# Loss of Internal 1 → 6 Substituted Monosaccharide Residues from Underivatized and Per-*O*-Methylated Trisaccharides

L. P. Brüll, W. Heerma, J. Thomas-Oates, and J. Haverkamp

Utrecht University, Bijvoet Center for Biomolecular Research, Department of Mass Spectrometry, F.A.F.C. Wentgebouw, Utrecht, The Netherlands

V. Kovácik

Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovak Republic

P. Kovác

NIDDK, National Institutes of Health, Bethesda, Maryland, USA

The fragmentation behavior of $[M + H]^+$ ions of a series of underivatized and per-*O*-methylated trisaccharides having 1 → 6 linked residues, of which one or two is a deoxy-fluoro or deoxy residue and thus has a unique mass, has been studied by using collision-induced dissociation fast-atom bombardment mass spectrometry. In addition to the usual fragment ions resulting from glycosidic bond cleavage, fragment ions were observed which must have been generated following an unusual rearrangement process which can be rationalized in terms of the loss of an internal monosaccharide residue. © *1997 American Society for Mass Spectrometry (J Am Soc Mass Spectrom 1997, 8, 43–49)*

Fast-atom bombardment mass spectrometry (FAB-MS) [1] is a well-established method for the identification and structural characterization of oligosaccharides and glycoconjugates [2, 3]. With this soft ionization technique it is not only possible to determine the molecular mass of underivatized oligosaccharides, but it may also be used to derive structural information from fragment ions.

Derivatization of oligosaccharides significantly improves the structural information available from oligosaccharides [3, 4] since it directs cleavage, dramatically improves sensitivity, and allows unambiguous sequence and branching determination since the difference between single and double cleavage events is readily identified. Fragmentation may be enhanced following high energy collision-induced dissociation (CID) of native and derivatized [5–7] oligosaccharides.

The mass spectrometric fragmentation behavior of native and derivatized oligosaccharides is well defined [2]. The formation of $B_i$- and $Y_i$-fragment ions (nomenclature of Domon and Costello [7]) characterizes the mass spectrometric fragmentation of oligosaccharides. These ions are considered to be the result of fragmentation of the glycosidic bond to yield oxonium (B) ions and ions corresponding to smaller protonated oligosaccharides (Y ions).

The nature of the monosaccharides present in an oligosaccharide substantially affects the fragmentation of the compound. This is illustrated by the preferential charge retention on an *N*-acetylhexosamine residue in oligosaccharides, which favors cleavage of the glycosidic bond adjacent to this residue, thus resulting in the formation of abundant B ions, depending on the position of the *N*-acetylhexosamine residues in oligosaccharides [3].

An unusual type of fragmentation of oligosaccharides was recently described by Kovácik et al. [8]. Y* ions resulting from the loss of internal $\alpha - 1 \rightarrow 2$ substituted galactose residues from underivatized tri- and tetrasaccharides are observed in abundance. The unique masses of the monosaccharide residues in these model compounds allowed us to identify the loss as

Address reprint request to L. P. Brüll, Utrecht University, Department of Mass Spectrometry, F. A. F. C. Wentgebouw, Sorbonnelaan 16, 3584 CA, Utrecht, The Netherlands.


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that of an internal residue. An analogous loss of an internal monosaccharide residue from per-*O*-alkylated oligosaccharide-alditols under conditions of chemical ionization mass spectrometry has also been reported by McNeil [9], who stated that the loss of an internal residue is not related to the anomericity or linkage position of the internal residue. A comparable process has been reported for protonated peptides [10] involving the "internal loss" of the C-terminal amino acid and transfer of the C-terminal hydroxyl group.

To determine whether the internal residue loss observed in CID mass spectra of oligosaccharides is limited to α − 1 → 2 substituted monosaccharide residues, we now present the results of our study of synthetic trisaccharides that have 1 → 6 linked monosaccharides residues and are composed of galactose, glucose, deoxy-galactose, deoxy-fluoro-galactose, and deoxy-fluoro-glucose, and their per-*O*-methylated derivatives. A variety of factors, such as the position and nature of a specific residue in the oligosaccharide chain, the identity and position of fluorination or deoxygenation in the residue, and anomeric configuration might all be able to influence the abundance of the fragment ions formed. The aim of this study is a careful screening of the experimental results to determine what factors affect internal monosaccharide residue loss.

## Experimental

The compounds investigated are summarized in Figure 1.

