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# Fragmentation pattern of regioselectively *O*-methylated maltooligosaccharides in electrospray ionisation-mass spectrometry/collision induced dissociation

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**Abstract**

Maltose, maltotriose and maltotetraose derivatives consisting of regioselectively *O*-methylated glucosyl units have been obtained via synthesis and partial hydrolysis of the corresponding β-cyclodextrin derivatives. The fragmentation mode of the sodium adducts in electrospray ionisation-mass spectrometry (ESI-MS)/collision induced dissociation (CID; ESI-$MS^2$) was investigated and interpreted with regard to the positions of the methyl groups. Nearly no influence of the status of position 6 in the glucosyl units was recognised. While *O*-2-methylation strongly favoured cross ring fragmentation, cleavage of glucosidic linkages is preferred for the *O*-3-methylated maltooligomers yielding dimeric (from trioses) or also trimeric (from tetraoses) Y and B ions of similar intensities. When both secondary OH groups were blocked, formation of $Y_{n-1}$ became the preferred process.


*Keywords:* Electrospray ionisation-collision induced dissociation/mass spectrometry (ESI-CID/MS); Regioselectively *O*-methylated maltooligosaccharides; *O*-Methyl-β-cyclodextrins; Mechanism of daughter ion formation

## 1. Introduction

During the last 15 years, combination of soft ionisation methods in mass spectrometry like FAB-MS, MALDI-TOF-MS or ESI-MS, with post source decay (PSD) or collision induced dissociation (CID) have been developed as a powerful technique to analyse the sequence and linkage positions of oligosaccharides. Mainly samples from natural sources like glycoconjugates have been studied since these often are only available in small amounts and therefore require a very sensitive analytical approach. Sequential loss of sugar units of different masses allow the determination of the sugar connectivities. Since the linkage positions influence the fragmentation behaviour, these can also be deduced from the ion motifs. Tseng et al. [1] have established a catalog-library and Gaucher et al. [2] developed a web-based computational program STAT (saccharide topology analysis tool) for an automatical and fast elucidation of $MS^n$ data of oligosaccharides. Permethylated maltooligosaccharides have been investigated as model compounds by some authors to enhance the sensitivity [3,4]. In the structure analysis of starch and cellulose derivatives the gross structure of the polysaccharide backbone is known, but it is still a great challenge to determine the distribution pattern on a higher structural level. Arisz et al. [5], Mischnick and Kühn [6], Richardson et al. [7], and Van der Burgt et al. [8] have applied FAB, MALDI or ESI-MS on degradation products from starch or cellulose ethers, to gain information about the substituent distribution along the polymer chain, but to the best of our knowledge, no systematic studies are known about the influence of substituents in defined positions on the $MS^n$ fragmentation behaviour of oligosaccharides. In contrast to glycosidically linked sugar substituents, these ether type substituents are usually stable and therefore cause different fragmentation pathways.

During our investigations of *O*-(2-hydroxy-3-trimethylammonium)propyl starches we recognised that the ESI-$MS^2$ spectra of enzyme degradation products are characteristic for the derivatisation procedure, e.g., a slurry, a dry, a paste or an extruder process [9]. However, we could not interpret these correlations of synthesis conditions and analytical data on a

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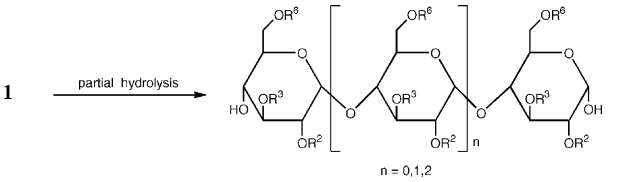
molecular structure level. Since we want to further develop the application of mass spectrometry in the analysis of starch and cellulose ether derivatives we started to investigate defined maltooligomer ethers. Chemical modification of 1,4-linked glucans usually yields a copolymer with eight various monomers: un-, 2-, 3-, and 6-*O*-mono-, 2,3-, 2,6-, and 3,6-di-*O*-, and 2,3,6-tri-*O*-substituted glucosyl units. We synthesised all these patterns, which were not otherwise available, by regioselective methylation of β-cyclodextrin which was subsequently hydrolysed to the corresponding maltooligomers. Uniformly *O*-methylated maltoses, maltotri- and tetraoses were investigated by ESI-MS and ESI-MS$^n$.

## 2. Results and discussion

Regioselectively methylated β-cyclodextrins (CD) were synthesised according to the literature [10,11]. Briefly, 2-*O*-methyl-CD is available via 2,6-di-*O*-THxDMS-CD, methylation under quantitative rearrangement of the TBDMS group into the 3-O-position [12], and subsequent deprotection. 3-*O*-Methyl-CD was obtained by the sequence 6-*O*-TBDMS-CD, 2-*O*-Bn-6-*O*-TBDMS, 3-*O*-methylation, and deprotection. Analogously, the 3,6-di-*O*-methyl-CD could also be obtained when 6-*O*-TBDMS was cleaved prior to methylation by tetrabutylammonium fluoride, while direct methylation of the 6-*O*-TBDMS ether with subsequent desilylation yields the 2,3-di-*O*-methylated isomer. 2,6-Di-*O*-methyl-CD was commercially available and the remaining 2,3,6-tri-*O*-substituted CD was obtained by direct permethylation. The heptakis[*O*-methyl-cyclomaltoheptaose] derivatives were then partially hydrolysed with 2 M trifluoroacetic acid at 120 °C for 12–20 min (Table 1). The oligomeric mixture obtained was dissolved in methanol and submitted to ESI-MS. The sodium adducts $[M + Na]^+$ were detected in all cases. The ions of the trimers (dimers, tetramers) were isolated in the ion trap and submitted to CID (Fig. 1). The daughter ion spectra were recorded at an amplitude where the relative ion intensities are constant. To check this, the resonance amplitude which excites the collision gas in the ion trap, was increased until no further change of the relative fragment intensities was observed, but only the mother ion was further decreased. This was the case when the intensity of the mother ion was smaller than that of the most intense daughter ion (Fig. 2).

