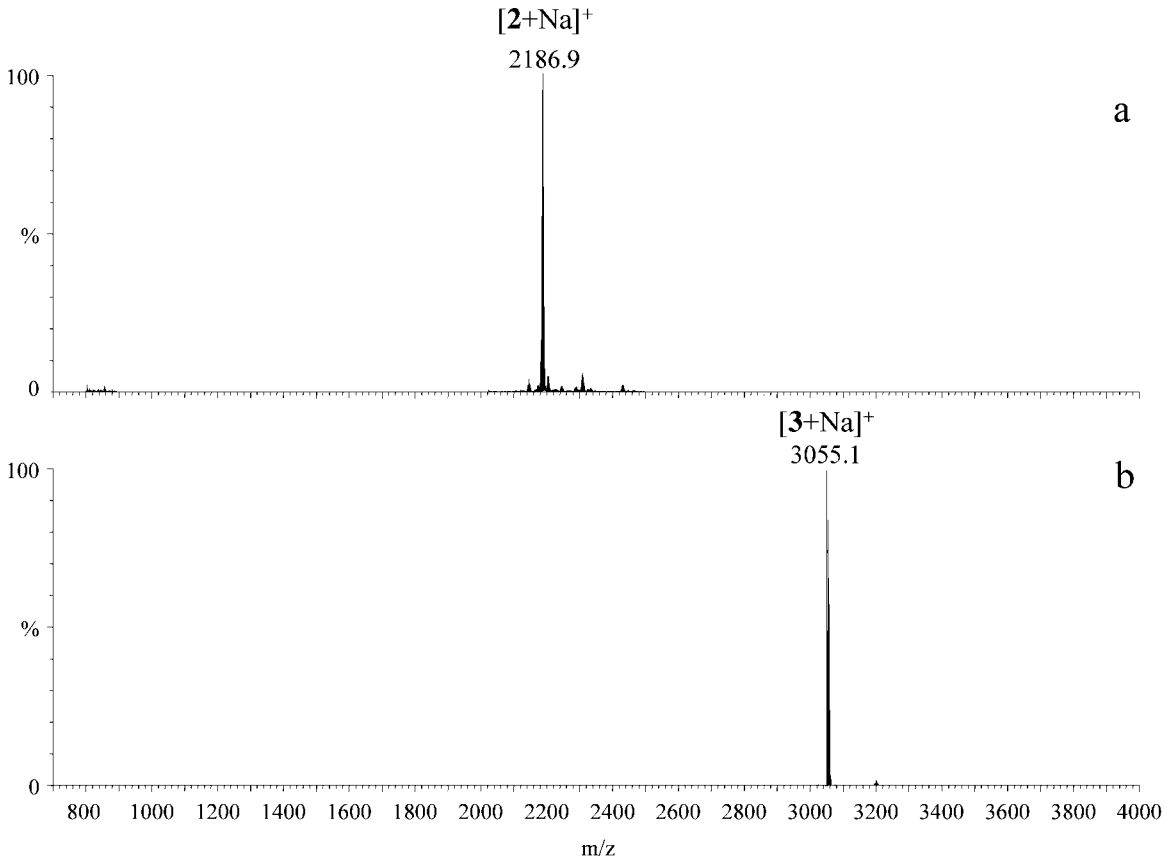


Figure 1. ESI-MS spectra of the esterified and brominated-β-CDs **2** (a) and **3** (b).

Table 1. Accurate mass measurements of the $[M+Na]^+$ ions of the target 6-deoxymaltoheptaose derivatives **4–10** using the Q-TOF *Ultima Global* instrument equipped with a LockMass sprayer

Compound	Mass	Calc. mass	ppm	Formula
4	2288.9446	2288.9401	1.9	$C_{74}H_{97}O_{45}{}^{79}Br_3{}^{81}Br_4Na$
5	3157.1616	3157.1592	0.8	$C_{144}H_{125}O_{45}{}^{79}Br_3{}^{81}Br_4Na$
6	2616.8428	2616.8513	3.3	$C_{74}H_{97}O_{45}I_7Na$
7	3485.0750	3485.0704	1.3	$C_{144}H_{125}O_{45}I_7Na$
8	2022.5773	2022.5845	3.6	$C_{74}H_{97}O_{45}N_{21}Na$
9	2890.8115	2890.8036	2.7	$C_{144}H_{125}O_{45}N_{21}Na$
10	2961.0513	2961.0416	3.3	$C_{123}H_{195}O_{59}N_7S_7Na$

