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A novel method for the analysis of the substitution pattern of *O*-methyl-α- and β-1,4-glucans by means of electrospray ionisation-mass spectrometry/collision induced dissociation

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Abstract

The substitution pattern of *O*-methyl amylose and *O*-methyl cellulose was analysed after per-*O*-methylation (Me-d_3), and partial hydrolysis by subsequent ESI-MS/CID of the sodium (MS^2) and the lithium adducts (MS^3). Based on previous studies about the influence of regioselective *O*-methylation on the fragmentation pathways of malto- and cello-oligosaccharides, we could calculate the contribution of a certain methyl pattern to a distinct signal in the reproducible ESI-MS^2 daughter spectrum. Signal intensities obtained from each *O*-methyl-*O*-methyl-d_3 disaccharide were distributed on the corresponding methyl patterns and accumulated for all peaks of the mother mass spectrum. Data from ESI-MS^2 were not sufficient for disaccharides bearing methyl and deuteromethyl groups in the combination 2 and 4, 3 and 3, or 4 and 2. Further independent information was obtained by ESI-MS^3 of the lithium adducts. Monomer composition of methyl celluloses and methyl amyloses obtained by this novel approach were in very good agreement with reference data from GLC of the partially methylated glucitol acetates after complete hydrolysis, reduction and acetylation.

Keywords: Electrospray ionisation-collision induced dissociation/mass spectrometry (ESI-CID/MS); *O*-Methyl glucans; Substitution pattern; Monomer composition

1. Introduction

The physicochemical and biological functions of polysaccharide derivatives are greatly affected by the number and pattern of their substituents. While location on certain positions in the glycosyl units might be essential in molecular recognition processes, the distribution on the structural level of the polymer molecules is of predominant importance for solubility or chain–chain interactions. Analysis of the substituent distribution in the glucosyl units of starch and cellulose derivatives has been performed by NMR spectroscopic methods or by chromatographic or electrophoretic separation of appropriate monomer derivatives.

^{13}C NMR suffers from its poor sensitivity, especially in the case of polymer solutions. The degree of 2- and 6-*O*-substitution can be calculated from the partly shifted signals of C-1 (C-1′) and C-6 (C-6′), while the partial DS of position 3 is less reliable estimated. To get a more uniform polymer peracylation is often performed prior to NMR analysis [1]. By means of HPLC of hydrolysates, un-, mono-, di-, and trisubstituted fractions can be separated and quantified [2]. HPAEC/PAD (High-pH anion exchange chromatography/pulsed amporometric detection) has been applied for anionic ethers like sulfoethyl- and carboxymethyl cellulose [3], but also for the water soluble monomers from glucan methyl ethers [4]. Calibration with isolated or synthesised standards is required for the PAD, and unfortunately, calibration curve is not linear. Recently, capillary electrophoresis (CE/UV) with alkaline borate buffer has been applied for

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the same spectrum of derivatives after reductive amination with aminobenzonitrile [5]. The most widely used line of approach to determine the complete monomer composition uses the high separation efficiency of gas chromatography with its well established coupling with mass spectrometry to identify even minor products and components of very complex mixtures, e.g., from hydroxyalkyl methyl celluloses. Quantification without standard compounds is also favoured by the effective carbon response concept for flame ionisation detectors [6]. Sample preparation usually includes several steps as permethylation, hydrolysis and subsequent reduction or direct reductive cleavage, and finally acetylation of hydroxy groups formed or liberated [7]. Methyl ethers can also be hydrolysed directly. Cationic or anionic ethers as well as alkali labile esters require special sample preparation procedures [8–10]. The general problems of all these procedures are the requirement of quantitative reactions on the polymer level and the risk of discrimination on the monomer level due to various polarities and volatilities in the range from unsubstituted and trisubstituted residues. Therefore, an independent reference method would be valuable.

To analyse the substitution pattern along the polymer chain, starch or cellulose derivatives are usually converted to mixed *O*-methyl-/*O*-methyl-d_3 ethers [7,9,11,12] in one or several steps. Mass spectrometry of di- and trisaccharides obtained after partial random degradation shows the substituent distribution in these diades or triades. The results are then compared with the data calculated for a random substitution.

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 [13–22]. To use this technique for further structure analysis of glucan derivatives, we recently investigated regioselectively *O*-methylated maltooligosaccharides by means of ESI-MS/CID [23]. This was the situation when we wondered whether it would be possible to deduce the complete monomer composition of a methyl glucan from the mother and daughter mass spectra of the samples prepared for oligomer analysis. That this indeed can successfully be