Table 1
Methyl pattern of maltooligosaccharides prepared from regioselectively methylated β-cyclodextrins, only the α-form is shown




| β·CD | $R^2$ | $R^3$ | $R^6$ |
|---|---|---|---|
| **1** | H | H | H |
| **2** | $CH_3$ | H | $CH_3$ |
| **11** | H | H | $CH_3$ |
| **15** | H | $CH_3$ | H |
| **17** | H | $CH_3$ | $CH_3$ |
| **20** | $CH_3$ | $CH_3$ | H |
| **21** | $CH_3$ | H | H |
| **22** | $CH_3$ | $CH_3$ | $CH_3$ |

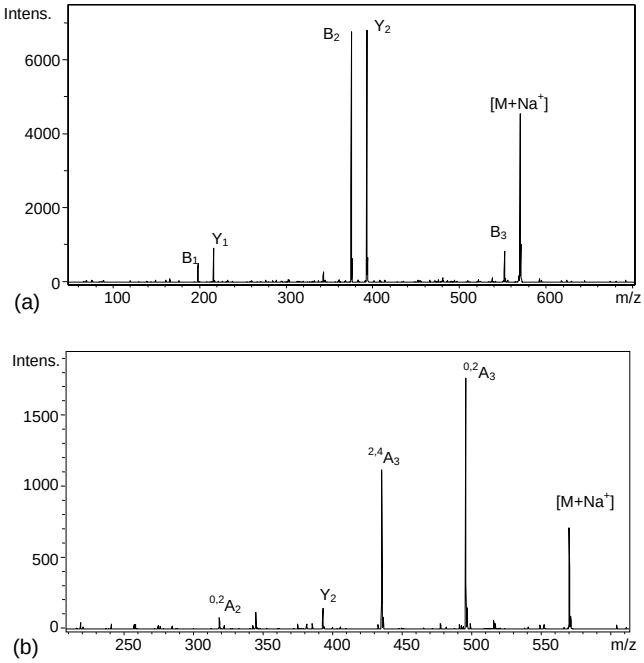



Fig. 1. ESI-MS$^2$ spectrum of (a) (3-*O*-Me-α-glc)$_3$ and (b) (2-*O*-Me-α-glc)$_3$; mother ion: *m/z* 569.

### 2.1. Maltotriose derivatives

The ion intensities were normalised to 100% and are presented in Fig. 3 for the maltotrioses which consist of a reducing, a non-reducing, and one internal glucosyl unit. In the following we will discuss the fragmentation pathways of these model compounds with regard to the methylation pattern. The nomenclature of Domon and Costello is used [13] and demonstrated in Scheme 1. The corresponding *m/z* values are listed in Table 2. Two patterns are always combined in one graphical illustration: unsubstituted maltotriose glc$_3$ and (6-*O*-Me-α-glc)$_3$ (Fig. 3a), (2-*O*-Me-α-glc)$_3$ and (2,6-di-*O*-Me-α-glc)$_3$ (Fig. 3b), (3-*O*-Me-α-glc)$_3$ and (3,6-di-*O*-Me-α-glc)$_3$ (Fig. 3c), and (2,3-di-*O*-Me-α-glc)$_3$ and (2,3,6-tri-*O*-Me-α-glc)$_3$ (Fig. 3d). It is obvious that there is nearly no influence of the status of position 6. That means that the 6-OH is not involved in ion stabilisation and proton transfer processes during fragmentation. Y and B ions, which are formed by cleavage of the glycosidic bond between C-1 and the glycosidic oxygen are the dominating ones for all derivatives with a free 2-OH (Fig. 3a and c). C and Z fragments which would have the same masses

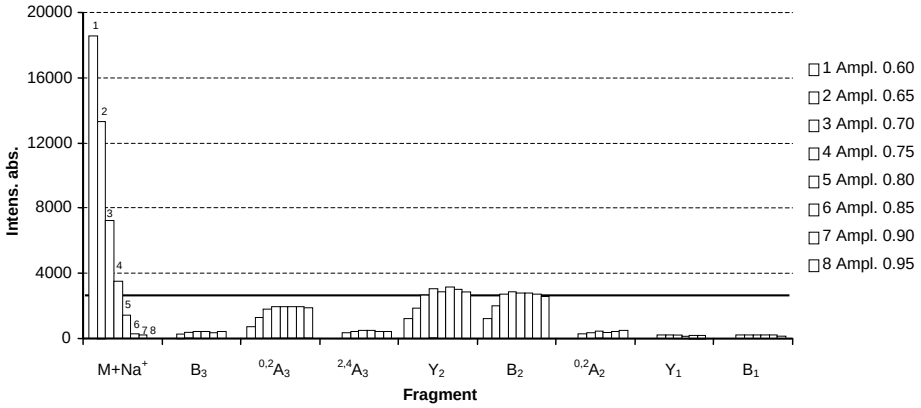



Fig. 2. Absolute intensities of fragment ions in comparison to the mother ion in dependence of the excitation energy, given as relative amplitude. The intensities of the fragment ions become constant when the intensity of the residual mother ion is about that of the most intense daughter ions (line). The amplitude required was separately determined for each sample.

as Y and B fragments, respectively (see Scheme 1) could be excluded by $^{18}$O-labelling at the reducing end. This is in agreement with the observations of Hofmeister et al. [14] and of Friedl et al. [15] and was expected, since the glycosidic C1-O-linkage is more easily cleaved than the C4-O-ether linkage. Z fragments could further be excluded by ESI-MS$^3$ experiments. Since Z, but not the B fragment has still a reducing end, cross-ring cleavage is only expected for MS$^3$ of a Z fragment. Since no ring fragmention was observed in MS$^3$ the signals were assigned to the B series. The cleavage of Y- and B-fragments includes the transfer of a proton to the leaving aglycon (O-4′). This is provided from the free 2-OH by the kinetically favoured formation of an 1,2-anhydro sugar (Scheme 2a). Depending on the location of the coordinated sodium ion, the B or Y fragment is observed. Since $B_2$ and $Y_2$ ions are observed with nearly equal intensity for the "2-OH-compounds" at a relative ratio of about 10:1 to the corresponding $Y_1$ and $B_1$ ions, sodium is obviously better coordinated by two sugar units than by one and therefore retained on the disaccharide fragment. In addition, cross-ring fragments $^{0,2}A_3$, and to a lower extent $^{2,4}A_3$ and $^{0,2}A_2$ are observed for maltotriose and the 6-*O*-methyl derivative (Fig. 3a). Cross-ring cleavage requires a reducing end [16]. Therefore, $^{0,2}A_{n-1}$ ions require a $Y(C)_{n-1}$ precursor or are formed from the corresponding $A_n$ ions by the loss of an anhydro glucose from the non-reducing end (→Y cleavage). Cleavage of the C-2–C-3 linkage (→ $^{0,2}A_n$) can be explained by a retro-aldol type reaction [17,18] of the open chain aldehyde form of the sugar and is initiated by deprotonation of OH-3 (see Scheme 3). Since these fragmentations, in contrast to the cleavage of glycosidic linkages ("charge-site-process"), are independent of the adduct ion ("charge-remote-process," [19]) the oxygens of the sugar, here presumably O-5, must promote this process. A new aldehyde (at C-3) with a β-hydroxy group (at C-5) is thus formed which can undergo a further retro-aldol-cleavage between C-4 and C-5 (→ $^{2,4}A_n$).