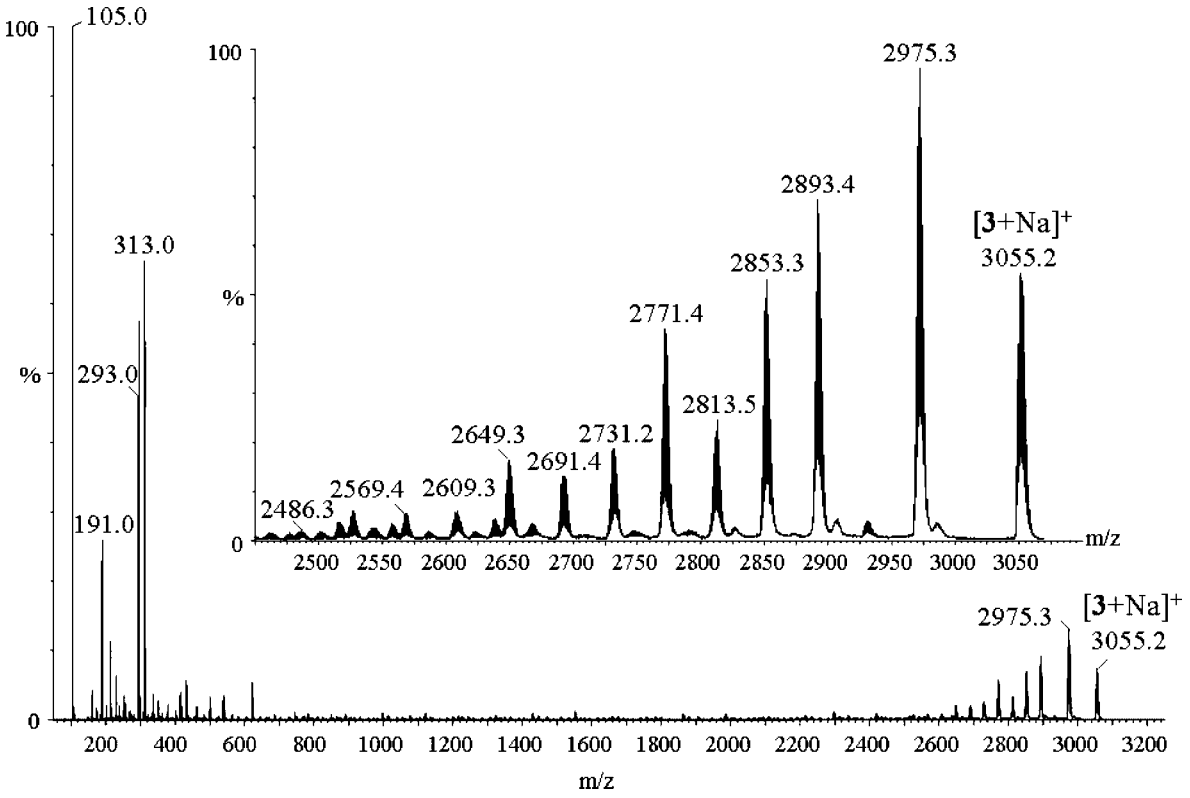
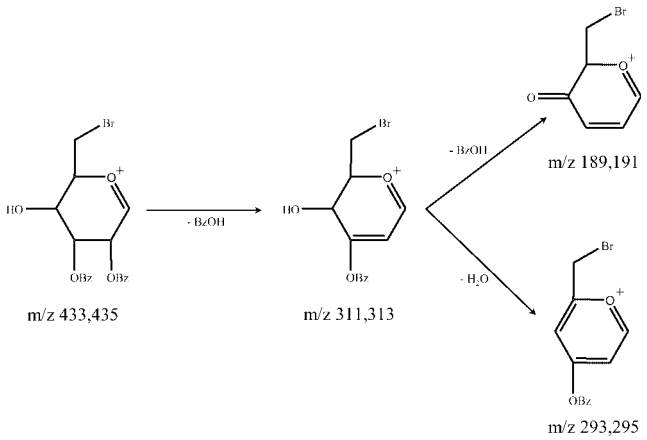