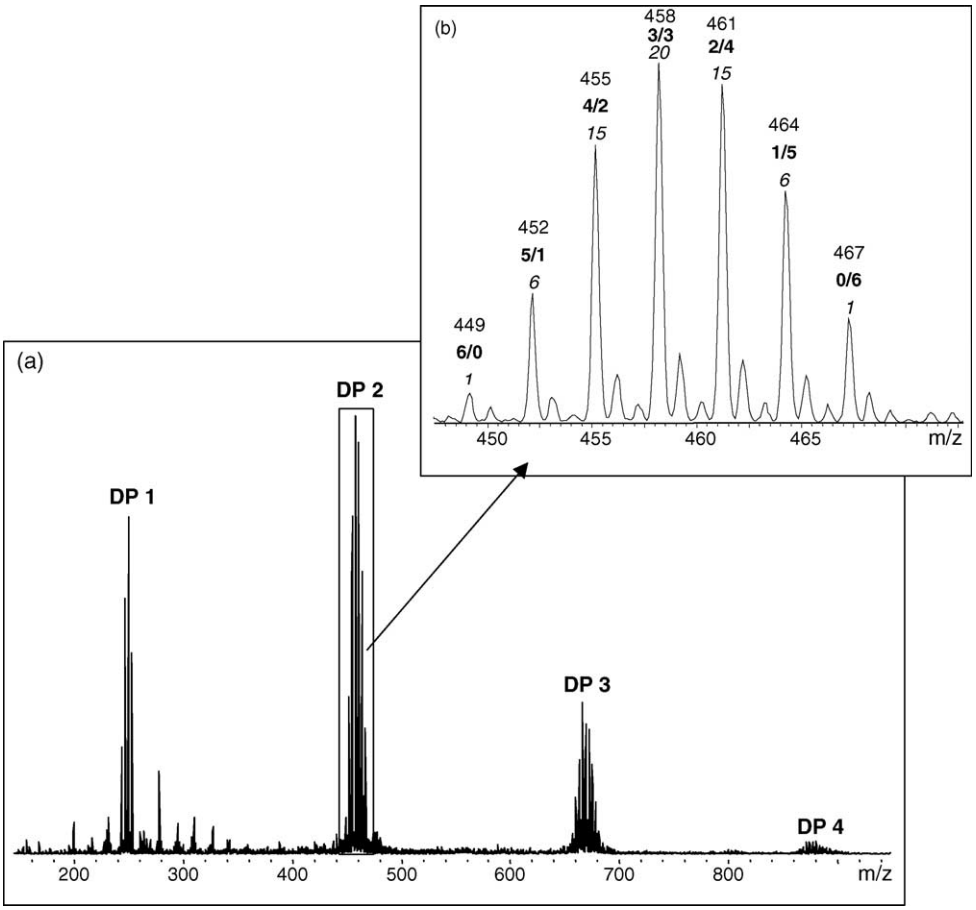


Fig. 1. ESI-MS mass spectrum of oligosaccharides obtained from methyl cellulose after perdeuteromethylation and random hydrolysis (a). Signals from the DP2 fraction are shown as enlarged detail and assigned with their *m*/*z* value, the ratio of Me and Me-d_3 groups, and the number of isomers (b).

Table 1
Substitution patterns of *O*Me/*O*Me-d_3 disaccharides, *m*/*z* values of the sodium adducts of un- to hexasubstituted fractions (C_0–C_6), and number of possible isomers

Fraction	*m*/*z* $[M+Na]^+$	Number of CH_3 groups in position			Variants total	Statistical probability (%)	Variants of B
		Total	A	B			
C_0	467	0	0	0	1	100	1
C_1	464	1	1	0	3	50	1
			0	1	3	50	3
C_2	461	2	2	0	3	20	1
			1	1	9	60	3
			0	2	3	20	3
C_3	458	3	3	0	1	5	1
			2	1	9	45	3
			1	2	9	45	3
			0	3	1	5	1
C_4	455	4	3	1	3	20	3
			2	2	9	60	3
			1	3	3	20	1
C_5	452	5	3	2	3	50	3
			2	3	3	50	1
C_6	449	6	3	3	1	100	1

done even from such complex oligosaccharide mixtures is demonstrated in the following.

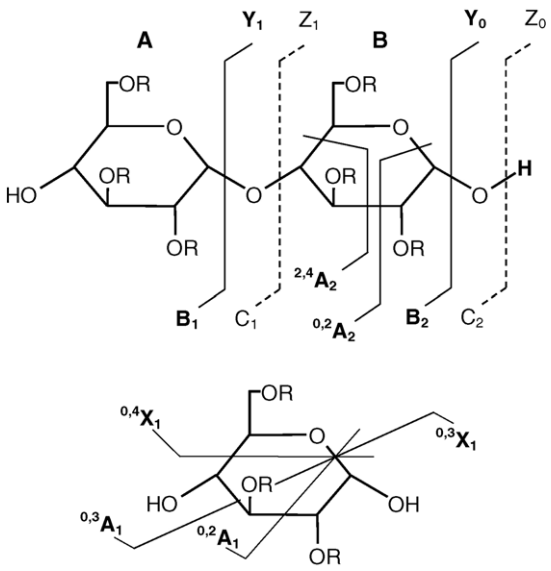
2. Results and discussion

Methyl amyloses and methyl celluloses were used for these studies. All samples were permethylated with MeI-d_3 and submitted to partial hydrolysis From the resulting oligosaccharide mixture, the distribution of methyl groups along the polymer chain [12] and, as will be demonstrated in this paper, the complete monomer composition can be determined by additional tandem-ESI-MS experiments. Thus, total hydrolysis, reduction, acetylation and gas chromatographic analysis can be omitted. The first idea was to deduce the monomer composition from the fragmentation of the DP 1 fraction of the partially hydrolysed perdeuteromethylated methyl glucans. However, the formation of $[M+Na]^+$ from these small molecules is comparably poor and stability under CID conditions relatively high. Therefore we focussed on the DP 2 fraction. An ESI-MS mother spectrum of the mixed *O*-methyl/*O*-methyl-d_3 disaccharides is shown in Fig. 1. Signals from *m*/*z* 449 to 467 with a difference of 3 are observed, representing $[M+Na]^+$ of disaccharides with six methyl groups (two trisubstituted glucosyl units, C_6) to those with six deuteromethyl groups (two originally unsubstituted glucosyl units, C_0) and all mixtures between (C_5 ... C_1) [24]. While the signals at both ends of the pattern represent only one isomer each, their composition becomes increasingly complex towards its center. The number of regioisomers contributing to a signal of certain *m*/*z* is listed in Table 1 and is assigned to the signals in Fig. 1b. By means of isolation and fragmentation of each of these complex disaccharide regioisomers in the ion trap of the mass spectrometer, further information about their composition is obtained. An important prerequisite is the assumption that the substituent pattern is symmetrical, i.e., the composition of monomers is represented by each glucosyl unit the disaccharide as well. This is a consequence of a random degradation where each glycosidic linkage is cleaved with the same probability independent of the substitution pattern.

2.1. ESI-MS^2—interpretation of the daughter spectra

Daughter mass spectra are recorded from the signals at *m*/*z* 452 (C_5), 455 (C_4), 458 (C_3), 461 (C_2), and 464 (C_1) representing more than one isomer. Fragments are assigned according to the nomenclature of Domon and Costello as illustrated in Scheme 1 for a 1,4-linked glucodisaccharide [13]. The fragmentation mechanisms have been elaborated in our previous studies on regioselectively methylated/deuteromethylated maltooligosaccharides [23].

Therefore, we know the position of methyl groups which are still present in a certain fragment ion and that there is no influence of the nature of the methyl group, i.e., whether isotopically labelled or not, on the relative intensities of the fragment ions as obvious from Fig. 2. Due to the symmetry of the disaccharides, we focus on the reducing end. Valuable information is obtained from Y_1 formed by cleavage of the glucosidic linkage, its consecutive fragment Y_1-MeOH (MeO from position 3), and the ring cleavage fragment ions $^{0,2}A_2$, resulting from retro-aldol cleavage of the reducing glucosyl residue. These are the most abundant signals.