Table 2
Fragment ions of partially methylated maltotriose derivatives observed as sodium adducts in ESI-MS$^2$

| Fragment | Position of methyl group | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | – | 2 | 3 | 6 | 2, 3 | 2, 6 | 3, 6 | 2, 3, 6 |
| | *m/z* | | | | | | | |
| M | 527 | 569 | 569 | 569 | 611 | 611 | 611 | 653 |
| $B_3$ | 509 | – | 551 | 551 | 593 | 593 | 593 | – |
| $^{0,2}A_3$ | 467 | 495 | – | 509 | 537 | 537 | – | 579 |
| $^{2,4}A_3$ | 407 | 435 | – | 435 | – | 463 | – | – |
| $Y_2$ | 365 | 393 | 393 | 393 | 421 | 421 | 421 | 449 |
| $B_2$ | 347 | – | 375 | 375 | 403 | 403 | 403 | 431 |
| $^{0,2}A_2$ | 305 | 319 | – | 333 | 347 | 347 | – | 375 |
| $^{2,4}A_2$ | 245 | 259 | – | 259 | 287 | 273 | – | 301 |
| $Y_1$ | 203 | – | 217 | 217 | 231 | – | 231 | 245 |
| $B_1$ | 185 | – | 199 | 199 | 213 | – | – | 227 |

When position 2 is blocked by methylation, but 3-OH is available [(2-*O*-Me-α-glc)$_3$ and (2,6-di-*O*-Me-α-glc)$_3$], $B_2$ and $Y_2$ are only detected at very low relative intensities (Fig. 3b). This proves that the 2-hydroxy group is the preferred proton donor for the leaving sugar residue. It is assumed that small amounts of B and Y fragments can be explained by an energetically less favoured alternative: deprotonation of 2-CH yielding an 1,2-unsaturated cyclic ether (2H-dihydropyran, see Scheme 2b). Surprisingly, availability of 6-OH in (2-*O*-Me-α-glc)$_3$ does not result in enhanced B/Y ion intensities, although 1,6-anhydrosugar formation seems to be likely. Thus, for the 2,6- as well as for the 2-*O*-methyl trimers cross-ring cleavage becomes

Those maltooligomers which are methylated at O-3, but not at O-2 (Fig. 3c), show preferred formation of Y- and B-fragments in a similar ratio as for unsubstituted maltotriose. The just described cross-ring cleavage mechanism is no longer possible, since O-3 is blocked by the methyl group. Although OH in position 5 is still available, the $^{2,4}A_n$ fragment is no longer observed since it is a result of consecutive retro-aldol-reactions, the first of which is inhibited now.

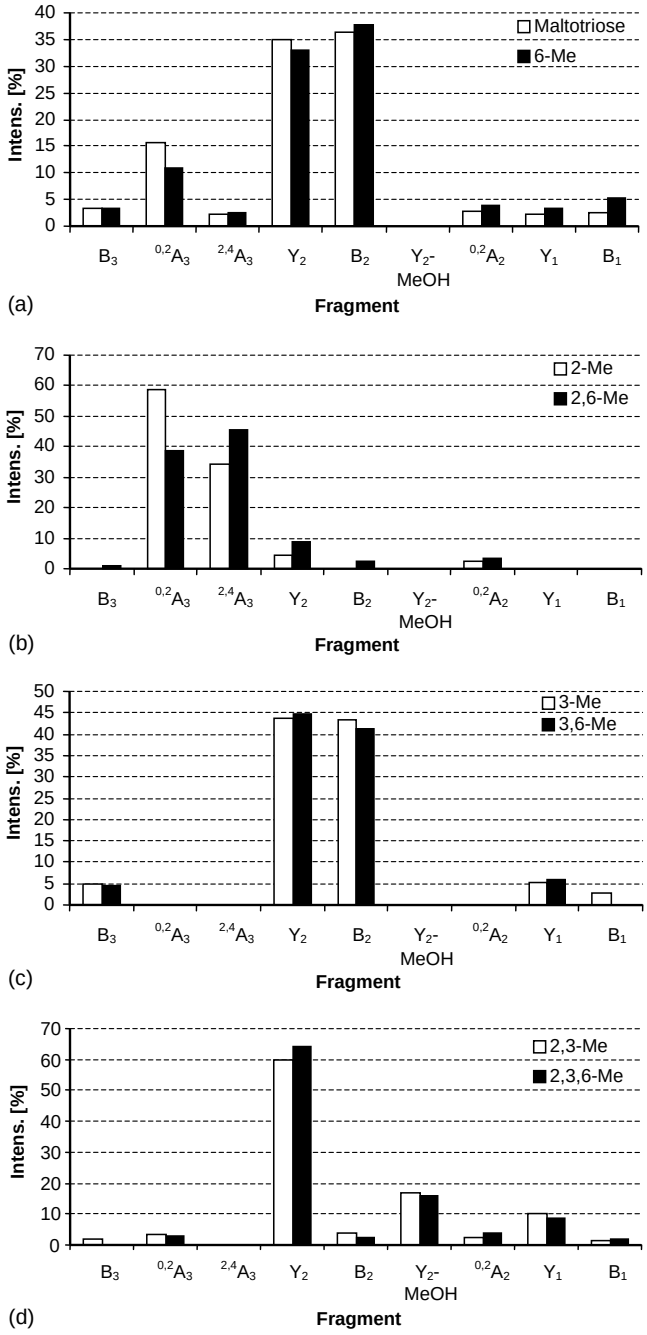
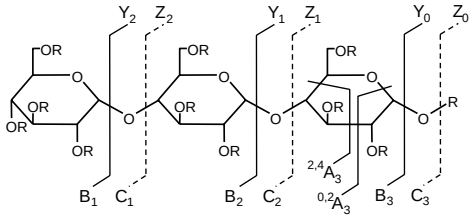



Fig. 3. Relative intensities of ESI-MS$^2$ fragment ions of all methyl patterns of maltotriose derivatives; *x*- and *x*,6-patterns are directly compared; (a) maltotriose and (6-*O*-Me-α-glc)$_3$; (b) (2-*O*-Me-α-glc)$_3$ and (2,6-di-*O*-Me-α-glc)$_3$; (c) (3-*O*-Me-α-glc)$_3$ and (3,6-di-*O*-Me-α-glc)$_3$; (d) (2,3-di-*O*-Me-α-glc)$_3$ and (2,3,6-tri-*O*-Me-α-glc)$_3$.