Figure 2. ESI-MS/MS spectrum of the $[M+Na]^+$ ion (*m/z* 3055) of the benzoylated and brominated-β-CD **3**.

respectively. The unchanged CDs **2** and **3** could be recovered in 78 and 58% yields, showing that no degradation occurred despite the harsh reaction conditions used. At this stage of the synthesis, compounds **4** and **5** could not be obtained totally pure. Even after purification by means of Kiesegel column chromatography, some traces of the corresponding maltohexaose (DP6) derivative could be detected. This phenomenon is illustrated for compound **4** in the ESI-MS spectrum presented in Fig. 3(a) ($[DP6+Na]^+$ ion observed at *m/z* 1979). In contrast when the Vasella acetolysis methodology (**iv**, Scheme 1) was applied to cleave compound **2**, it was found that the desired maltoheptaose derivative **4** was obtained in an optimum yield of 30%, but with a lower purity. In this case, abundant ions were observed at *m/z* 1979 and 1671, corresponding to the $[M+Na]^+$ ions of the DP_6 and the DP_5 derivatives, respectively (Fig. 3(b)).

For the two heptabromo products **4** and **5**, good agreement was found between the experimental isotopic patterns of the obtained $[M+Na]^+$ ions and those predicted by theoretical means, as shown for **5** in Fig. 4. The results of the elemental



Scheme 2. Fragmentation pathway of one modified hexose unit of the benzoylated and brominated-β-CD **3**.