Scheme 1. Nomenclature of fragments obtained from disaccharides and those obtained by MS^3 from Y_1 ions according to [13].

That methanol is selectively eliminated from position 3 of Y_1 is obvious from the loss of 35 mass units for the 2,6-di-*O*-Me-3-*O*-Me-d_3- and the 2,3,6-tri-*O*-Me-d_3 standards, and the loss of only 32 masses from 2,3,6-tri-*O*-Me- and 2,3-di-*O*-Me-6-Me-d_3 substituted disaccharides (Fig. 3). Minor signals as $^{2,4}A_2$ (see Fig. 2a) are not considered since small deviations of their intensities would cause relatively large ones in the calculation of the monomer composition. As mentioned in the beginning, we investigated malto- and cello-oligosaccharides. Structures of the fragment ions were identical, while the relative intensities were different, but reproducible, and could be regarded in the evaluation. Therefore, it was possible to analyse both, α- and β-glucans.

The principle of our strategy is explained by the fragmentation of *m*/*z* 464 (C_1), consisting of one originally unsubstituted and one mono-*O*-methylated glucosyl residue. Three isomers are possible for the monosubstituted units: 2, 3, and 6. Table 2 lists the fragment ions which are observed in $ESI\text{-}MS^2$. First of all the intensities of all Y_1 signals (*m*/*z* 251 and *m*/*z* 254), all Y_1-R^3OH signals (*m*/*z* 216 and *m*/*z* 219), and the $^{0,2}A_2$ (*m*/*z* 387 and *m*/*z* 390) are summarised and the relative intensities of these signal groups are calculated. They are in the range of 65:13:22 for α-1,4-glucans (see Fig. 2a), and in the range of 29:47:24 for β-1,4-glucans. Using these

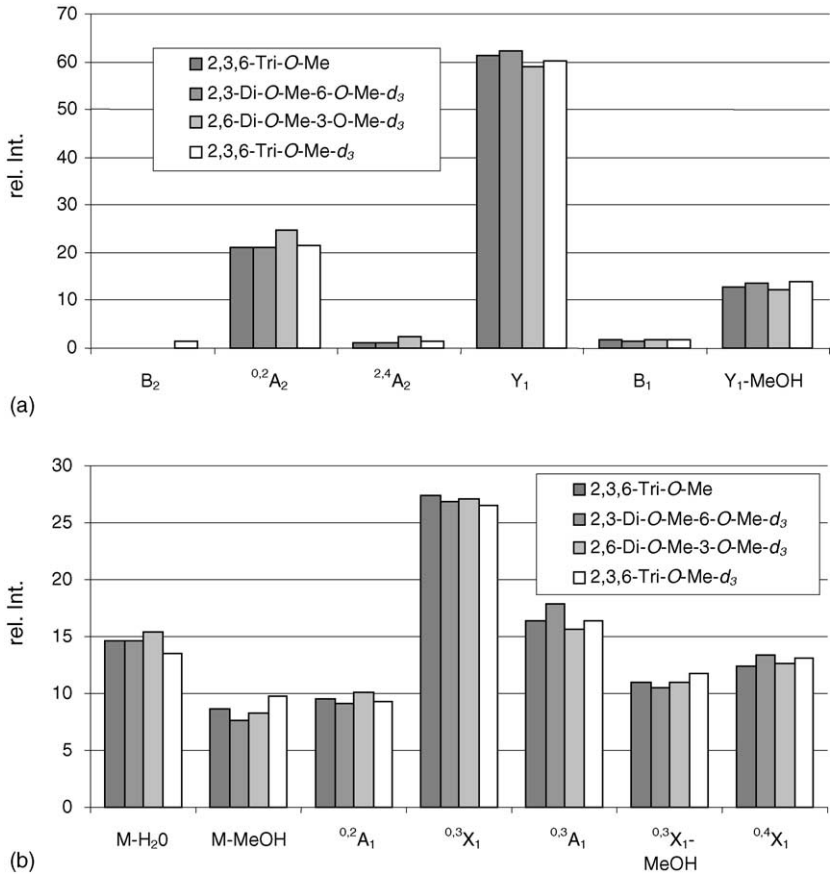


Fig. 2. Relative intensities of the fragment ions obtained from regioselectively methylated/deuteromethylated maltoses by $ESI\text{-}MS^2$ (a) and by $ESI\text{-}MS^3$ of Y_1 ions (b).

Table 2
Fragment ions in ESI-MS^2 mass spectra of *O*Me/*O*Me-d_3 1,4-linked glucodisaccharides; *m/z* values corresponding to the substitution pattern of the reducing part (B in Table 1) of originally un- (C_0) to hexasubstituted disaccharides (C_6) are listed

	R^2	R^3	R^6	*m/z*			
				$[M+Na]^+$	$^{0,2}A_2$	Y_1	Y_1-MeOH
C_0	○	○	○	467	390	254	219
C_1	○	○	○	464	387	254	219
	○	○	●		387	251	216
	●	○	○		390	251	216
	○	●	○		387	251	219
C_2	○	○	○	461	384	254	219
	○	○	●		384	251	216
	●	○	○		387	251	216
	○	●	○		384	251	219
	●	●	○		387	248	216
	○	●	●		384	248	216
	●	○	●		387	248	213
C_3	○	○	○	458	381	254	219
	○	○	●		381	251	216
	●	○	○		384	251	216
	○	●	○		381	251	219
	●	●	○		384	248	216
	○	●	●		381	248	216
	●	○	●		384	248	213
	●	●	●		384	245	213
C_4	○	○	●	455	378	251	216
		○	○		381	251	216
	○	●	○		378	251	219
	●	●	○		381	248	216
	○	●	●		378	248	216
	●	○	●		381	248	213
	●	●	●		381	245	213
C_5	●	●	○	452	378	248	216
	○	●	●		375	248	216
	●	○	●		378	248	213
	●	●	●		378	245	213
C_6	●	●	●	449	375	245	213

R^2, R^3, R^6: black = Me, white = Me-d_3.