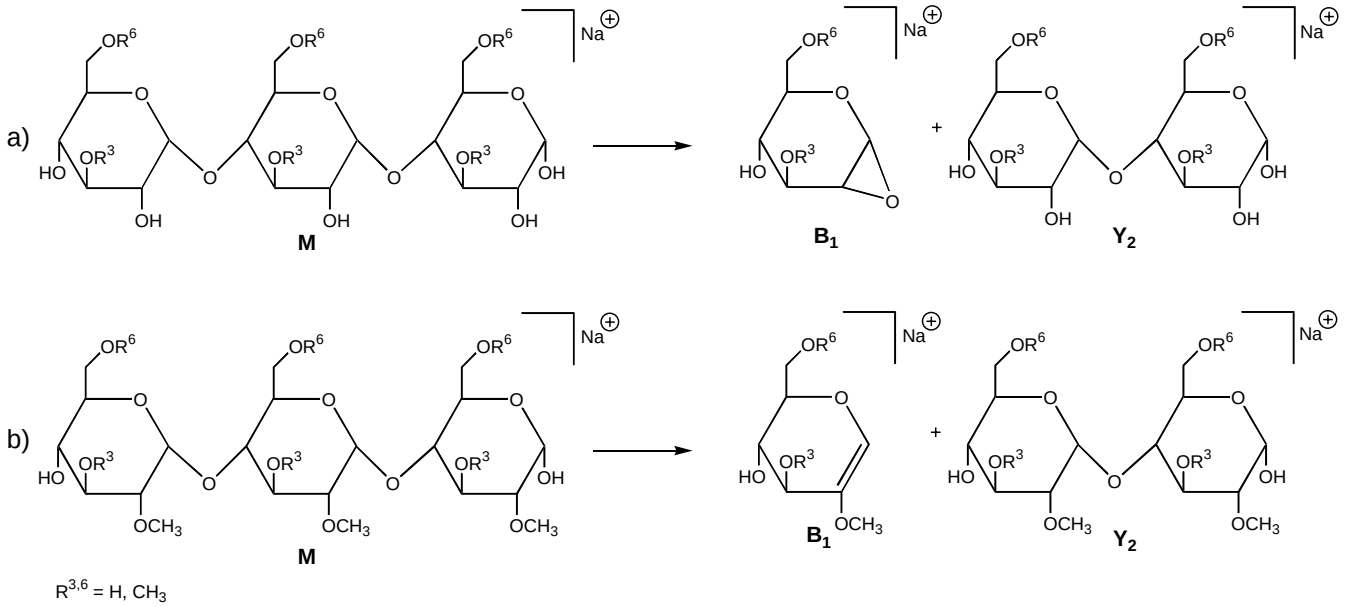

Scheme 1. Fragmentation of oligosaccharides nomenclature according to Domon and Costello [13].

the preferred process. $^{0,2}A_3$ and consecutively $^{2,4}A_3$ are formed in a ratio of 1.7 (2-Me) and 0.85 (2,6-Me), respectively. This is the only significant difference caused by *O*-6-methylation. In contrast, the ratio of $^{0,2}A_3$ to $^{2,4}A_3$ is more similar for unsubstituted maltotriose and the 6-*O*-methyl derivative. In addition, these compounds are also able to form B and Y fragments. Therefore, sugar ring fragmentation is less pronounced in comparison to the corresponding *O*-2-methylated derivatives.

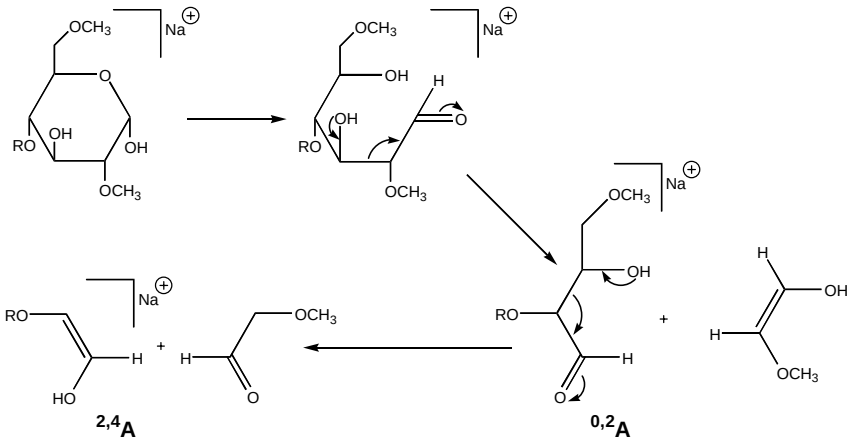
When both the 2- and 3-position are blocked, the fragmentation pathways described are inhibited. Now $Y_2$ is the predominating fragment ion with 60% for (2,3-di-*O*-Me-α-glc)$_3$ and 64% for (2,3,6-tri-*O*-Me-α-glc)$_3$ of the total intensity, followed by $Y_1$ with 10 and 9%, respectively (Fig. 3d). B fragments as well as ring cleavage products are no longer or only weakly detected. It is surprising that even now the 6-OH does not serve as an alternative source for proton transfer. The high preference of $Y_2$ (=$Y_{n-1}$, with $n$ = number of glucosyl units) might be explained by participation of the terminal 4-OH. The terminal residue could be lost as 1,4-anhydro-glucopyranose, while the proton is transferred to the O-4′ of the leaving maltose (Scheme 4). $Y_2$ from (2-*O*-Me-α-glc)$_3$ and (2,6-di-*O*-Me-α-glc)$_3$ (4.4 and 8.6% relative intensity, respectively) could be formed in the same way. The $Y_{n-1}$ ions also dominate the MS$^2$ spectra of 2,3-di-*O*-methyl maltose and maltotetraoses.

