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Enhanced structural information on oligosaccharides by scan of linked magnetic and electrostatic fields (B/E) and neutral gas collision fast-atom-bombardment mass spectrometry (FABMS) *

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ABSTRACT

Homo- and hetero-oligosaccharides **1–27**, composed of hexopyranose, deoxyhexopyranose, deoxyfluorohexopyranose, and 2-acetamido-2-deoxyhexopyranose units, have been examined by fast-atom-bombardment mass spectrometry. Scans by linked magnetic and electrostatic (B/E) fields of quasi-molecular $[M+H]^+$ ions, or scans combined with helium collision, gave rise to structurally significant ions. The information thus obtained aids significantly in the sequence analysis of oligosaccharides without derivatization.

INTRODUCTION

Mass spectrometry plays an important role in structural analysis of complex carbohydrates. The available methods, which involve the ionization in the gas phase, e.g., electron-impact (EI) and chemical-ionization (CI) mass spectrometry, provide useful information for the confirmation of structures of substances that are sufficiently volatile or mixtures of substances amenable to gas–liquid or liquid chromatography. The low volatility of many substances makes their analysis by mass spectrometry impractical and requires prior chemical manipulation to produce a sufficiently volatile material. Such procedures may be difficult to apply with substances that are unstable or undergo side reactions or are available in only minute amounts.

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Newer, so-called "soft ionization methods"[2], such as field-desorption (FD), plasma desorption ^{252}Cf (PD), laser desorption (LD) or fast-atom-bombardment (FAB) techniques circumvent the requirement of high volatility of samples. In particular, FAB[3–5] has been widely used in the carbohydrate field since the method is quite sensitive and experimentally easy to apply. The spectra obtained from a native sample primarily contain the molecular mass information in the form of one or more quasimolecular ions[6].

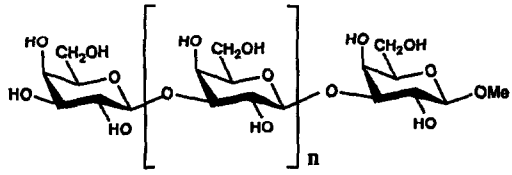
Molecular weight information is almost always obtainable by FAB mass spectrometry. However, structurally informative fragment ions may be absent in FAB spectra, or they may be so weak that they are obscured by the background signals of the liquid matrix. Moreover, underivatized oligosaccharides frequently afford fragment ions that result from at least two cleavage events, thereby making the structural assignment of the sequence ambiguous. Therefore, unambiguous sequencing by FABMS requires prior derivatization, e.g., acetylation[7] or methylation[8].

In order to determine the structure of oligosaccharides without derivatization, we have measured mass spectra of a large number of substances of this class by so-called "soft ionization techniques" carried out under various conditions. The techniques used were positive- and negative-ion FAB measurements using various matrices and plasma-desorption ^{252}Cf mass spectra. Linked scan, the technique in which the magnetic field strength "B" and electrostatic sector field voltage "E" are maintained in a fixed relationship throughout the scan, is applicable to the analysis of specific daughter- or parent-ions and of specific losses[2]. In our experiments the $[M + H]^+$ quasimolecular ions have been chosen for daughter-ion analysis. To enhance the fragmentation, helium collision gas was also introduced into the first field-free region (1st FFR) of the mass spectrometer.

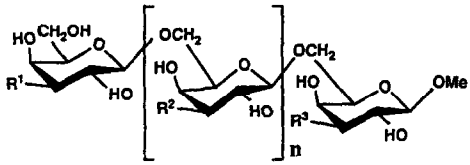
EXPERIMENTAL

The compounds investigated, **1–24**, were synthesized as described earlier[9–12]. Cellobiose [β-D-glucopyranosyl-(1 → 4)-D-glucopyranose (**25**)], methyl α-D-mannopyranosyl-(1 → 3)-α-D-mannopyranosyl-(6 ← 1)-α-D-mannopyranoside (**26**) and melizitose [α-D-glucopyranosyl-(1 → 3)-β-D-fructofuranosyl-(2 ← 1)-α-D-glucopyranoside (**27**)] were obtained from Sigma Chemical Company and were used as supplied. The newly synthetized oligosaccharides **1–6** and **8–24** are introduced in the list of structural formulas. Compound **7** is the octasaccharide, methyl α-D-glucopyranosyl-(1 → 6)-α-D-glucopyranosyl-(1 → 6)-α-D-glucopyranosyl-(1 → 6)-α-α-D-glucopyranosyl-(1 → 6)-α-D-glucopyranosyl-(1 → 6)-α-D-glucopyranosyl-(1 → 6)-α-D-glucopyranosyl-(1 → 6)-α-D-glucopyranoside.