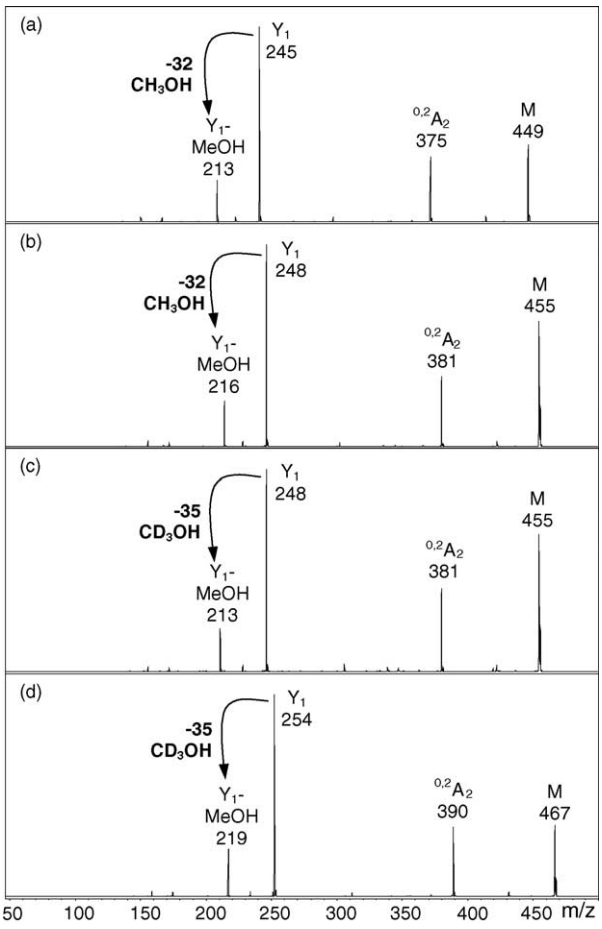


Fig. 3. ESI-MS^2 mass spectra of regioselectively methylated/deuteromethylated maltoses ($[M+Na]^+$). Fragment ions used for quantitative evaluation are assigned: (a) 2,3,6-tri-*O*-Me; (b) 2,3-di-*O*-Me-6-*O*-Me-d_3; (c) 2,6-di-*O*-Me-3-*O*-Me-d_3; (d) 2,3,6-tri-*O*-Me-d_3. By loss of MeOH with 35 or 32 mass units from Y_1 origin of methyl from *O*-3 is confirmed.

constant proportions, the contribution of a certain methyl pattern to a mixed daughter ion is calculated. This is illustrated in Fig. 4. Fig. 4a shows the relative intensities of the relevant daughter ions as columns. As can be seen in Table 2, Y_1 at *m/z* 254 represents an originally unsubstituted reducing residue (Unit B in Table 1), while *m/z* 251 is formed from the disaccharides originally monosubstituted at unit B. The intensities of $^{0,2}A_2$ (*m/z* 387) and Y_1-R^3OH (*m/z* 219) corresponding to the signal intensity of *m/z* 254 are calculated and shown as grey columns in Fig. 4b. $^{0,2}A_2$ at *m/z* 390 is unique for *O*-2-methylated B units, since it contains Me-d_3 in positions 3 and 6, while the original methyl group at position 2 is lost. So, this ion gives direct information on the amount of 2-*O*-Me glucosyl units in the regioisomeric mixture of C_1 and can be used to calculate the corresponding contributions to the related ions at *m/z* 251 and *m/z* 216 (see Table 2 and Fig. 4c). From Table 2, it is also obvious that Y_1-R^3OH at *m/z* 219 is only formed from C_1 disaccharides originally unsubstituted or *O*-3-methylated at the reducing glucosyl unit. The remaining intensity of *m/z* 219 can, therefore, be assigned to the 3-*O*-methyl pattern, and the corresponding intensities of Y_1 at *m/z* 251 and $^{0,2}A_2$ at *m/z* 387 can be calculated and subtracted from the total intensity of these signals (Fig. 4d). The residual intensities of the three signals represent methylation at O-6. (Fig. 4e). The results of these evaluation steps illustrated in Fig. 4b–e are summarised in Fig. 4f. Now, the intensity of *m/z* 464 in the mother mass spectrum can be divided up on the involved methyl patterns as shown in Fig. 4h. In the same way, the mirror-image signal at *m/z* 452 (C_5) can be divided up in tri-*O*-methyl patterns (50%) and the three di-*O*-methyl patterns, 23, 26, and 36. The remaining signals of the mother spectrum are much more difficult to evaluate since they comprise 15 (C_2 and C_4) or even 20 (C_3) possible isomers (Table 1). As an example, the procedure to puzzle out C_3 shall be presented. The trisubstituted disaccharides C_3 comprise 20 regioisomers as listed in Table 1. Those which are built up from one originally un- and one trisubstituted