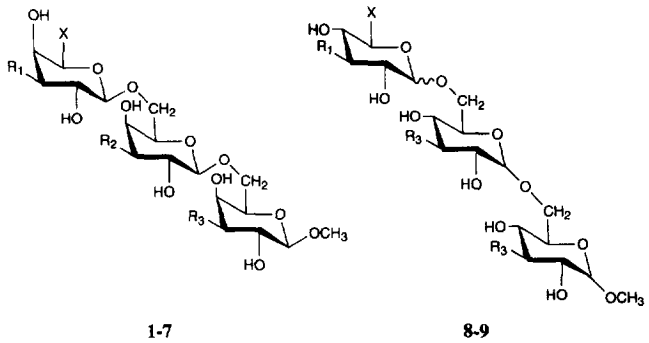



**Figure 1.** Compounds investigated. List of oligosaccharides: **1.** Methyl *O*-(3-deoxy-3-fluoro-β-D-galactopyranosyl-(1 → 6)-(β-D-galactopyranosyl)-(1 → 6)-β-D-galactopyranoside [13], $R_1$ = F, $R_2 = R_3$ = OH, X = $CH_2OH$. **2.** Methyl *O*-(β-D-galactopyranosyl)-(1 → 6) - (3-deoxy - 3-fluoro- β -D-galactopyranosyl - (1 → 6) - β-D-galactopyranoside [14], $R_2$ = F, $R_1 = R_3$ = OH, X = $CH_2OH$. **3.** Methyl *O*-(β-D-galactopyranosyl) - (1 → 6) - (β-D-galactopyranosyl) - (1 → 6) - 3-deoxy - 3-fluoro -β -D-galactopyranoside [15], $R_3$ = F, $R_1 = R_2$ = OH, X = $CH_2OH$. **4.** Methyl *O*-(3-deoxy-3-fluoro-β-D-galactopyranosyl)-(1 → 6)-(β-D-galactopyranosyl)-(1 → 6)-3-deoxy-3-fluoro-β-D-galactopyranoside [16], $R_1 = R_3$ = F, $R_2$ = OH, X = $CH_2OH$. **5.** Methyl *O*-(3-deoxy-β-D-xylo-hexopyranosyl)-(1 → 6)-(β-D-galactopyranosyl)-(1 → 6)-β-D-galactopyranoside [17], $R_1$ = H, $R_2 = R_3$ = OH, X = $CH_2OH$. **6.** Methyl *O*-(β-D-galactopyranosyl)-(1 → 6)-(3-deoxy-β-D-xylo-hexopyranosyl)-(1 → 6)-*O*-D-galactopyranoside [17], $R_2$ = H, $R_1 = R_3$ = OH, X = $CH_2OH$. **7.** Methyl *O*-(β-D-galactopyranosyl)-(1 → 6)-(β-D-galactopyranosyl) - (1 → 6)-3-deoxy-β-D-xylo-hexopyranoside [17], $R_3$ = H, $R_1 = R_2$ = OH, X = $CH_2OH$. **8.** Methyl *O*-(6-deoxy-6-fluoro-α-D-glucopyranosyl) - (1 → 6) - (α-D-glucopyranosyl)-(1 → 6)-α-D-glucopyranoside [18], $R_1 = R_2 = R_3$ = OH, X = $CH_2F$. **9.** Methyl *O*-(6-deoxy-6-fluoro-β-D-glucopyranosyl) - (1 → 6) - (α-D-glucopyranosyl) - (1 → 6)-α-D-glucopyranoside [16], $R_1 = R_2 = R_3$ = OH, X = $CH_2F$.

### *Peracetylation*

Peracetylation of oligosaccharides **1**–**9** was performed under conditions of acid catalysis [11] in 250 μL of a 2:1 (v/v) mixture of trifluoroacetic anhydride (Aldrich Chemical Co., Milwaukee, WI) glacial acetic acid (Merck, St. Louis, MO) for 10 min at room temperature, when the samples were dried under vacuum.

### *Per-O-Methylation and Per-(Trideutero)-O-Methylation*

Oligosaccharides **1**–**9** were per-*O*-methylated by using a modification of the method of Ciucanu and Kerek [12] by adding 300-mg freshly ground sodium hydroxide to the samples dissolved in 200-μL dimethylsulfoxide (Aldrich); 250-μL methyl iodide (Aldrich) was added after 0, 10, and 30 min. The reaction was stopped after 20 min by adding 1-mL sodium thiosulfate solution (100 mg $mL^{-1}$) and 1-mL chloroform. After mixing and centrifugation, the water layer was removed and the remaining organic layer was washed three times with water, after which the organic layer was evaporated to dryness under nitrogen. The same derivatization method was used for per-(trideutero)-*O*-methylation of the oligosaccharides by using trideuteromethyl iodide (Aldrich) instead of methyl iodide.