Principally, $Y_2$ could also be the result of an internal loss of a glucosyl unit [20]. This fragmentation mode has been described by many authors, and the fragment has been named Y*, however exclusively for protonated molecule adducts $[M+H]^+$, but never for $[M+Na]^+$. Therefore, we assume that internal loss can be excluded in our case. For an additionally *O*-4-methylated trisaccharide formation of $Y_2$ according to our model suggested in Scheme 4 would no longer be possible, but internal loss should give an Y* ion with +14 Da. This shift is not detected. The main fragment in this mass spectrum is $Y_2$ with free 4-OH and might been formed according to Scheme 2b. In addition to the fragmentations of Scheme 1 loss of methanol is observed from M, $Y_1$ and $Y_2$ in case of the 2,3-di-*O*-methyl and 2,3,6-tri-*O*-methyl compounds. Labelling experiments with Me-$d_3$ clearly indicate for $Y_1$ and $Y_2$ that methanol is eliminated from position 3, while it could not be undoubtedly located for [M − MeOH] due to very low intensity of this ion. No loss of methanol from the monomethylated derivatives is observed, since here the energetically more favoured fragmentations are preferred. In Fig. 3 only [$Y_2$ − MeOH] is included because [M − MeOH] and [$Y_1$ − MeOH] are observed as minor fragments of about 1% relative intensity only.

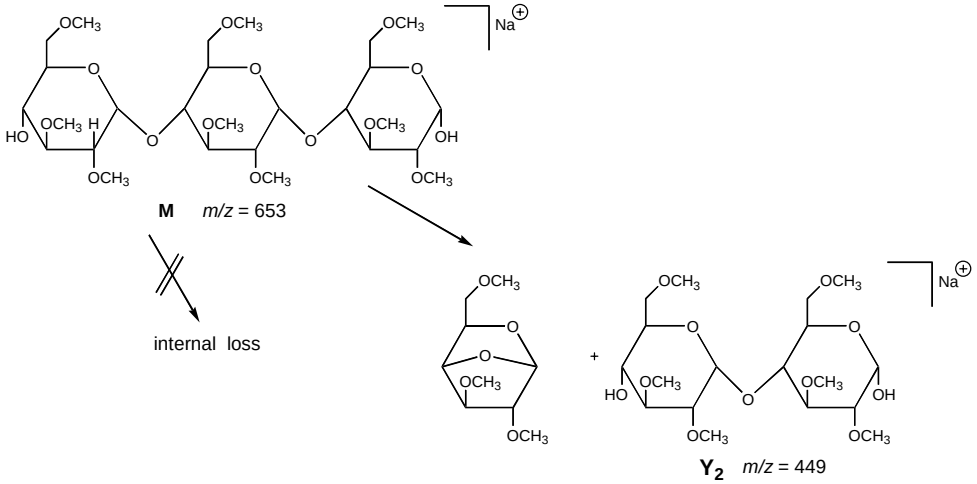
Mass shifts observed for the Me-$d_3$-labelled compounds (2,3-Me-6-Me-$d_3$-α-glc)$_3$, (2,6-Me-3-Me-$d_3$-α-glc)$_3$, and




Scheme 2. Formation of $B_1$ and $Y_2$ fragments for oligosaccharides with free 2-OH (a) and for 2-*O*-methyl derivatives (b); both possibilities of sodium adduct formation are shown. In the same way, $B_2$ and $Y_1$ could be formed.




Scheme 3. Formation of A-fragments by cross-ring cleavage of oligosaccharides with free 3-OH.




Scheme 4. Possible pathway to explain preferred $Y_2$ formation of (2,3,6-tri-*O*-α-Me-glc)$_3$.

(2,3,6-Me-$d_3$-α-glc)$_3$ are in full agreement with the fragmentation pathways discussed.

### 2.2. Comparison with the fragmentation mode of regioselectively O-methylated maltose and maltotetraose

The similarity of the MS$^2$ spectra of corresponding (6-OH-α-glc)$_n$- and (6-*O*-Me-α-glc)$_n$ derivatives is also observed for maltose and maltotetraose. Maltotetraose differs from maltotriose by two instead of one internal residue. So the corresponding daughter ion spectra are a little bit more complex (Fig. 4a–d). Y and B fragments are observed for all patterns with a free 2-OH with slight differences in intensities in the order $Y_3 > B_2 = B_3 > Y_2$ while $Y_1$ and $B_1$ are not detected. This indicates the stronger binding of the sodium ion by a dimer or trimer with more coordination sites. Since the (6-*O*-Me-α-glc)$_4$ and maltotetraose itself can also undergo cross-ring fragmentation, the relative intensities of B and Y ions are decreased. The compounds blocked at O-2 nearly exclusively show A-fragments and only minor contributions of the B and Y fragments as mentioned above with a higher tendency to be cleaved at the glycosidic linkage for (2,6-di-*O*-Me-α-glc)$_3$ compared to (2-*O*-Me-α-glc)$_3$. Again, $Y_{n-1}$ is the preferred ion of glucosidic linkage cleavage, presumably due to the participation of terminal 4-OH as discussed for maltotriose. Y ions are the predominating fragments for the (2,3-di-*O*-Me-α-glc)$_4$ and (2,3,6-tri-*O*-Me-α-glc)$_4$ with 78% (2,3-Me$_2$) and 83%