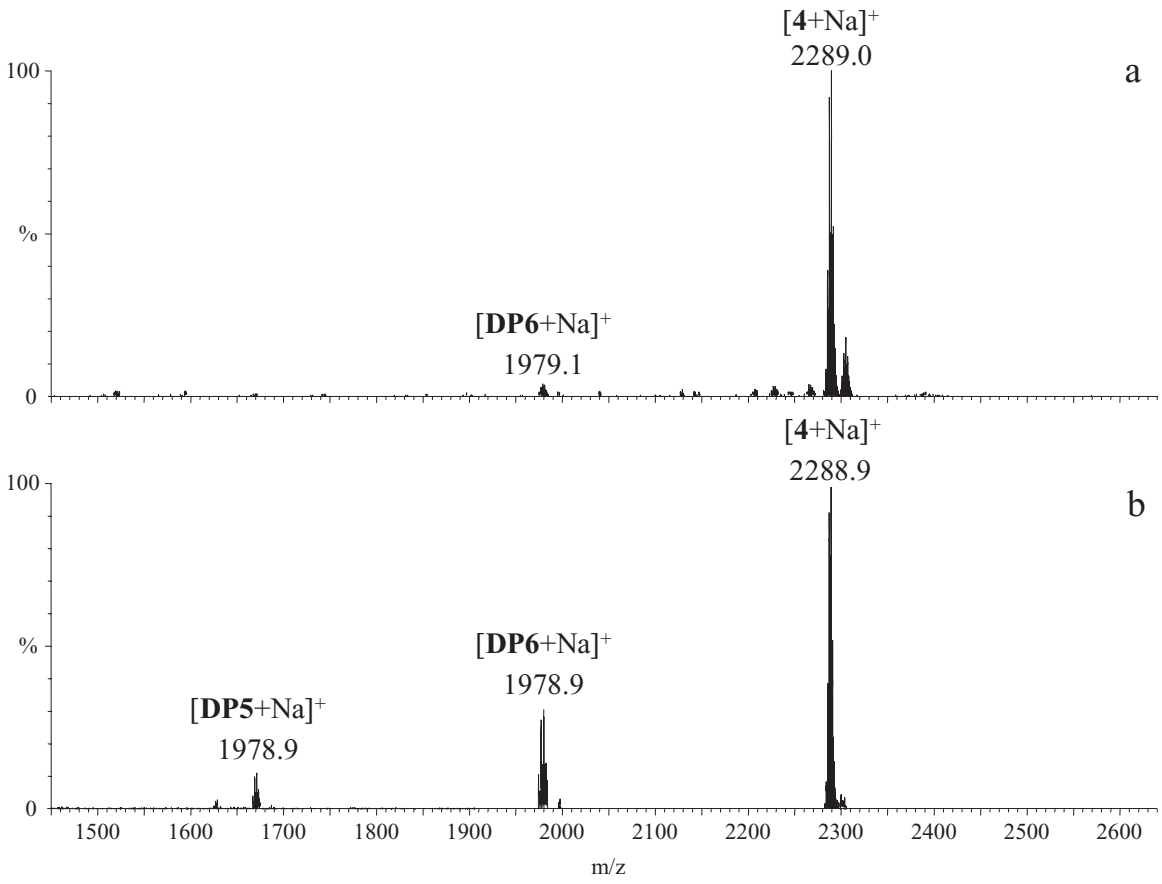


Figure 3. ESI-MS spectra of the heptabromo maltooligosaccharide **4** obtained using the Kuzuhara (a) and Vasella (b) acetolysis methods.

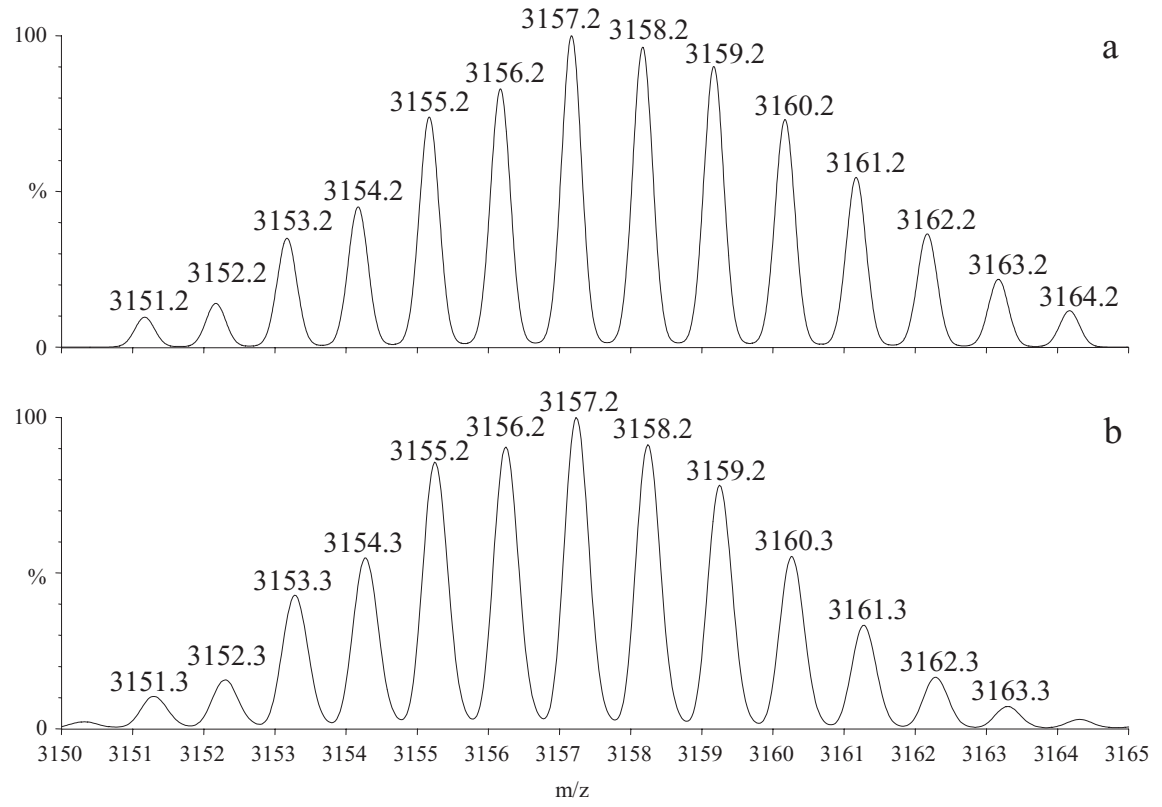


Figure 4. Theorical (a) and experimental (b) isotopic patterns for the $[M+Na]^+$ ion of the heptabromo maltooligosaccharide **5**.

composition determinations of these $[M+Na]^+$ ions are presented in Table 1.