### *Instrumentation*

Positive-ion fast-atom bombardment (FAB) mass spectra and precursor ion scans were obtained on a JEOL (Tokyo, Japan) JMS-SX/SX102A tandem mass spectrometer (BEBE geometry) by using 10-kV accelerating voltage. Xenon was used as the bombarding gas and the FAB gun was run at 6-kV accelerating voltage and an emission current of 10 mA. The CID tandem mass spectra were obtained by using the collision cell in the third field-free region (FFR) of the mass spectrometer, with air as collision gas at sufficient pressure to reduce the intensity of the selected ion beam by approximately 50%. As the collision cell was kept at ground potential the collision energy in the tandem mass spectrometry (MS-MS) experiments was 10 keV. Precursor-ion scans were carried out following fragmentation by using helium gas in the first FFR, using $B^2/E$ linked scanning [19]. The derivatized and native samples were dissolved in dichloromethane and water, respectively, and aliquots of 1 μL containing ~ 10-μg compound

were analyzed in a matrix of glycerol/thioglycerol (1 : 1, v/v).

## Results and Discussion

The methyl glycosides of trisaccharides used in this study, (Figure 1) are composed of monosaccharide residues with different residue masses; these include 162, 146, and 164 u for the underivatized galactose or glucose, the 3-deoxy-galactose, and the 3-deoxy-3-fluoro-galactose or 6-deoxy-6-fluoro-glucose residues, respectively. These trisaccharides were carefully chosen since their compositions allow the identification of internal residue loss,† a process that in most cases gives rise to the formation of fragment ions with mass values that may also be assigned to regular B and/or Y ions.

A requirement for this investigation is that the compounds be pure and that sequence isomers be absent. The structures of the compounds were confirmed by NMR spectrometry [13–18] and their purity was further confirmed by examining the CID tandem mass spectra of the $[M + H]^+$ ions of the peracetylated compounds. These derivatives fragment in a very predictable manner to given almost exclusively oxonium-type fragment ions and thus allow an unambiguous identification of the sequence.

Having verified that the trisaccharides are pure, they were examined underivatized by using collision-induced tandem mass spectrometry (CID-MS-MS) (Figure 2). It is obvious that, in contrast with the results observed for the peracetylated compounds, glycosidic bond cleavage in the native trisaccharides results in the formation of both B and Y ions that allow the sequence to be determined. However, the spectra also contain signals corresponding to ions which cannot be formed on simple direct glycosidic bond cleavages. The ions marked $Y_2^*$ at $m/z$ 359, 357, and 361 (Figure 2a, b, and d) can only be generated following a rearrangement reaction which results in the loss of the internal residue [8]. An analogous rearrangement product cannot be identified in the spectrum of **3** (Figure 2c), since the terminal glycosyl‡ residue and the internal residue are identical. As there is no reason to assume that the internal residue loss will not occur in this compound the $m/z$ 359 peak in Figure 2c should also be considered as resulting from the combined intensities of the $Y_2$ and $Y_2^*$ ions, which could also explain its relatively high abundance.

Another explanation for the occurrence of the $m/z$ 359 ions (Figure 2a, b, and d) might be "internal loss"

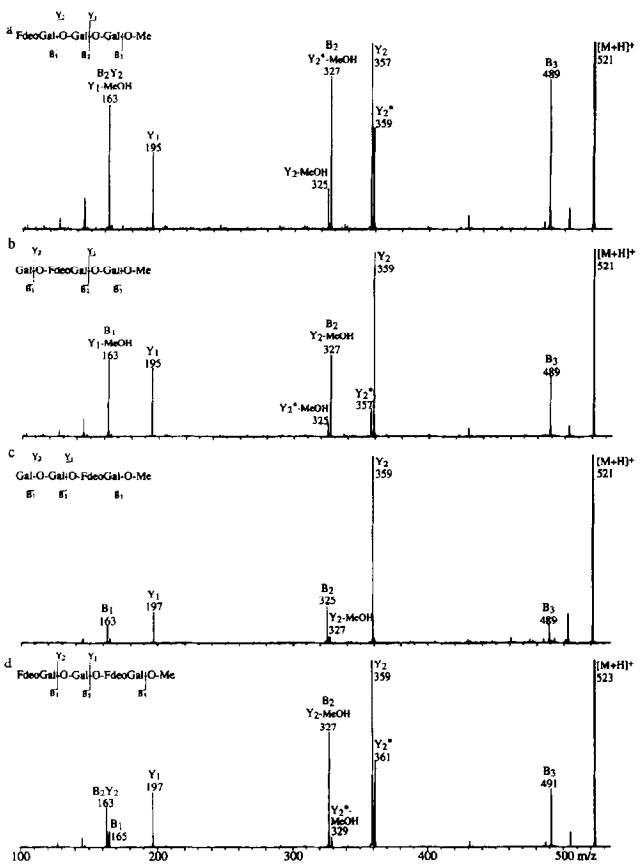



**Figure 2.** (a)–(d) CID mass spectra of compounds **1**–**4**, respectively. The $y$ axis represents relative abundance. The spectra are normalized to the most abundant product ion.

of the terminal glycoside residue accompanied by transfer of the C-1 methoxy group. In the CID spectrum of compound **3** (Figure 2c) an $m/z$ 357 ion may be expected to originate from an analogous rearrangement, but is not observed. However, the $Y_2^*$ ions in the spectra of **2** and **4** (Figure 2b and d) cannot be the result of such a rearrangement.