The FAB and B/E FAB linked mass spectra were obtained on a JMS SX-102 (JEOL) double-foccussing instrument possessing reversed geometry. The samples were dissolved in water, mixed with matrices on the target, and subjected to Xe (6 kV) bombardment. With every substance, three scans were run, and He was



	n
1	0
2	1
3	2
4	3
5	4
6	5



Compound	n	R^1	R^2	R^3
8	0	OH		F
9	0	F		OH
10	1	F	OH	OH
11	1	OH	F	OH
12	1	OH	OH	F
13	1	F	OH	F
14	2	OH	OH	F
15	0	H		OH
16	0	OH		H
17	1	H	OH	OH
18	1	0H	H	OH
19	1	0H	OH	H

introduced into the 1st FFR of the mass spectrometer. Finaly, additional three linked scans of $[M + H]^+$ ions have been measured. The following matrices have been examined: glycerol, thioglycerol, *p*-nitrobenzylalcohol–glycerol, and 9:1 dithiothreitol–dithioerythritol ("magic bullet").

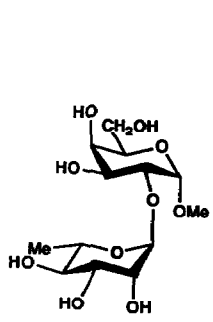
To perform the *O*-deuteration experiment, compound **1** was concentrated to dryness, the residue was dissolved in D_2O and mixed on the target with *O*-deuterated glycerol. The degree of deuteration achieved was 95.5%.

The PD spectra were obtained on a ^{252}Cf PD instrument. For electrospraying on aluminized mylar film, samples were dissolved in MeOH. Data aquisition time was ~ 240 min.

RESULTS AND DISCUSSION

To study the utility of FAB and FAB B/E linked scans in the sequence analysis of oligosaccharides, substances **1–27** were used as model compounds.

Since the experimental factors, such as the mode of ionization, choice of liquid matrix, amount of the compound analyzed and the presence of salts, dramatically effect the ratio of the quasimolecular ions to sequence-related ions[13], we have examined the utility of various matrices. The positive-ion FAB spectrum (Fig. 1 for compound **8**), obtained using glycerol as the matrix, exhibited a minor peak for quasimolecular $[M + H]^+$ ions. In addition, intense peaks originating from the glycerol matrix were also present. The use of thioglycerol and *p*-nitrobenzyl



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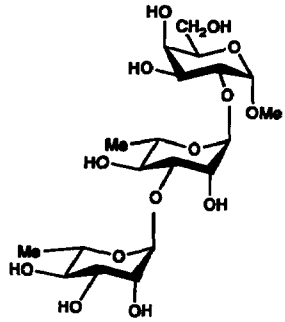

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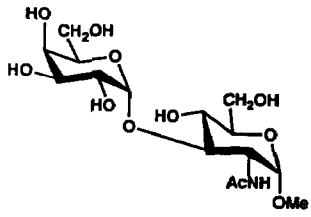

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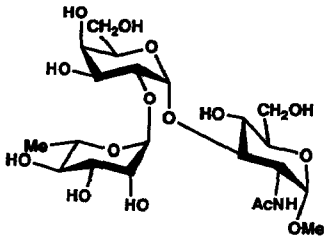

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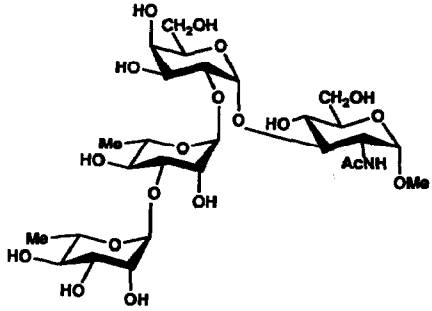