$1^{I},2^{I–VII},3^{I–VII},4^{VII}$-Hexadeca-*O*-acetyl-$6^{I–VII}$-heptadeoxy-$6^{I–VII}$-heptaiodo-α-maltoheptaose (6), $1^{I},4^{VII}$-di-*O*-acetyl-$2^{I–VII},3^{I–VII}$-tetradeca-*O*-benzoyl-$6^{I–VII}$-heptadeoxy-$6^{I–VII}$-heptaiodo-α-maltoheptaose (7), $1^{I},2^{I–VII},3^{I–VII},4^{VII}$-hexadeca-*O*-acetyl-$6^{I–VII}$-heptaazido-$6^{I–VII}$-heptadeoxy-α-maltoheptaose (8), $1^{I},4^{VII}$-di-*O*-acetyl-$6^{I–VII}$-heptazido-$2^{I–VII},3^{I–VII}$-tetradeca-*O*-benzoyl-$6^{I–VII}$-heptadeoxy-α-maltoheptaose (9) and $1^{I},2^{I–VII},3^{I–VII},4^{VII}$-hexadeca-*O*-acetyl-$6^{I–VII}$-*S*-[hepta-(*tert*-butoxycarbonyl-2-amino)ethyl]-$6^{I–VII}$-heptathio-α-maltoheptaose (10): purity control of final batches and complete characterization

With the key acyclic maltooligosaccharides **4** and **5** now available, nucleophilic substitution of the bromide groups was examined. As depicted in Scheme 1, the bromide groups were easily substituted sequentially, by iodide, azide and 2-(Boc-amino)ethanethiolate to afford, accordingly, the compounds **6**, **7**, **8**, **9** and **10** in yields ranging from 70–90%.

Interestingly, **6**, **7**, **8** and **9** could be readily separated from the contaminating maltohexaose derivatives by flash chromatography. In contrast, similar attempts to remove **10** from the contaminating maltohexaose derivative proved unsuccessful. However, an alternative procedure was found to give the desired product **10** in high-purity form from the precursor **6** in 89% yield (**viii**, Scheme 1).The ESI-MS spectra of the purified final batches of maltooligosaccharides products **6**, **7**, **8**, **9** and **10** are presented in Fig. 5. These spectra indicated a good level of purity for all of these compounds. ESI-MS/MS experiments were performed on the respective $[M+Na]^+$ ions allowing the different structures to be assigned, as exemplified for the acetylated compounds **6**, **8** and **10** in Scheme 3 and Fig. 6. For compound **6**, we observed successive losses of acetic acid (60 Da) and hydriodic acid (128 Da) leading, for example, to the products ions at *m/z* 2557 and 2429; the classical fragment ions from B-type inter-glycosidic cleavage (according to the Domon and Costello nomenclature[14]), which are characteristic of the oligosaccharide structure, are also clearly identified (Fig. 6(a), Scheme 3(a)). The CID fragmentations of the sodiated molecules of compounds **8** and **10** are quite different. Indeed, the maltooligosaccharide **8** carrying seven azido groups shows successive losses of seven nitrogen molecules (28 Da) to the intermediate product ion (*m/z* 1826) depicted in Scheme 3(b). This ion then generates successive losses of acetic acid (to *m/z* 1766, 1706, 1646, 1586…) and also B_i and $B_i'(B_i–C_2H_2O)$ fragment ions (Fig. 6(b), Scheme 3(b)). In the same way, the main fragmentation pathways of the $[M+Na]^+$ ion of compound **10** involves the successive losses of seven Boc groups (100 Da) giving abundant products ions at *m/z* 2863, 2762, 2662, 2562, 2462, 2362 and 2262, as depicted in Scheme 3(c). The *m/z* 2262 ion then loses two molecules of acetic acid (60 Da) to give *m/z* 2202 and 2142, and it is also at the origin of the formation of Bi and B_i' fragment ions (Fig. 6(c), Scheme 3(c)). It is noteworthy that highly substituted oligosaccharides show more complicated product ion mass spectra than the corresponding free linear