Precursor-ion scans of $m/z$ 359 (**1**), $m/z$ 357 (**2**), and $m/z$ 361 (**4**) revealed that these ions originate directly from their $[M + H]^+$ ions and must therefore be considered to result from a rearrangement process. Their formation, exemplified by the generation of $m/z$ 361 ions in the CID mass spectrum of **4** (Figure 2d), is proposed to occur as depicted in Scheme I. It should be noted that from the experimental results, it is not clear which of the two glycosidic oxygen atoms is retained in the $Y_2^*$ ion.

The double cleavage $[Y_n - MeOH]$ ions coincide in mass occasionally with $B_n$ ions (e.g., $B_2$ and $[Y_2 - MeOH]$, $B_1$ and $[Y_1 - MeOH]$ in Figure 2b and d). The $B_1$ oxonium ion is very weak or absent if the terminal glycosyl residue contains a fluorine atom (Figure 2a and d). In these cases, the presence of a double cleavage ion ($B_2Y_2$) dominates.

The formation of B and Y ions from protonated oligosaccharides involves cleavage of the glycosidic bond with retention of the glycosidic oxygen atom by

†An internal residue is an "in-chain" residue, that is, one bound on both sides by glycosidic linkages. The resulting product ions are assigned as $Y^*_{n,m}$ ions, with the subscripts $n$ and $m$ referring to the number(s) of the lost residue(s) starting the numbering at the reducing terminus [8].

‡Monosaccharides glycosidically linked to other monosaccharide residues are termed glycosyls, while those bearing an aglycon are termed glycosides.

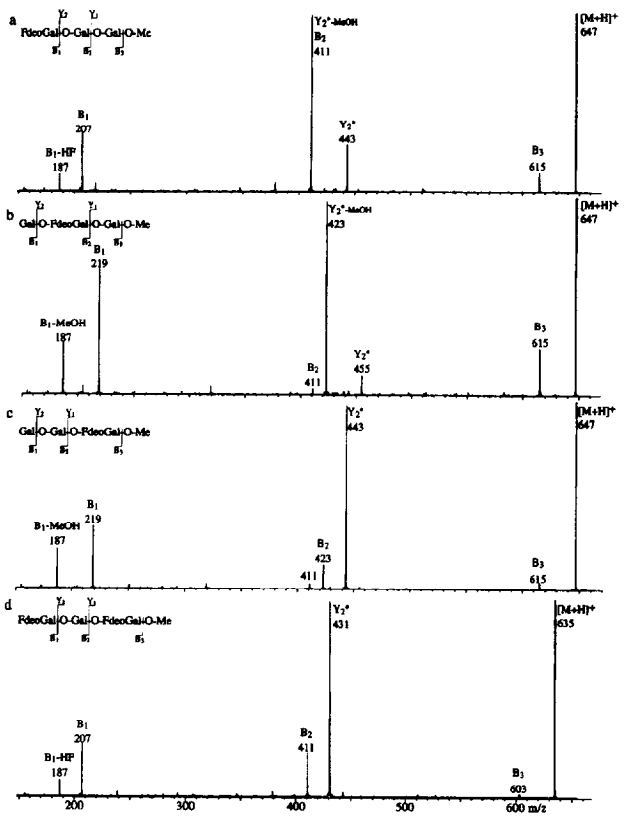


**Figure 3.** (a)–(d) CID mass spectra of per-*O*-methylated compounds 1–4, respectively. The *Y* axis represents relative abundance. The spectra are normalized to the most abundant product ion.

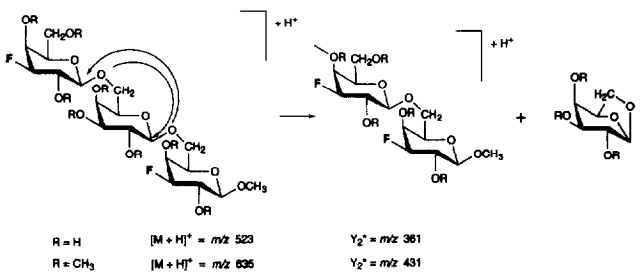
the terminal glycoside fragment. In the case of Y ions, an additional hydrogen transfer to the glycosidic oxygen is required. Following derivatization of an oligosaccharide, ions resulting from double cleavage events are readily identifiable since each $\beta$-cleavage event [2] generates a free hydroxyl group. In the spectra of the per-*O*-methylated derivatives **1**–**4**, while no Y ions are observed, ions corresponding in mass to fully methylated disaccharides are intense, or are even the base peaks; we assign these as $Y_2^*$ ions (Figure 3).