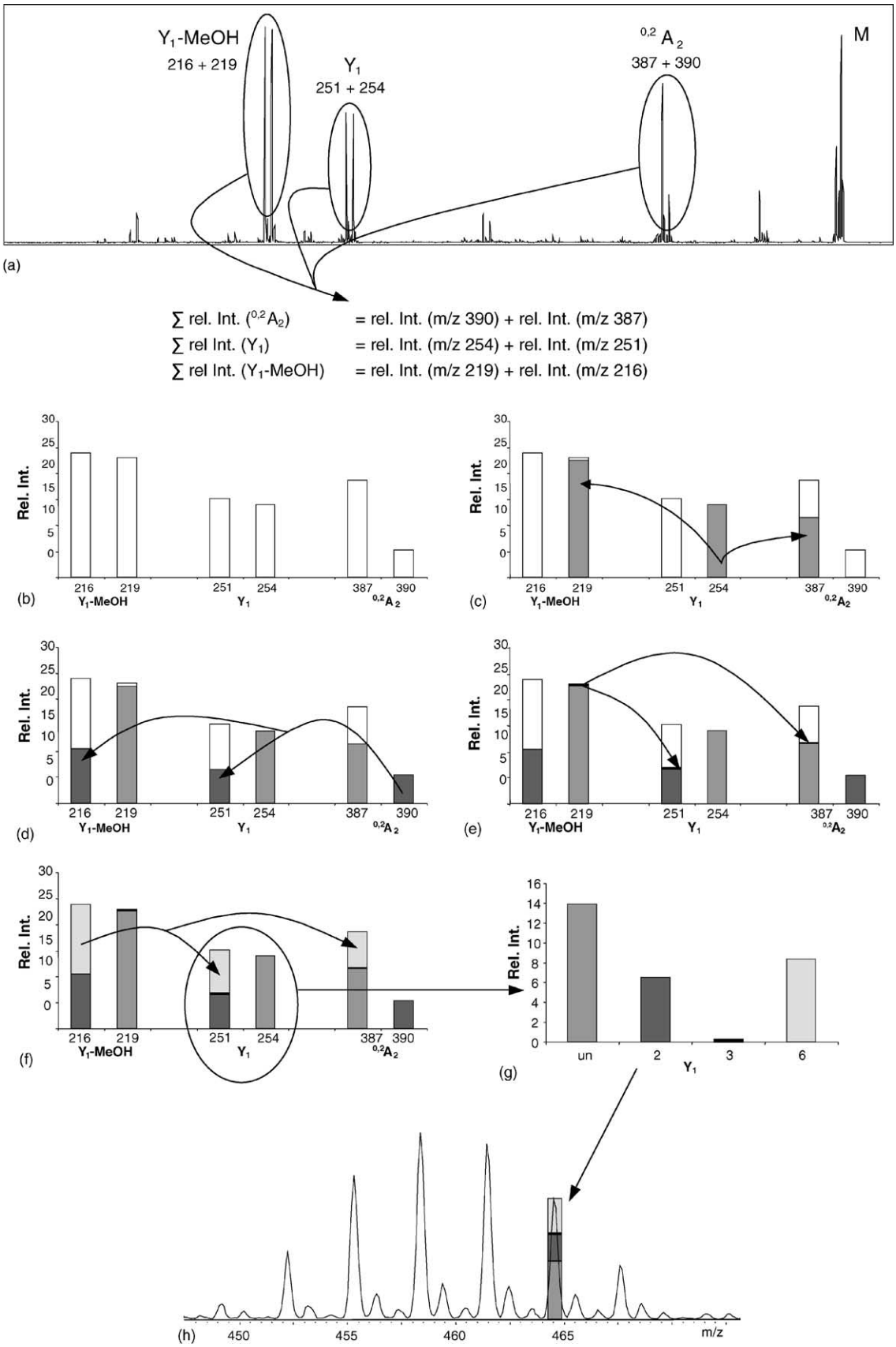


Fig. 4. Evaluation of the monomer composition of the C_1 fraction of *O*Me/*O*Me-d_3 disaccharides obtained from methyl amylose. Intensities of fragment ions in ESI-MS^2 mass spectrum are step by step divided to the comprising glucosyl substitution pattern. For details, see text.

glucosyl unit are unique. Therefore, their share of the mother ion intensity (m/z $[M+Na]^+ = 458$) is deduced from the ESI-MS^2 mass spectrum. Here, four possible Y_1 ions at m/z 245, 248, 251, and 254 occur with a statistical probability of 1:9:9:1, additionally weight with respect to the relative ratios of the un-, mono-, di-, and trisubstituted glucosyl units (c_0, c_1, c_2, c_3). Resulting intensities of Y_1 at m/z 245 and m/z 254 represent the portion of tri- and un-substituted glucosyl units of C_3. As described above, the corresponding intensities of the related $^{0,2}A_2$ and the Y_1-R^3OH ions can be calculated and subtracted from the total intensities of these ions. Two further patterns, 3-*O*-Me and 2,6-di-*O*-Me, can be calculated from the MS^2 spectra of the sodium adducts. From Table 2, it is obvious that m/z 219 is only formed from disaccharides with an originally un- (254-35) or *O*-3-methylated reducing end (251-32). Therefore, the residual intensity of Y_1-R^3OH can be allocated to 3-*O*-methyl-glucose and the corresponding intensities of m/z 251 and m/z 381 can be calculated and subtracted. In the same way, the contribution of 2,6-di-*O*-methyl-glucose can be calculated from Y_1-MeOH-d_3 at m/z 213, which is only formed from disaccharides originally tri- (245-32) or 2,6-di-*O*-methylated (248-35) at the reducing end. The remaining methyl patterns cannot be deduced from the MS^2 mass spectrum. The number of independent informations available from ESI-MS^2 are no longer sufficient to divide the remaining signal intensities up to these patterns. Therefore, we extended our mass spectral analysis to MS^3.

2.2. ESI-MS^3—interpretation of the granddaughter mass spectra

Y_1 ions are isolated and further fragmented. When we isolated Y_1 obtained from $[M+Na]^+$ mother ions we recognised that dissociation of sodium adducts is presumably more favoured than fragmentation (Fig. 5a and b). Therefore, mass spectra could not be evaluated and we changed to the more stable lithium adducts $[M+Li]^+$ [20,21]. The consecutive generations of ESI-MS^n mass spectra are shown in Fig. 5a and b and c–f, respectively. Composition of the fragment ions was deduced from ESI-MS^3 of the regioselectively *O*-methylated and then *O*-deuteromethylated standard compounds (Figs. 6a–d and 2b). Methanol is preferably lost from position 2, but not exclusively as is obvious from the additional loss of 35 (CD_3OH) from the 2,3-di-*O*-Me-6-*O*-Me-d_3- and 2,6-di-*O*-Me-3-*O*-Me-d_3-derivatives (Fig. 6b and c). M-74/77 can be assigned as $^{0,2}A_1$ still containing R^3 and R^6, M-104/107 contains C1–C3, since a loss of 107 is only observed for the 6-*O*-Me-d_3 standard (Fig. 6b). Therefore, these ions at m/z 125/128/131 are assigned as $^{0,3}X_1$. The complementary fragment ions $^{0,3}A_1$ at M-118/121/124 contain R^6 only. Another fragment of diagnostic value is observed at m/z 81/84 and is assigned as $^{0,4}X_1$. It is formed from C5 and C6, since the higher mass of 84 is detected for the 6-*O*-Me-d_3 standard compounds only (Fig. 6b and d). These fragment ions are used for evaluation (Scheme 1b). Y_1 fragments at m/z 235 and 232 representing the three mono- and the three di-*O*-methyl patterns of the methyl glucans are isolated after MS^2 and further fragmented each (Fig. 5c–f). The much higher predominance of Y_1 fragments in the daughter spectra of lithium adducts compared to sodium adducts enhances sensitivity for MS^3, but does not allow a complete switch to lithium since the intensities of the other diagnostically valuable fragment ions in MS^2 are too poor (compare Fig. 5b and d). Again, relative intensities of the summarised relevant fragment ions of ESI-MS^3 are calculated first as described above for MS^2. MS^3 from Y_1 at m/z 232, containing the disubstituted glucosyl unit gives direct information on the amount of 2,3-di-*O*-methyl substitution. This is deduced from $^{0,3}X_1$ at m/z 125, containing R^2 and R^3, and the corresponding signal intensities of the other fragment ions are then calculated. $^{0,2}A_1$ at m/z 155 from Y_1 at m/z 232 is related to 3,6-di-*O*-methyl residues, while the residual part of m/z 158 is related to the 2,6-di-*O*-methyl pattern. However, as already mentioned, 2,6-di-*O*-methyl can also be calculated from the MS^2 mass spectra of the sodium adducts of the C_3 fraction. Finally, the MS^3 mass spectrum of Y_1 from C_3 at m/z 235, which represents the originally monosubstituted glucosyl residues, is evaluated. The portion of 6-*O*-substitution can directly be obtained from $^{0,3}A_1$ at m/z 111 or the complementary $^{0,3}X_1$ at m/z 131, while that of *O*-2-methylated glucosyl units is represented by $^{0,2}A_1$ at m/z 161 as result of only 74 units loss. Then, the amount of *O*-3-methylation can be calculated from the residual intensities of the mixed signals, but was also available from ESI-MS^2 of the sodium adducts, as already mentioned above. All portions were weighed with respect to their quantitative representation in the mother or corresponding daughter spectra, to accumulate their total contribution. So, finally the complete monomer composition was obtained.