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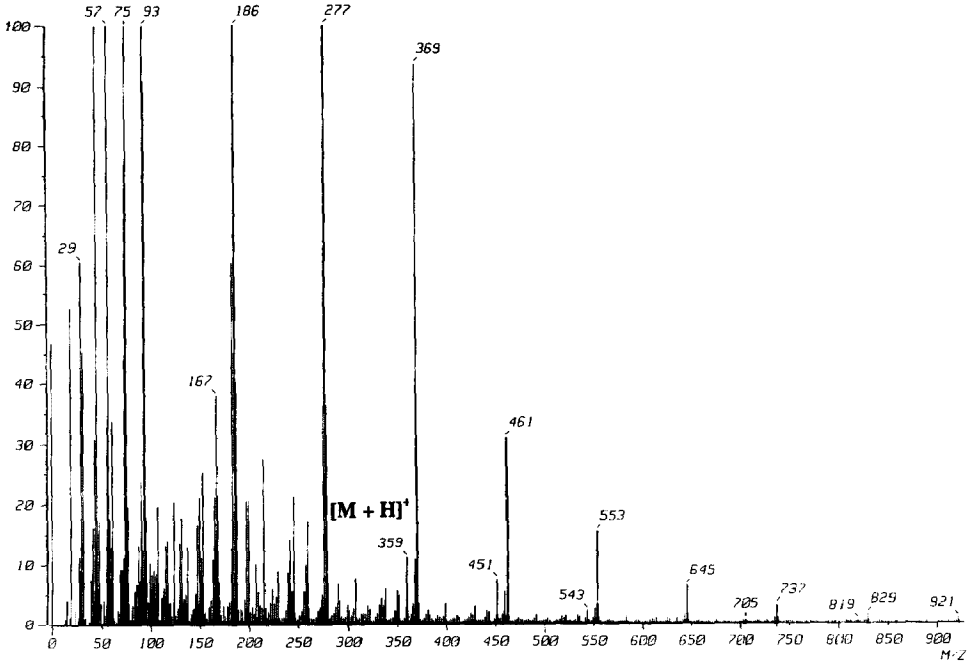


Fig. 1. FABMS (glycerol matrix) of methyl β-D-galactopyranosyl-(1 → 6)-3-deoxy-3-fluoro-β-D-galactopyranoside (**9**).

alcohol–glycerol matrices gave rise to spectra containing only negligible peaks of cluster ions. An even higher intensity of parent ions was achieved using the "magic bullet" matrix. Using this matrix, the spectrum of methyl β-D-galactopyranosyl-(1 → 6)-3-deoxy-3-fluoro-β-D-galactopyranoside (**8**, Fig. 2) contains more intense quasimolecular $[M + H]^+$ and $[M + Na]^+$ ions, as well as the fragment ions at m/z 197. The negative-ion FAB mass spectra exhibit only a negligible peak of $[M - H]^-$ ions. The only peak in the ^{252}Cf PD spectrum of methyl 3-deoxy-3-fluoro-β-D-galactopyranosyl-(1 → 6)-β-D-galactopyranosyl-(1 → 6)-β-D-galactopyranoside (**10**) was the $[M + Na]^+$ ion peak at m/z 543 (Fig. 3).

The method based on the B/E linked measurement of the spectra of $[M + H]^+$ ions obtained in the "magic bullet" matrix give additional information. As an example, Fig. 4 shows the linked B/E spectrum of $[M + H]^+$ ions of pentasaccharide **4**. The FAB B/E spectra of $[M + H]^+$ ions contain an intense peak of quasimolecular $[M + H]^+$ ions, as well as the peak of $[M + H - CH_3OH]^+$ ions. Two fragmentation routes leading to "sequence ions" can be observed for every glycosidic linkage. The respective fragment ions are also observed[14] in the normal FAB mass spectra and appear in the so-called region II. In that case, however, they are obscured by ions derived from the matrix molecule. Pathway A (Scheme 1), a protolytic cleavage of one of the glycosidic linkages, forms oxonium ions. Pathway B, a cleavage of the glycosidic linkage, results in a species charged at the