All the per-*O*-methylated compounds yield $B_1$, $B_2$, and $B_3$ ions. As in the spectra of the underivatized compounds, a low intensity ion is observed if the charge-bearing residue contains a fluorine atom, (e.g., $B_2$ in **2** and $B_3$ in **3** and **4**). $Y_2$ ions $m/z$ 441 are absent from the spectrum of **1**, while $Y_2^*$ ions ($m/z$ 443) are abundant (Figure 3a). The $m/z$ 443 ion formed from **3** (Figure 3c) could, in principle, also be considered to be a $Y_2$ ion resulting from glycosidic bond cleavage with concomitant methyl transfer. Such a phenomenon has been reported by Dell et al. [20]. Similarly the formation of $m/z$ 443 from **1** (Figure 3a) could be the result of "internal loss" of the glycoside terminal residue accompanied by transfer of the methoxy group from the terminal glycoside residue. However, analogous ions are not observed in the other spectra. The ions at $m/z$ 455 in **2** (Figure 3b) and $m/z$ 431 in **4** (Figure 3d; Scheme I) cannot result from single glycosidic bond cleavage or from a methyl-transfer reaction. From these observations it can be concluded that per-*O*-methylated compounds **1**–**4** also produce $Y_2^*$ fragment ions (Figure 3). Their precursor ion scans demonstrate (data not shown) that they originate directly from the $[M + H]^+$ ions, and therefore must be considered to arise by rearrangement processes. It should be noted that $Y_2^*$ ions constitute the base peak in the CID spectra of **3** and **4** in which the terminal glycoside residue is fluoridated. Per-*O*-methylated oligosaccharides contain no free hydroxyl groups so that it may be logical that the formation of regular $Y_1$ or $Y_2$ ions is unlikely since it has been reported that formation of Y ions involves the transfer of an exchangeable (hydroxyl) hydrogen [21]. An analogous transfer of a methyl group could not be demonstrated from a study of the CID spectra of these compounds.

To determine whether the presence of a fluorine atom in the saccharide is essential for the observation of the rearrangement which yields Y* ions, the spectra of a series of trisaccharides with 1 → 6 linked residues, but containing one deoxy-galactose and two galactose residues were examined (Figure 4).

Signals corresponding to ions resulting from internal residue loss are clearly present in the spectra of **5** ($m/z$ 341; Figure 4a) and **6** ($m/z$ 357; Figure 4b). Although the $m/z$ 341 ion in **5** could also be generated by an "internal loss" of the terminal glycoside residue accompanied by transfer of the methylglycoside, the elimination of the internal residue in **6** is beyond any doubt. The identity of the terminal glycosyl and internal residues in **7** prevents identification of the rearrangement ion, since $Y_2$ and $Y_2^*$ ions are isobaric. Since the glycosidic bonds formed by deoxyhexosyl residues are known to be chemically weak, bond cleavage at this residue is abundant, resulting in the $Y_2$ in **5**, $B_2$ in **6**, and $B_3$ in **7**, which represent the base peaks in the CID spectra of these compounds.

The assignment of Y* ions as the products of a rearrangement process can be supported since rearrangement processes are promoted under conditions of unimolecular decomposition [22]. Consequently, the spectra of all samples were also recorded without the use of collision gas, under which conditions ions arising following rearrangement processes may be ex-



**Scheme I.** Proposed rearrangement reaction leading to internal residue loss from underivatized and per-*O*-methylated **4**.