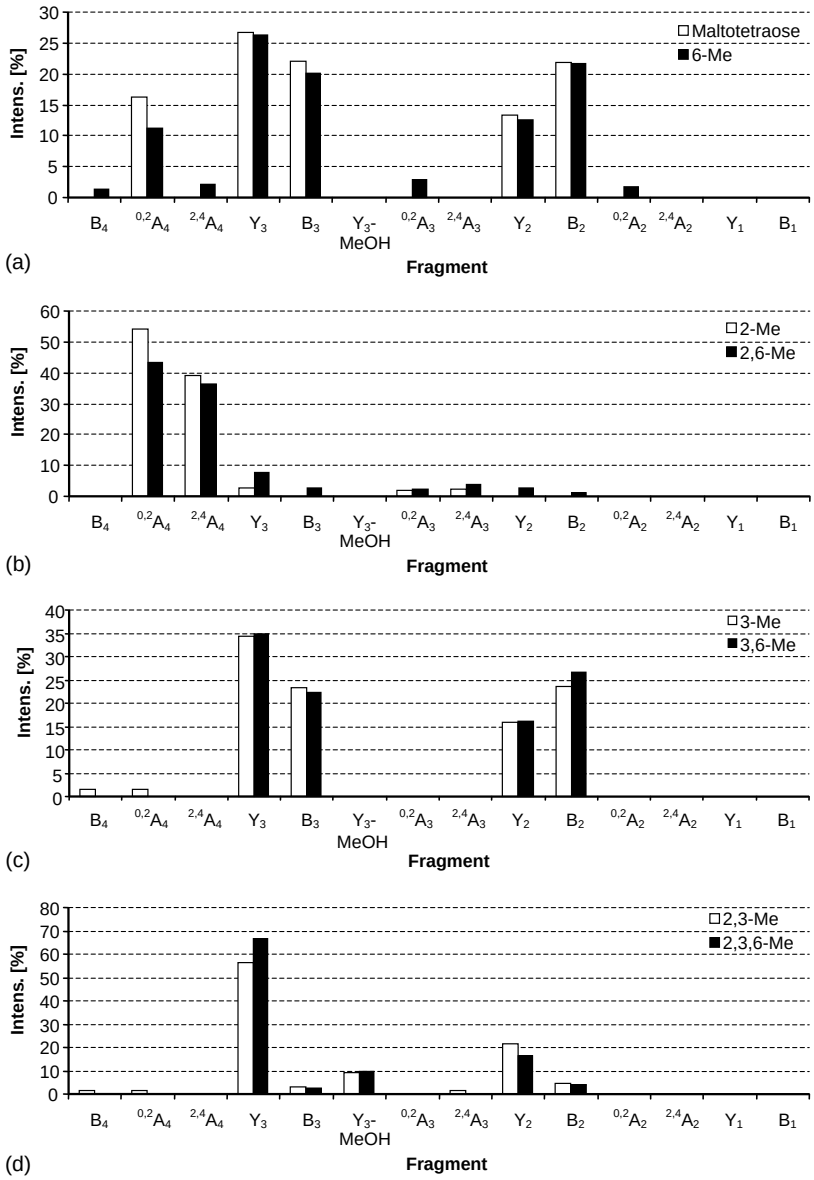



Fig. 4. Relative intensities of ESI-MS$^2$ fragment ions of all methyl patterns of maltotetraose derivatives; *x*- and *x*,6-patterns are directly compared; (a) maltotetraose and (6-*O*-Me-α-glc)$_4$; (b) (2-*O*-Me-α-glc)$_4$ and (2,6-di-*O*-Me-α-glc)$_4$; (c) (3-*O*-Me-α-glc)$_4$ and (3,6-di-*O*-Me-α-glc)$_4$; (d) (2,3-di-*O*-Me-α-glc)$_4$ and (2,3,6-tri-*O*-Me-α-glc)$_4$.

(2,3,6-$Me_3$) relative intensity and a $Y_3/Y_2$ ratio of 2.6 for the 2,3-di-*O*-, and of 3.9 for the 2,3,6-tri-*O*-Me derivative. $Y_2$ might be formed by a consecutive loss of a glucosyl unit from $Y_3$ as precursor. The higher relative probability of $Y_2$ formation for the 2,3-di-*O*-methyl derivative may be an indication of at least a partial contribution of the 6-OH. Again, loss of methanol is observed for M, $Y_3$, and $Y_2$ of the 2,3-di-*O*-methyl and 2,3,6-tri-*O*-methyl compounds. The labelling experiments with Me-$d_3$ confirm the elimination from position 3 for Y ions as mentioned for maltotriose derivative. In Fig. 4 only the most intense fragment [$Y_3$-MeOH] is included.

Maltose (Fig. 5a–d) shows some specialities, since only monomeric products can be formed by cleavage of the glucosidic linkage: $Y_1$ dominates the $MS^2$ spectra of all 3-*O*-methyl derivatives. For the 2,3-di-*O*-methyl and 2,3,6-tri-*O*-methyl compounds the already mentioned loss of methanol is observed. Now, the origin of OMe could undoubtedly be located by comparison of the regioselectively Me-$d_3$ labelled 2,3,6-tri-O-Me-α-glc)$_3$ isomers. While MeOH is eliminated from C-3 for $Y_1$ as precursor, it surprisingly originates from C-2 for eliminations from the mother ion. Only [$Y_1$ − MeOH] is included in Fig. 5, since [M-MeOH] again is only a minor fragment. Those maltose derivatives which bear a free 2-OH yield 18% (6-Me), 25% (3-Me and 3,6-Me) or 29% (maltose) $B_2$, which means elimination of water, but only 4-5% $B_1$, again indicating the higher ability of sodium adduct formation for the dimer. Cross-ring fragmentation requires a reducing end and is therefore nearly independent of the DP, which only determines the possibilities of consecutive cross-ring cleavages of Y fragments, while the probability of glycosidic linkage cleavages increases with increasing DP (degree of polymerisation). $^{0,2}A_2$ is the main fragment of 2- and (2,6-di-*O*-Me-α-glc)$_2$ and only small signals of the subsequent $^{2,4}A_2$ ion are detected.