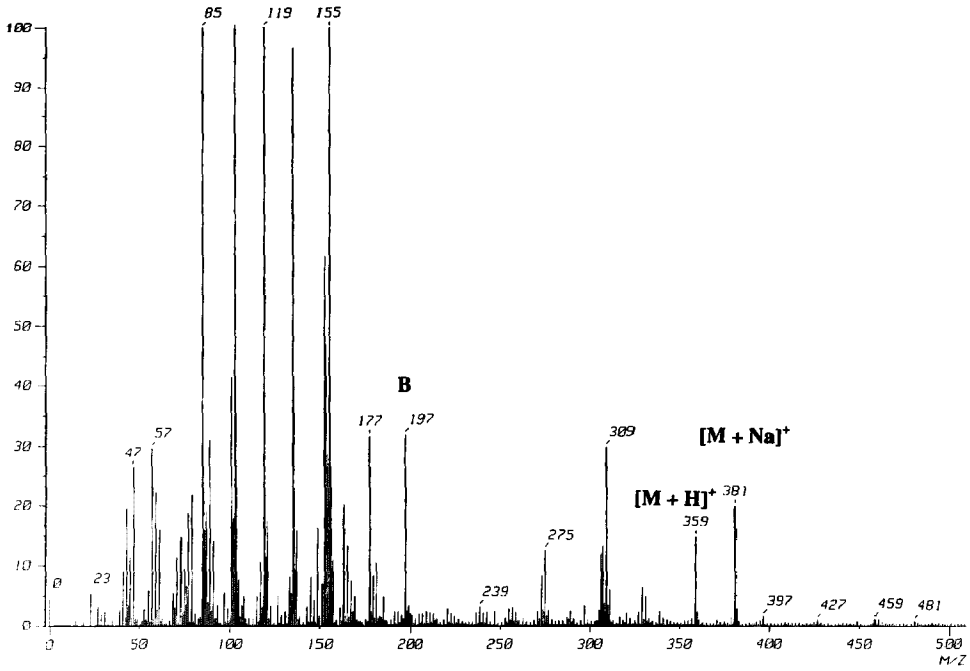


Fig. 2. FABMS ("magic bullet" matrix) of methyl β-D-galactopyranosyl-(1 → 6)-3-deoxy-3-fluoro-β-D-galactopyranoside (**9**).

aglycon terminus. The *O*-deuteration experiment carried out with compound **8** proved that the hydrogen atom of one of the hydroxyl groups present in the nonreducing end of the molecule takes part in the rearrangement process. The *m/z* value of the B-type ions (197) changed to 201. A plausible mechanism of the formation of the A- and B-type ions is shown in Scheme 1.

Taking, as an example, the molecule of hexasaccharides **5** constructed from cycles **a**–**f**, the following equations can be constructed for the verification of masses of the cycles (Scheme 2).

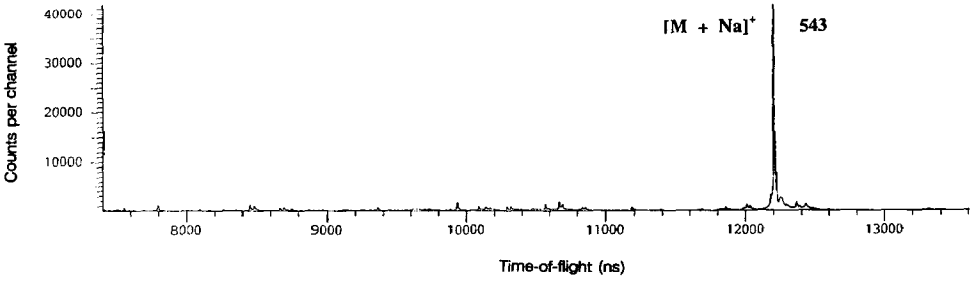


Fig. 3. ^{252}Cf PD mass spectrum of methyl 3-deoxy-3-fluoro-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside (**10**).