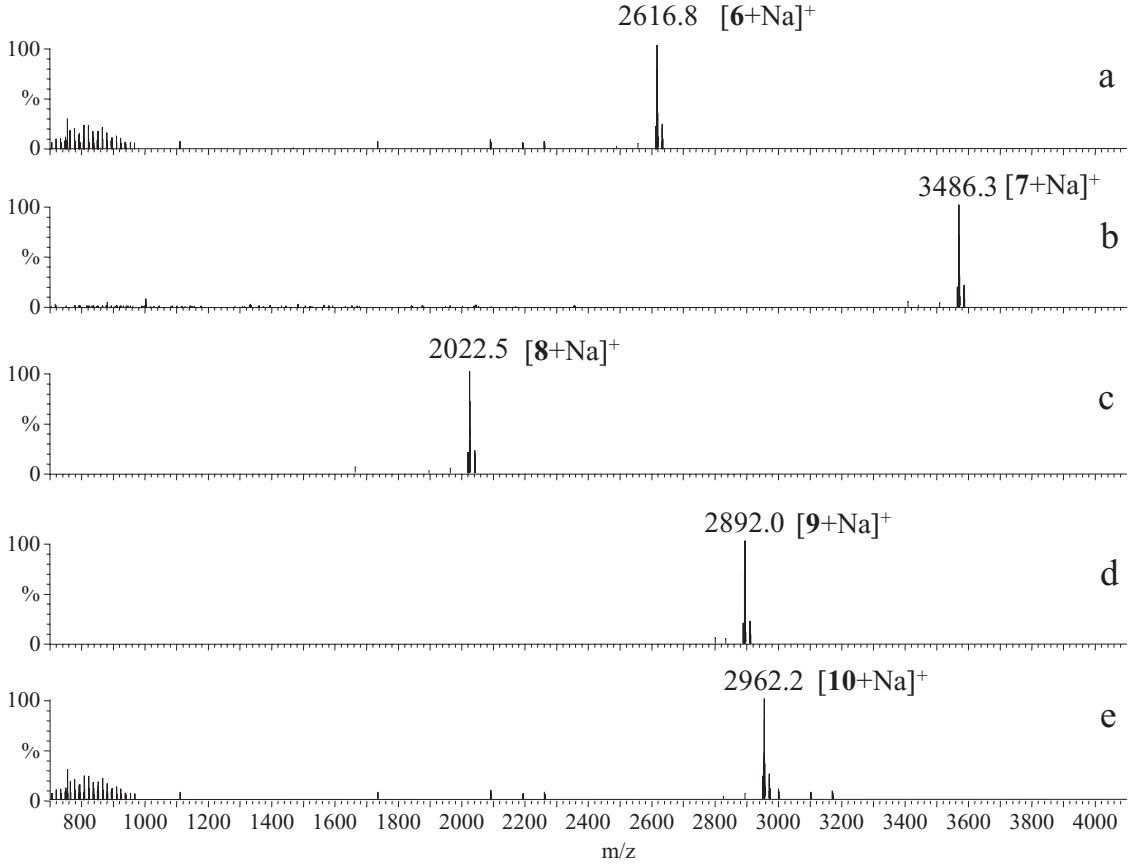
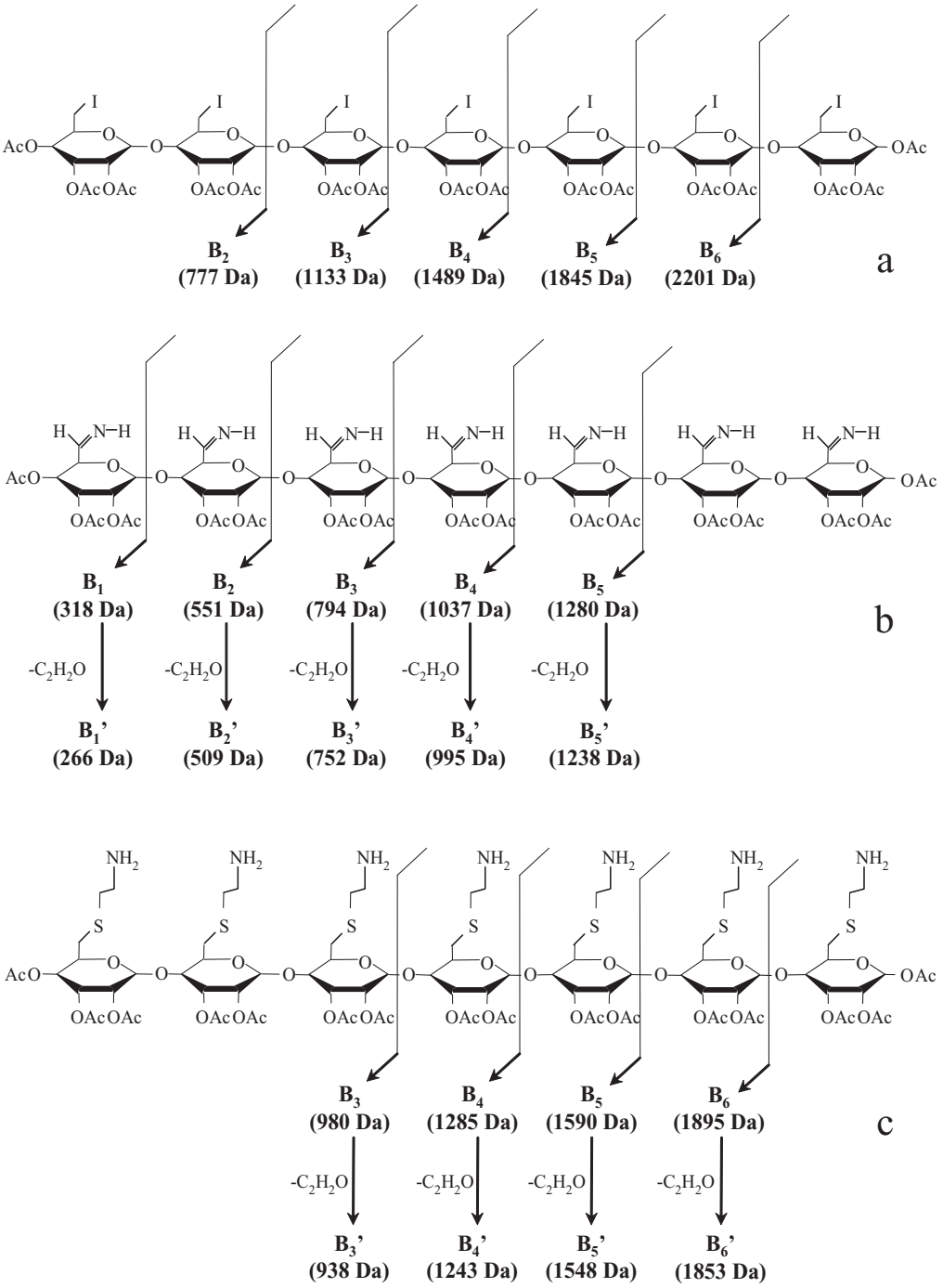