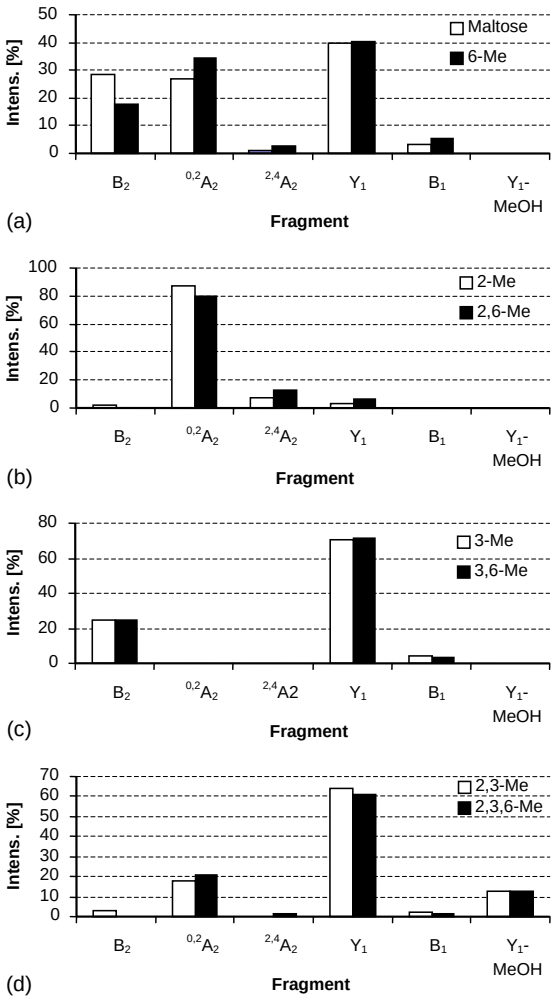



Fig. 5. Relative intensities of ESI-$MS^2$ fragment ions of all methyl patterns of maltose derivatives; *x*- and *x*,6-patterns are directly compared; (a) maltose and (6-*O*-Me-α-glc)$_2$; (b) (2-*O*-Me-α-glc)$_2$ and (2,6-di-*O*-Me-α-glc)$_2$; (c) (3-*O*-Me-α-glc)$_2$ and (3,6-di-*O*-Me-α-glc)$_2$; (d) (2,3-di-*O*-Me-α-glc)$_2$ and (2,3,6-tri-*O*-Me-α-glc)$_2$.

With a relative intensity of about 20% a fragment ion could be observed for (2,3-di-*O*-Me-α-glc)$_2$ and (2,3,6-tri-*O*-Me-α-glc)$_2$ with *m/z* corresponding to $^{0,2}A_2$. From the $MS^2$ spectra of the regioselectively Me-$d_3$ labelled permethylated isomers it can be deduced that methyl from O-2 is lost. Smaller amounts (2–4% relative intensity) of these supposed A fragments have also been observed for the corresponding trimers and tetramers. Their formation is inconsistent with the mechanisms discussed above (Scheme 3). It must be mentioned that fragmentation of (2,3,6-tri-*O*-Me-α-glc)$_2$ requires a higher excitation energy (amplitude 0.8–0.85 V) than the other derivatives (0.65–0.7 V). Since the "normal" fragmentations are energetically less favoured due to the low DP and the inhibition by methylation, less probable fragmentations become more competitive.

### 2.3. Influence of the configuration of the glucosidic bond

Cellotriose and (2,3-di-*O*-Me-β-glc)$_3$ were easily available from commercial cellooligosaccharides and 2,3-di-*O*-methyl-cellulose [21] by partial hydrolysis. (2,3,6-tri-*O*-Me-β-glc)$_3$ was obtained by permethylation of commercial methyl cellulose (DS = 1.9) and partial hydrolysis. They were also investigated by means of ESI-$MS^2$ to find out whether there is an influence of the configuration of the glucosidic bond. Although the same fragments are observed, relative intensities significantly deviate from the corresponding α-isomers (Fig. 6a–c). B fragments are much more favoured in the β-glucans (cellotriose) compared to the α-series. In addition, bigger differences between the daughter mass spectra of (2,3-di-*O*-Me-β-glc)$_3$ and (2,3,6-tri-*O*-Me-β-glc)$_3$ are observed as for the maltotriose derivatives. This indicates a higher contribution of the 6-position of β-configurated glucans compared to α, which corresponds with the higher relative reactivity of the 6-OH in cellulose compared to amylose. Due to these surprising great differences we will further investigate the other β-isomers.

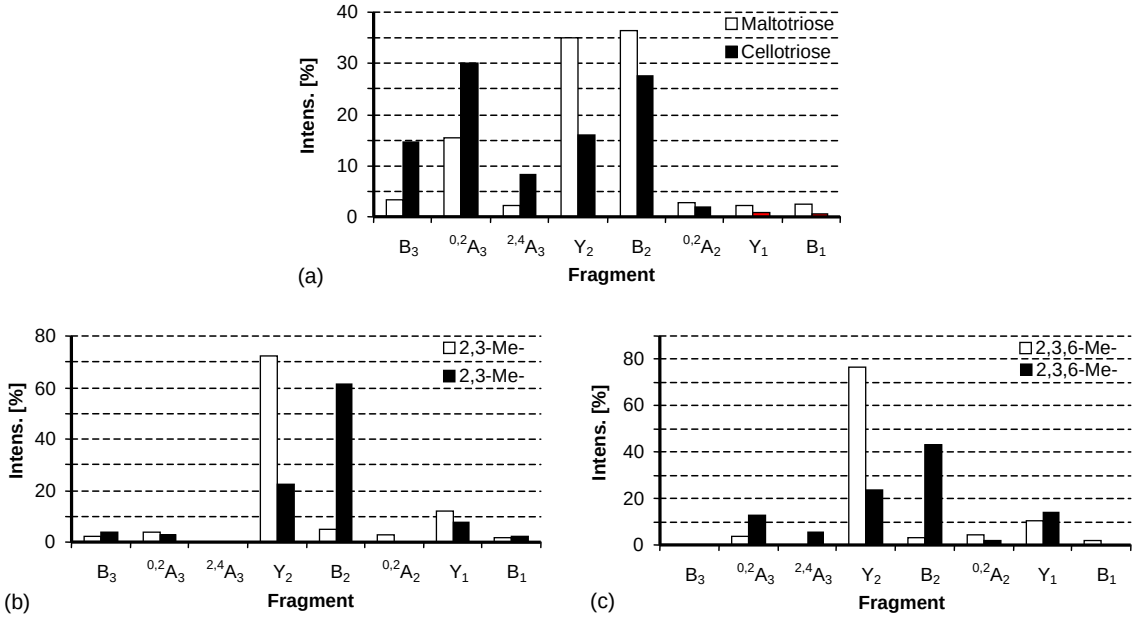



Fig. 6. Influence of the configuration of the glucosidic bonds in 1,4-linked gluco-trimers on the ESI-MS$^2$ spectrum. Comparison of relative intensities of (a) unsubstituted malto- and cellotriose, (b) (2,3-di-*O*-Me-glc)$_3$, and (c) (2,3,6-tri-*O*-Me-glc)$_3$.

## 3. Experimental

### 3.1. General

All reagents were at highest purity available and purchased from Fluka, Aldrich or Merck. β-Cyclodextrin (**1**) was purchased from Aldrich, heptakis[2,6-di-*O*-methyl]-β-cyclodextrin (**2**) from Cyclolab (Budapest, Ungarn). Heptakis[2-*O*-methyl-3,6-di-*O*-THxDMS]-β-cyclodextrin (**3**) [22] and 2,3-di-*O*-methyl-cellulose (**4**) [21] have been described elsewhere. Methyl cellulose (DS = 1.9) (**5**) was purchased from Aldrich and cellooligosaccharides (**6**) from Merck. The solvent $CDCl_3$ was purchased from ARMAR (Döttingen, CH). For purification silica gel 60 (63–200 μm, Merck) was used.