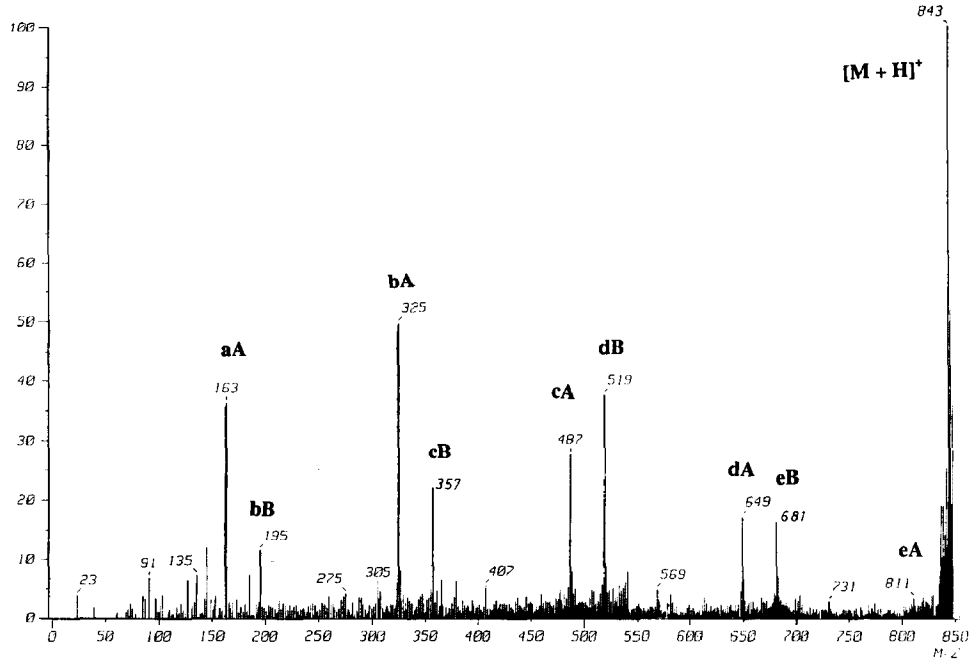
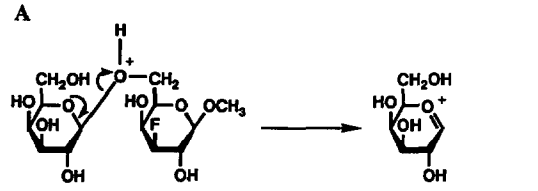
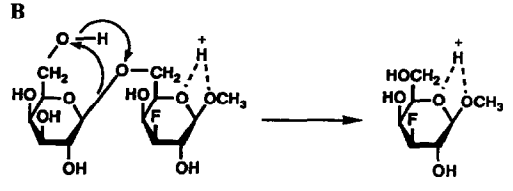


Fig. 4. The B/E FABMS of methyl β-D-galactopyranosyl-(1 → 6)-β-D-galactopyranosyl-(1 → 6)-β-D-galactopyranosyl-(1 → 6)-β-D-galactopyranosyl-(1 → 6)-β-D-galactopyranoside (4).

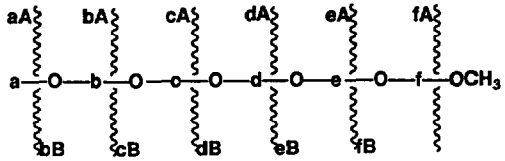


8 $[M + H]^+$ *m/z* 359 (366) aA *m/z* 163 (167)



$[M + H]^+$ *m/z* 359 (366) bB *m/z* 197 (201)

Scheme 1.



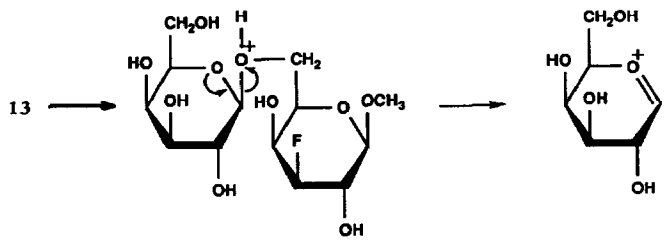
$$aA + bB - 1 = [M+H]^+ \quad (1)$$
$$bA + cB - 1 = [M+H]^+ \quad (2)$$
$$cA + dB - 1 = [M+H]^+ \quad (3)$$
$$dA + eB - 1 = [M+H]^+ \quad (4)$$
$$eA + fB - 1 = [M+H]^+ \quad (5)$$

Scheme 2.