Figure 5. ESI-MS mass spectra of the final maltooligosaccharides products **6** (a), **7** (b), **8** (c), **9** (d), and **10** (e).



Scheme 3. ESI-MS/MS fragmentation pathways of the $[M+Na]^+$ ions of the acetylated maltooligosaccharides **6** (a), **8** (b), and **10** (c) (all the indicated B and B′ ions are sodiated).

oligosaccharides.[15] That seems to be because, for substituted oligosaccharides, the main CID fragmentation process involved principally the elimination of the protecting groups and in that way inter-glycosidic product ions of lower abundance are generated. Finally, for a full characterization of these final products, accurate mass measurements of their $[M+Na]^+$ ions were performed (Table 1).

CONCLUSIONS

The aim of this work was to develop an efficient route to regioselectively derivatized maltooligosaccharides coupled with the capability to perform rapid chemical process control and structural identification using electrospray ionization mass spectrometry (ESI-MS). We have shown that acetolysis of target derivatized cyclodextrins (CDs) opened the way to potentially interesting compounds such as anionic, cationic, zwitterionic or amphiphilic oligosaccharides in excellent yield and high purity. We have also demonstrated the practicality of using ESI-HRMS and ESI-MS/MS to enable reactions to be followed, to effect accurate and rapid structural elucidation, and to identify and characterize highly substituted maltooligosaccharides of more than 2000 Da molecular weight.