2.3. Application and comparison with GC analysis of glucitol acetates

This new strategy was applied to two methyl amyloses (MA 1, DS 0.67, and MA 2, DS 0.83) and five methyl celluloses (MC 1–5, DS 1.08–1.92), which have been analysed by GLC/FID after hydrolysis, reduction and acetylation. The degree of substitution (DS) is defined as the number of modified OH groups/glucosyl unit. Fig. 7 shows the monomer composition as determined by both methods. Deviation of the average DS is in the range of no to 4.6%.

How reliable is this method? Of course, relative intensities of the signals are scattering slightly as is obvious from Fig. 2. However, there is no systematic trend of discrimination with respect to the methyl pattern. In some cases, there is more than one possibility to calculate the portion of a certain methyl pattern in an isomeric mixture. This is illustrated for MC 1 by MS^3 of Y_1 with m/z 232, obtained from m/z 445 in the mother spectrum (C_2). This daughter ion comprises 2,3-, 2,6-, and 3,6-di-*O*-methyl glucoses. As can be seen in Table 3, $^{0,3}X_1$ at m/z 125, $^{0,3}A_1$ at m/z 114, and $^{0,4}X_1$ at m/z 84 are all related to the 2,3-di-*O*-methyl pattern, only. When taking the intensity of m/z 125, and calculating the corre-