### 3.2. Instrumental

Electrospray ionisation mass spectra (ESI-MS, positive mode) were recorded on an Esquire LC (Bruker Daltonics, Bremen, Germany). The samples were dissolved in MeOH-$CHCl_3$ (1:1, v/v), and introduced directly via a syringe at a flow of 240 μl/h. The mass spectra for MS$^2$ experiments consist of an average of 200 scans. Nitrogen is used as drying gas (4 l/min, 300 °C) and as nebuliser gas (10 psi). The following Voltages were used: capillary 4500 V, end plate offset −500 V, capillary exit 120.0 V, skim 140.0 V and skim 2 10.0 V. The amplitude of the resonance frequency which excites the ions before fragmentation in the ion trap was optimised for every ion and was between 0.55 and 0.9 V. The isolation width for MS$^2$ experiments was 4 *m*/*z*.

NMR spectra were measured with a Bruker AMX 300 instrument ($^1$H: 300 MHz, $^{13}$C: 75 MHz) or a Bruker AMX 400 ($^1$H: 400 MHz; $^{13}$C: 100 MHz).

### 3.3. Sample preparation

The regioselectively methylated cyclodextrins (**2**, **11**, **15**, **17**, **20**, **21**, **22**), the unsubstituted cyclodextrin **1**, the regioselectively methylated celluloses (**4**, **23**) and the unsubstituted cellooligosaccharides **6** (2–5 mg) were stirred in a 1 ml V-vial with 2 M trifluoroacetic acid (1 ml) at 120 °C for 12 min. After cooling the acid was removed in a stream of nitrogen. Residues of the acid were removed by co-distillation with toluene (five times).

The regioselectively methylated β-cyclodextrins were synthesised according to Takeo et al. [10,11] via the following intermediates: heptakis[6-*O*-TBDMS]-β-cyclodextrin (**7**), heptakis[2,3-di-*O*-acetyl-6-O-TBDMS]-β-cyclodextrin (**8**), heptakis[2,3-di-*O*-acetyl]-β-cyclodextrin (**9**), heptakis-[2,3-di-*O*-acetyl-6-*O*-methyl]-β-cyclodextrin (**10**), heptakis-[6-*O*-methyl]-β-cyclodextrin (**11**), heptakis[2-*O*-benzyl-6-*O*-TBDMS]-β-cyclodextrin (**12**), heptakis[2-*O*-benzyl-3-*O*-methyl-6-*O*-TBDMS]-β-cyclodextrin (**13**), heptakis[2-*O*-benzyl-3-*O*-methyl]-β-cyclodextrin (**14**), heptakis[3-*O*-methyl]-β-cyclodextrin (**15**), heptakis[2-*O*-benzyl-3,6-di-*O*-methyl]-β-cyclodextrin (**16**), heptakis[3,6-di-*O*-methyl]-β-cyclodextrin (**17**), heptakis[2,3-di-*O*-methyl-6-*O*-TBDMS]-β-cyclodextrin (**18**), heptakis[2,3-di-*O*-methyl-6-*O*-acetyl]-β-cyclodextrin (**19**) and heptakis[2,3-di-*O*-methyl]-β-cyclodextrin (**20**). All compounds were purified by column chromatography and characterised by NMR spectroscopy and ESI-MS. Spectroscopic data were in accordance with

the literature. Heptakis[2-*O*-methyl]-β-cyclodextrin (**21**) was obtained by deprotection of heptakis[2-*O*-methyl-3,6-di-*O*-THxDMS]-β-cyclodextrin (**3**) according to the procedure reported by Icheln et al. [12]. NMR spectra were in accordance with the literature. Heptakis[2,3,6-tri-*O*-methyl]-β-cyclodextrin (**22**) was obtained by permethylation of **1**. 2,3,6-Tri-*O*-methyl-cellulose (**23**) was prepared by permethylation of methyl cellulose of DS 1.9 (**5**). Heptakis[2,3-di-*O*-methyl-6-*O*-methyl-$d_3$]- (**22**-6-Me-$d_3$), heptakis[2,6-di-*O*-methyl-3-*O*-methyl-$d_3$]- (**22**-3-Me-$d_3$) and heptakis[2,3,6-tri-*O*-methyl-$d_3$]-β-cyclodextrin (**22**-2,3,6-Me-$d_3$) were prepared by permethylation of **20**, **2**, and **1** with MeI-$d_3$, respectively. All methylations were performed according to Ciucanu and Kerek [23] with NaOH/MeI in DMSO.

## 4. Conclusion

Maltooligomers of all possible uniform methylation patterns present in partially methylated 1,4-α-glucans have been obtained from the corresponding β-cyclodextrins. ESI-$MS^2$ spectra of the $[M + Na]^+$ pseudomolecular ions show a strong influence of the status of O-2 and O-3, but nearly no effect of *O*-6-methylation. Ring-cross cleavage is the preferred process when position 2 is blocked, while it is prevented by *O*-3-methylation. If an exchangeable proton is available in an appropriate position, B and Y ions are formed by cleavage of a glucosidic bond and proton transfer. The proton is best delivered from the most acidic 2-OH, but if not available, terminal 2-CH and probably 4-OH can also contribute, while 6-OH surprisingly plays no or only a minor role as proton donor. Loss of methanol from position 3 of $Y_{n-1}$ is also observed. These less favoured alternatives are especially observed for the 2,3-di-*O*-methyl and 2,3,6-tri-*O*-methyl maltooligomers. While the daughter ion mass spectra of maltotetraose correspond to those of maltotriose, maltose derivatives show some specialities. Due to the fact that coordination of sodium ion preferably involves two or more glycosyl units, cleavage of the only glycosidic bond is less favoured and usually less probable fragmentations become more competitive. These systematic studies on defined model compounds will help to interpret the ESI-$MS^n$ spectra of maltooligosaccharides obtained from starch ethers by chemical or enzymatic degradation. First investigations on the corresponding cellooligomer derivatives show a significant influence of the configuration of the glucosidic linkage. Further investigations are in progress.

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