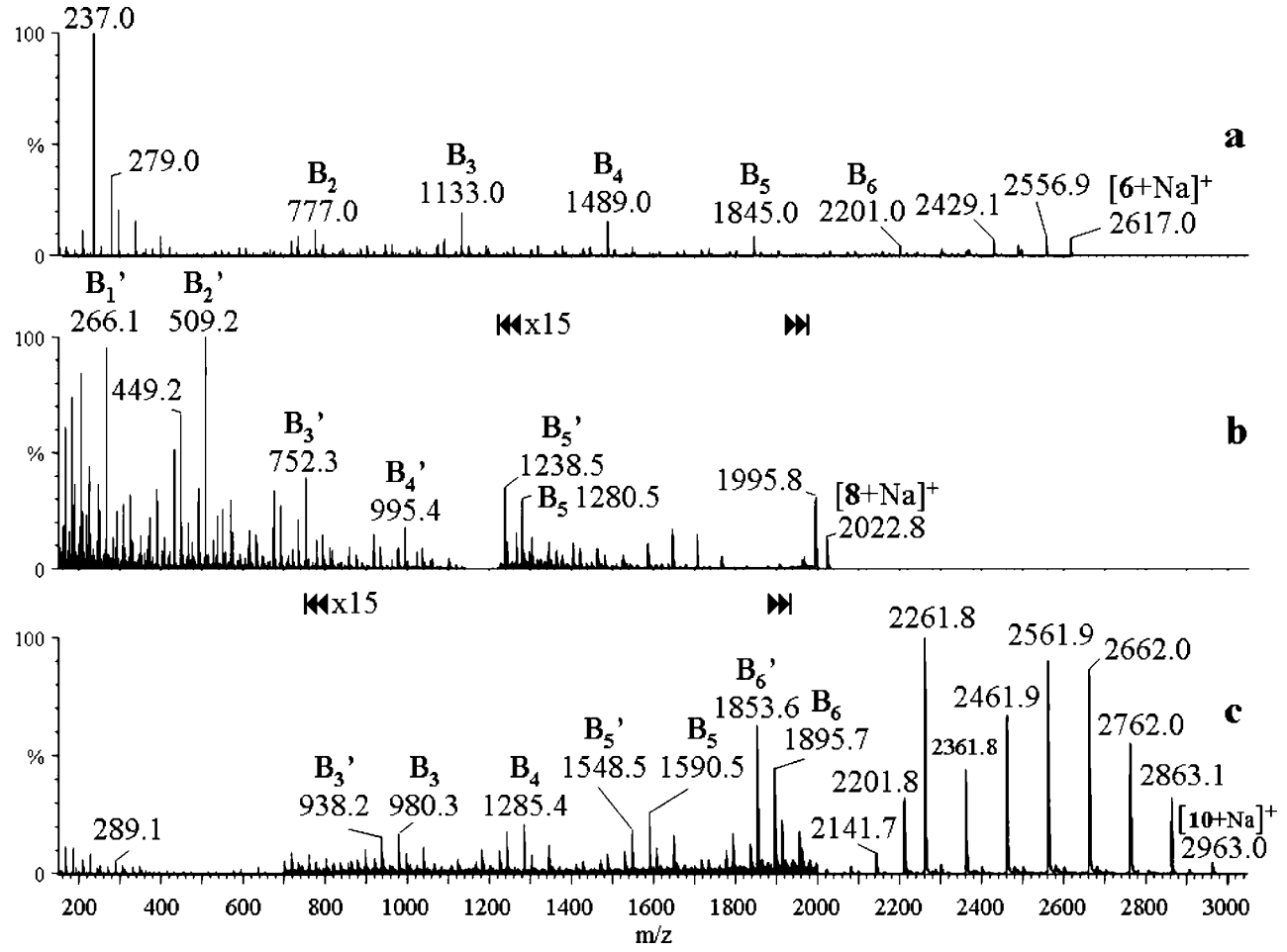


Figure 6. ESI-MS/MS mass spectra of the $[M+Na]^+$ ions (*m/z* 2962, 2022 and 2617) of the acetylated maltooligosaccharides **6** (a), **8** (b), and **10** (c).

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