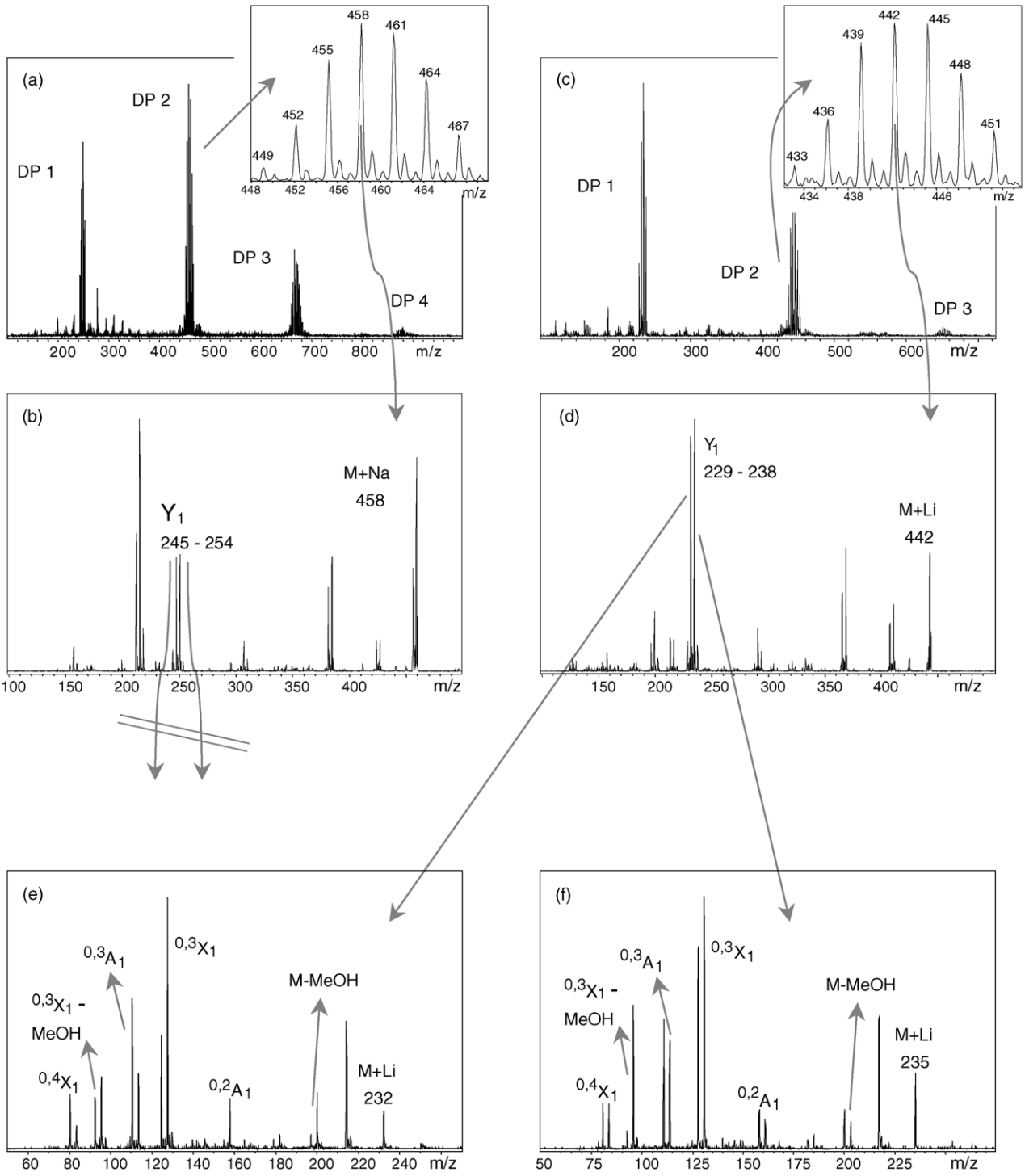


Fig. 5. ESI-MS^n of *O*Me/*O*Me-d_3 disaccharides obtained from methyl amylose. ESI-MS of $[M + Na]^+$ (a); ESI-MS^2 of the C_3 fraction (b); ESI-MS of $[M + Li]^+$ (c); ESI-MS^2 of the C_3 fraction (d); ESI-MS^3 of Y_1 at *m*/*z* 232 (e); and ESI-MS^3 of Y_1 at *m*/*z* 235 (f).

sponding intensities of *m*/*z* 114 and *m*/*z* 84, the values are a little bit lower in the first, and a little bit higher in the second case. That means, that slightly different results are obtained depending on which signal is selected for evaluation. For example, the contribution of 2,3-di-*O*-methyl to Y_1 at *m*/*z* 232 is calculated from *m*/*z* 125 to be 7.92%, from *m*/*z* 114 to be 8.67%, and from *m*/*z* 84 to be 7.61%, which means a final contribution to C_2 in the mother mass spectrum of 2.10%, 2.30%, and 2.02%, respectively. The final result after accumulation of all 2,3-di-*O*-methyl contributions of the relevant C_2–C_5 signals varies between 6.73% and 7.11% for MC 1. The fragment ion at *m*/*z* 125 is finally selected for further calculations, since this shows the highest intensity and therefore the best signal/noise ratio. The result is 6.91%. Deviation of

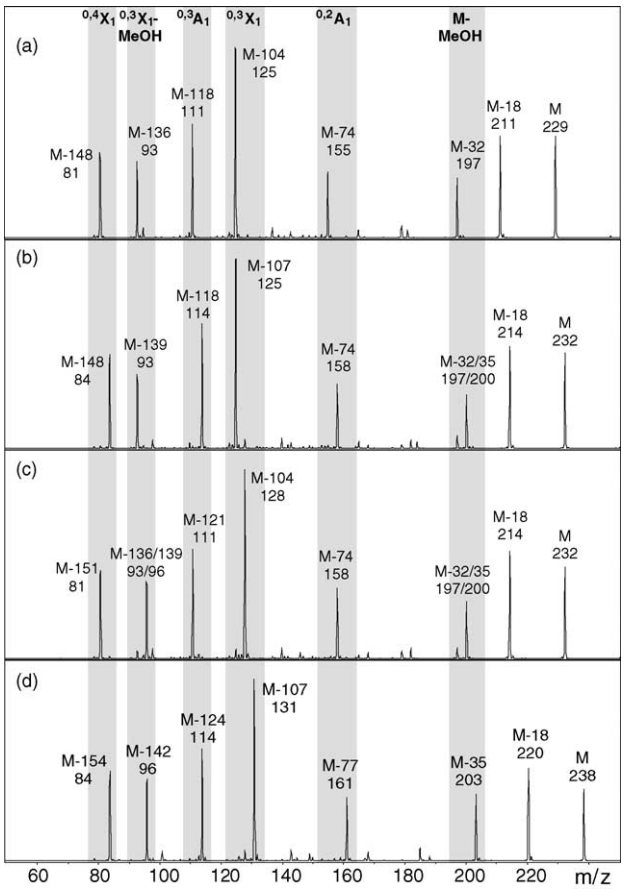


Fig. 6. ESI-MS^3 mass spectra of regioselectively methylated/deuteromethylated maltoses ($[M + Li]^+$). Fragment ions used for quantitative evaluation are assigned: (a) 2,3,6-tri-*O*-Me; (b) 2,3-di-*O*-Me-6-*O*-Me-d_3; (c) 2,6-di-*O*-Me-3-*O*-Me-d_3; and (d) 2,3,6-tri-*O*-Me-d_3.

alternative data, based on the intensity of *m*/*z* 114 or *m*/*z* 84, from this value is −0.18 (−2.6%), and +0.20 (+2.9%), respectively. This is in the same order of magnitude as for reference analysis. Relative deviation increases for minor constituents (<1 mol%) as is observed for gas chromatrographic analysis as well. Material of starch and cellulose derivatives is not restricted and therefore optimal sample concentration can be guaranteed. Quantitative mass spectrometric analysis of the monomer composition without perdeuteromethylation is limited, since ion yields depend on the polarity of the partially methylated oligosaccharides. Therefore, perdeuteromethylation is still a requirement for quantitative evaluation and additionally prevents non-random hydrolysis.

Table 3
Fragment ions in ESI-MS^3 mass spectra of Y_1 ions isolated from ESI-MS^2 of *O*Me/*O*Me-d_3 1,4-linked gluco-disaccharides $[M + Li]^+$; *m*/*z* values corresponding to mono- or disubstituted reducing glucosyl residues (B in Table 1 of originally two-, tri-, or tetrasubstituted (C_2–C_4) disaccharides are listed

R^2	R^3	R^6	*m*/*z*				
			Y_1	$^{0,2}A_1$	$^{0,3}X_1$	$^{0,3}A_1$	$^{0,4}X_1$
●	●	○	232	158	125	114	84
○	●	●		155	128	111	81
●	○	●		158	128	111	81
○	○	●	235	158	131	111	81
●	○	○		161	128	114	84
○	●	○		158	128	114	84

R^2, R^3, R^6: black = Me, white = Me-d_3.

3. Experimental

3.1. General

All reagents were at highest purity available and purchased from Fluka, Aldrich or Merck. MeI-d_3 was purchased from Deutero, methyl amyloses were prepared in our laboratory and methyl celluloses were technical products or purchased from Aldrich/Fluka.