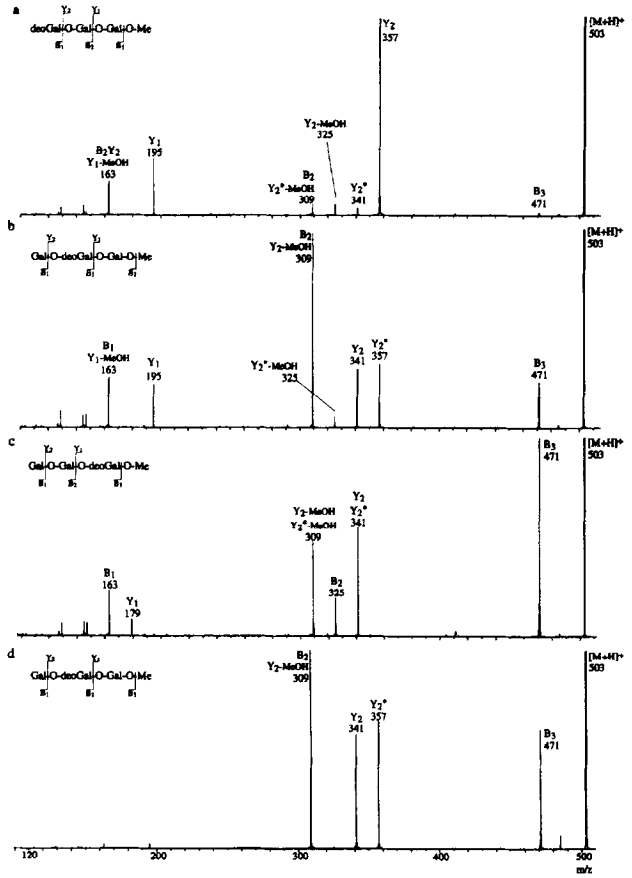


**Figure 4.** (a)–(c) CID mass spectra of compounds **5**–**7**, respectively. (d) Unimolecular mass spectrum of compound **6**. The *Y* axis represents relative abundance. The spectra are normalized to the most abundant product ion.

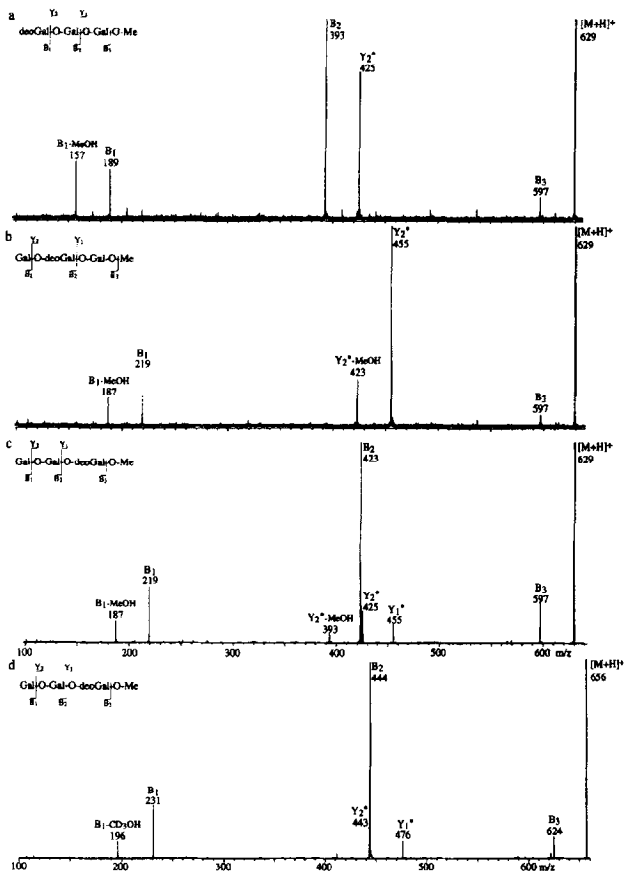


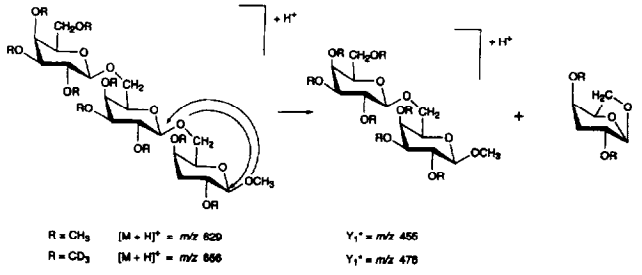
**Figure 5.** (a)–(c) CID mass spectra of per-*O*-methylated compounds **5**–**7**, respectively. (d) per-(trideutero)-*O*-methylated compound **7**. The *Y* axis represents relative abundance. The spectra are normalized to the most abundant product ion.

pected to become relatively more intense (Figure 4d). The relative abundances of the $Y_2$ and $Y_2^*$ ions with respect to the $B_2$ ion have increased considerably compared to those in the CID spectrum (Figure 4b), a phenomenon which is consistent with rearrangement rather than direct bond cleavage.

Compounds **5**–**7** were also analyzed by using CID-MS-MS following per-*O*-methylation. As for their fluorinated analogs, no regular Y ions were observed (Figure 5a–c) although a complete series of B ions, together with $Y_2^*$ ions is produced.

In the spectra of both per-*O*-methylated compound **6** (Figure 5b) and **7** (Figure 5c) $m/z$ 455 product ions are present, corresponding to the loss of a deoxyhexose residue bearing two methoxy groups. This residue is internal in **6** and corresponds to the terminal glycoside residue in **7**, so that the production of the $Y_1^*$ ion in the spectrum of **7** is postulated to arise by the "internal loss" of the terminal glycoside residue and transfer of the C-1 methoxy group.

We postulate that the formation of the $m/z$ 455 ion in the spectrum of **7** and the absence of analogous ions in the spectra of other compounds is probably due to the relatively weak glycosidic bond formed by deoxyhexoses. This assignment is supported following mass spectrometric analysis of the product obtained on per-(trideutero)-*O*-methylation of **7** (see Scheme II). Under these conditions the aglycon is retained and all other hydroxyl groups are converted to trideuteromethoxy groups. In the CID mass spectrum (Figure 5d) the $Y_1^*$ ion produced on loss of the terminal deoxygalactoside residue is now observed at $m/z$ 476, consistent with it containing a single methyl group and seven trideuteromethyl moieties, demonstrating that the C-1 methoxy group is transferred in this rearrangement reaction. It is interesting to compare our data with the observations of Yoon and Laine [23], who also gener-



**Scheme II.** Proposed fragmentation mechanism for transfer of the C-1 methoxy group and for the loss of the terminal glycoside residue from per-(trideutero)-*O*-methylated **7**.

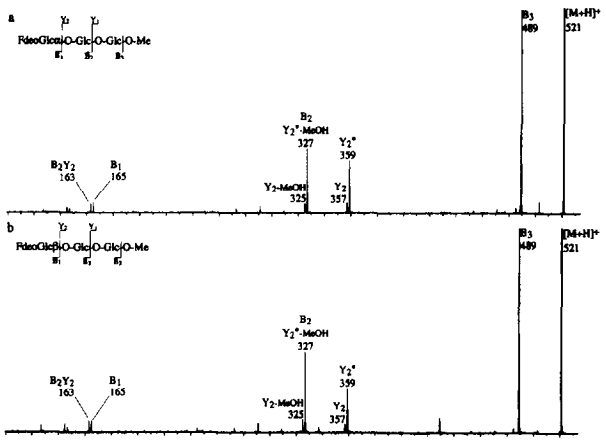


**Figure 6.** (a), (b) CID mass spectra of compounds **8** and **9**, respectively. The *Y* axis represents relative abundance. The spectra are normalized to the most abundant product ion.

ated an ion at $m/z$ 455 on CID analysis of per-*O*-methylated trisaccharides. They assign this ion as being generated on transfer of a C-3 or C-4 methoxy group from the terminal glycoside residue rather than as loss of an internal residue or methyl glycoside transfer.

To determine whether the anomericity of the monosaccharides affects the formation of $Y_2^*$ ions, the CID mass spectra of the $[M + H]^+$ ions of underivatized compounds **8** and **9**, which contain two $\alpha - 1 \rightarrow 6$ and one $\beta - 1 \rightarrow 6$ and one $\alpha - 1 \rightarrow 6$ anomers, were recorded (Figure 6). The spectra of compounds **8** and **9** are very similar, and $Y_2^*$ ions are formed in each case, showing that both the $\alpha - 1 \rightarrow 6$ and the $\beta - 1 \rightarrow 6$ linked internal residues can be lost. Interestingly, in the study of Yoon and Laine [23] the spectrum of trisaccharide Gal$\beta$1 → 4Glc$\beta$1 → 6Glc does not contain an ion at $m/z$ 455, which we would expect based on our observations to result from loss of either the $\beta - 1 \rightarrow 4$ substituted glucose or the $\beta - 1 \rightarrow 6$ substituted glucose residue. Curiously the spectra of **8** and **9** are dominated by abundant $B_3$ ions suggesting that the position of the fluorine atom in the terminal glycosyl residue significantly affects the abundance of the Y-type ions. A comparison of the spectrum obtained from **1** (Figure 2a) with those from **8** and **9** (Figure 6a and b) shows that the $Y_2$ ion ($m/z$ 357) is the most abundant fragment ion if the fluorine atom is located on C-3 of the deoxygalactose residue in **1**, while a relatively weak $Y_2$ ion ($m/z$ 357) is formed when the fluorine atom is on the C-6 position of the deoxyglucose residue (**8** and **9**). This reduction in intensity of the $Y_2$ ion in the spectra of the 6-fluoro-deoxyglucose-containing compounds is compensated by an increase in the intensity of the $Y_2^*$ ion ($m/z$ 359).