3.2. Instrumental

Electrospray ionisation-mass spectra (ESI-MS, positive mode) were recorded on an Esquire LC (Bruker Daltonics, Bremen, Germany). The partially degraded samples were dissolved in MeOH, and introduced directly via a syringe at a flow of 200 μL/h. For analysing the lithium adducts, samples were dissolved in 1 mM $LiClO_4$ in MeOH. The mass spectra for MS^n 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, 210.0 V. The amplitude of the resonance frequency which excites the ions for fragmentation in the ion trap was optimised for every ion and was between 0.85 and 0.95 V. The isolation width for MS^n experiments was 2 *m*/*z*.

3.3. Sample preparation

Methyl amyloses and methyl celluloses were alkylated with MeI-d_3 according to Ciucanu and Kerek [25] with NaOH/MeI-d_3 in DMSO. Completeness of the reaction was controlled by means of ATR-IR spectroscopy. For partial degradation, 2 mg of the deuteromethylated samples were stirred in a 1 mL V-vial with 2 M trifluoroacetic acid (1 mL) at 120 °C for 17 min. After cooling to room temperature, acid was removed in a stream of nitrogen. Residues of acid were removed by co-distillation with toluene (five times). The residue was dissolved in 4 mL of MeOH for ESI-MS and ESI-MS^2 of sodium adducts. From 1 mL of this solution, the solvent was removed and the sample dissolved again in 1 mL of 1 mM $LiClO_4$ in MeOH for ESI-MS of lithium adducts. For reference, monomer composition was determined by GC after hydrolysis, reduction and acetylation as described elsewhere [12].

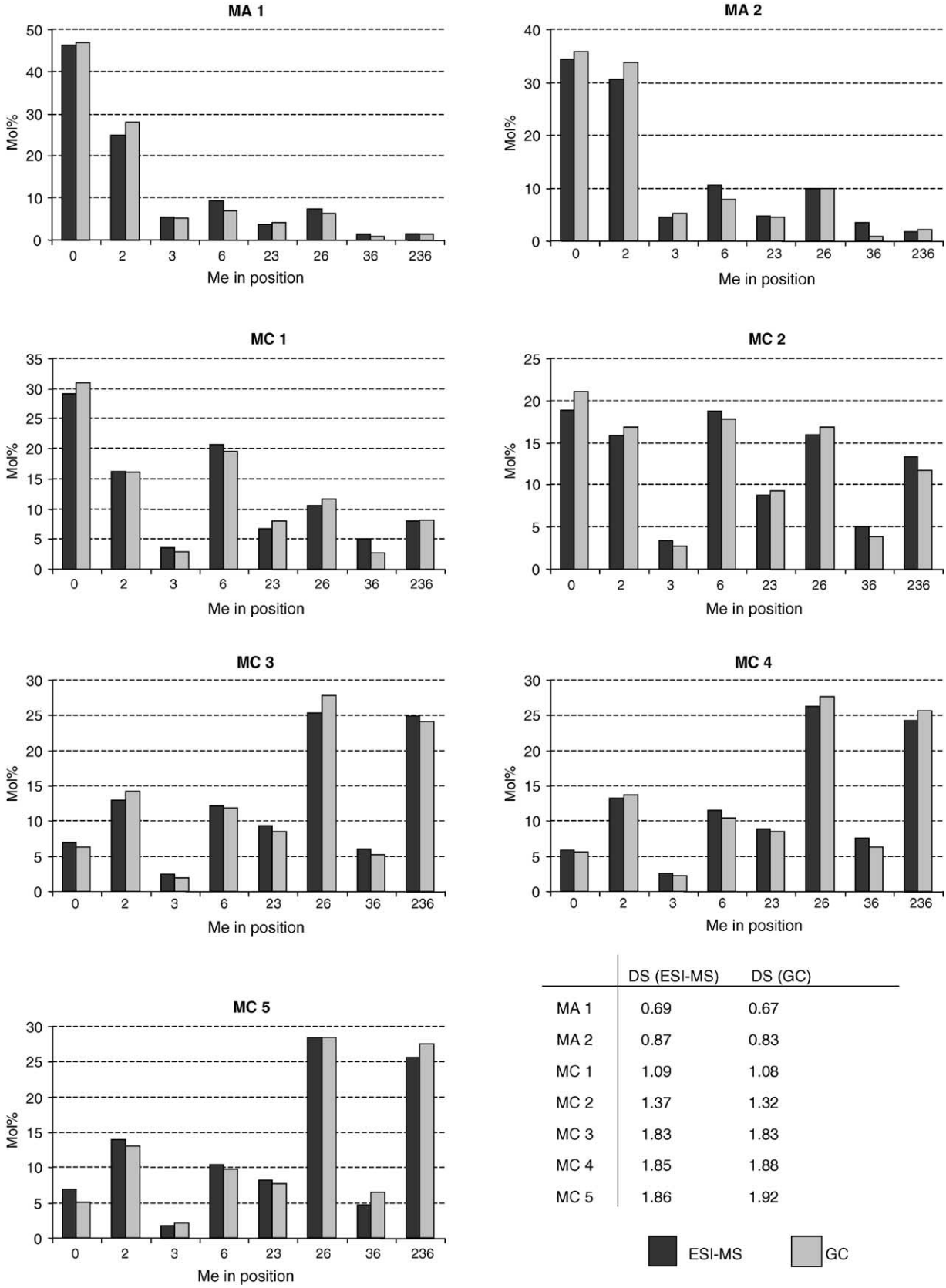


Fig. 7. Monomer composition (in mol%) of methyl amyloses MA 1 and MA 2 and methyl celluloses MC 1–5. Comparison of results obtained by ESI-MS/CID and GC of the partially methylated glucitol acetates.

4. Conclusion

It has been demonstrated that complete monomer composition of methyl amyloses and methyl celluloses can be determined by ESI-MS/CID. Sample preparation is the same as required to determine the methyl pattern along the glucan chains, which can be calculated from the mother ESI-MS mass spectra of corresponding oligosaccharides: samples were perdeuteromethylated and partially degraded by random hydrolysis. By ESI-MS^2 of all *O*Me/*O*Me-d_3 disaccharide signals, and by additional ESI-MS^3 of those Y_1 fragments that represent disaccharides bearing one mono- and one disubstituted glucosyl unit, sufficient information is obtained to divide the signal intensities up to distinct methyl patterns. Results are in good agreement with reference data obtained by hydrolysis, reduction and acetylation. Further studies with a wider range of methyl glucans, with lower and higher DS and various regioselectivities will test the efficiency of this new method.

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