## Conclusions

The internal residue loss that we previously reported as having observed in CID mass spectra of $1 \rightarrow 2$ substituted oligosaccharides [8] is not restricted to oligosaccharides containing this linkage. Underivatized as well as per-*O*-methylated trisaccharides in which the residues are $1 \rightarrow 6$ linked also exhibit product ions which arise from loss of an internal residue. The extent to which this rearrangement process occurs depends on the nature of the residue. The process seems to be independent of anomericity, and the rearrangement ions are not observed in the spectra of the peracetylated compounds used in this study.

Very abundant or base peak Y* ions are observed in the CID spectra of per-*O*-methylated deoxyhexose- and hexose-containing oligosaccharides which can cause confusion in sequence determination. Nevertheless it is very important to note that in spite of the presence of Y* ions, we have, to date, always been able to determine the sequence correctly, based on a ready identification of B and Y ions. Interpreting FAB and CID mass spectra requires the awareness that nonsequential fragmentation reactions can occur. From the results obtained with peracetylated compounds the suggestion seems to be justified that mass spectra of peracetyl-derivatized oligosaccharides are more reliable for structural analysis when *N*-acetylhexosamine residues are absent.

A further systematic study is in progress to investigate factors such as linkage, nature and position of substituents, derivatization, branching, and nature of eliminated and adjacent residues, which might influence the occurrence and extent of the elimination reaction of oligosaccharides.

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## References

1. Barbar, M.; Bordoli, R. S.; Sedgwick, R. D.; Tyler, A. N.; *J. Chem. Soc. Chem. Commun.* **1981**, 325–332.
2. Dell, A. *Adv. Carbohydr. Chem. Biochem.* **1987**, *45*, 19–72.
3. Dell, A.; Morris, H. R.; Egge, H.; Von Nicolai, H.; Strecker H. *Carbohydr. Res.* **1983**, *115*, 41–52.
4. Egge, H.; Dell, A.; Von Nicolai, H.; *Arch. Biomed. Biophys.* **1983**, *224*, 235–253.
5. Gillece-Castro, B. L.; Burlingame, A. L.; *Methods Enzymol.* **1990**, *193*, 689–712.
6. Müller, D. R.; Domon, B.; Richter, W. J.; *Methods Enzymol.* **1990**, *193*, 607–623.
7. Domon, B.; Costello, C. E. *Glycoconj. J.* **1988**, *5*, 397–409.
8. Kovácik, V.; Hirsch, J.; Kovác, P.; Heerma, W.; Thomas-Oates, J.; Haverkamp, J. *J. Mass Spectrom.* **1995**, *30*, 949–958.
9. McNeil, M. *Carbohydr. Res.* **1983**, *123*, 31–40.
10. Thorne, G. C.; Ballard, K. D.; Gaskell, S. J. *J. Am. Soc. Mass. Spectrom.* **1990**, *1*, 249–258.
11. Bourne, E. J.; Stacey, M.; Tatlow, J. C.; Tedder, J. M.; *J. Am. Chem. Soc.* **1949**, 2976–2979.
12. Ciucanu, I.; Kerek, F. *Carbohydr. Res.* **1986**, *131*, 1126–1134.

13. Kovác, P.; Ye, H. J. C.; Glaudemans, C. P. J. *Carbohydr. Res.* **1985**, *140*, 277–288.
14. Kovác, P.; Glaudemans, C. P. J. *J. Carbohydr. Chem.* **1985**, *4*, 613–626.
15. Kovác, P.; Glaudemans, C. P. J. *Carbohydr. Res.* **1985**, *140*, 289–298.
16. Kovác, P.; Glaudemans, C. P. J.; Guo, W.; Wong, T. C. *Carbohydr. Res.* **1985**, *140*, 299–311.
17. Kovác, P.; Edgar, K. J. *Carbohydr. Res.* **1990**, *201*, 79–93.
18. Kovác, P.; Sklenár, V.; Glaudemans, C. P. J. *Carbohydr. Res.* **1988**, 175, 201–213.
19. Boyd, R. K.; Porter, C. J.; Beynon, J. H. *Int. J. Mass Spectrom. Ion Phys.* **1982**, *44*, 199–214.
20. Dell, A.; Khoo, K.-H.; Panico, M.; McDowell, R. A.; Etienne, A. T.; Reason, A. J.; Moriss, H. R.; *Glycobiology, A Practical Approach*; Fukuda, M.; Kobata, A., Eds.; Oxford University Press: Oxford, 1993; p. 195.
21. Li, Q. M.; Claeys, M. *Biol. Mass Spectrom.* **1994**, *23*, 406–416.
22. McLafferty, F. W.; Ikuo, S. *Org. Mass Spectrom.* **1973** *7*, 971–987.
23. Yoon, E.; Laine, R. A. *Biol. Mass Spectrom.* **1992**, *21*, 479–485.