In the fragment notation, the small letter characterizes the cycle from which the fragment originated. In the case of a pentassachariде, the letter **f** and eq (5) do not apply. Similarly, with the tetrasaccharides, the letter **e** and eq (4) are absent. By analogy, the spectrum of a heptasaccharide can be verified by six equations, using the additional letter **g**, etc. Table I summarizes the m/z values of all characteristic sequence-ions taken from the spectra of compounds **1–27**. The peak intensities (10–80% of the intensity of $[M + H]^+$ ions) vary in dependance of presence and pressure of helium collision gas in the 1st FFR of the mass spectrometer.

For the interpretation of FAB and B/E experiments of an underivatized, unknown oligosaccharide, we recommend the following procedure. The candidate for the value of $[M + H]^+$ parent ions should be determined from FAB spectra using the "magic bullet" matrix. Then, after scanning the linked B/E scan of $[M + H]^+$ ions, the B-type ions should be preferentialy noticed. In the next step the m/z values of the A-type ions should be calculated using the foregoing equations. These are controlled by the A-type peaks present in the B/E spectrum. When a complete set of xA and yB ions is found, the interpretation is unambiguous.

The opposite approach, i.e., the search in the spectra for peaks corresponding to possible A-type ions, may in some cases lead to faulty conclusions. It is possible to erroneously ascribe the structure of A-type ions to a peak that does not actually represent the **a** terminal unit of the oligosaccharide. For example, the B/E FAB



Scheme 3.

TABLE I

Characteristic sequence-ions for compounds **1–27**

Compound	MH	aA	bB	bA	cB	cA	dB	dA	eB	eA	fB	fA	gB	gA	hB	hA
1	357	163	195	325												
2	519	163	357	325	195	487										
3	681	163	519	325	357	487	195	649								
4	843	163	681	325	519	487	357	649	195	811						
5	1005	163	843	325	681	487	519	549	357	811	195	973				
6	1167	163	1005	325	843	487	681	649	519	811	357	973	195	1135		
7	1329	163	1167	325	1005	487	843	649	681	811	519	973	357	1135	195	1297
8	359	163	197	327												
9	359	165	195	327												
10	521	165	357	327	195	489										
11	521	163	357	329	195	489										
12	521	163	357	329	197	489										
13	523	165	359	329	197	491										
14	683	163	521	325	359	487	197	651								
15	341	147	195	309												
16	341	163	179	309												
17	503	147	357	309	195	471										
18	503	163	341	309	195	471										
19	503	163	341	325	179	471										
20	341	147	195	309												
21	487	147	341	293	195	455										
22	398	163	236	366												
23	544	147	398	309	236	512										
24	690	147	544	293	398	455	236	658								
25	343	163	181	325												
26	519	163	357			477										
27	519	163	357	325		501										

spectrum of methyl 3-deoxy-3-fluoro-β-D-galactopyranosyl-(1 → 6)-β-D-galactopyranosyl-(1 → 6)-3-deoxy-3-fluoro-β-D-galactopyranoside (**13**) contains an intense peak of ions with m/z 163. We have proved by high-resolution mass spectrometry that the elemental composition of these ions is $C_6H_{11}O_5$, and, thus, they represent the secondary ion from unit **b** of the trisaccharide **13** (Scheme 3). Consequently, the peak at m/z 163 does not represent the ions of the **aA** type. In the case of compound **13**, they appear in the B/E scan spectrum as a peak at m/z 165 and have the elemental composition of $C_6H_{10}FO_4$. To avoid misinterpretation, it should be noted that the peak of **aA** ions is ocasionally of low intensity. Such was the case of compounds **22–24**. Characteristic of these substances is the formation of **aA** ions by the cleavage of the glycosidic bond of the acetamido saccharide residue. For this reason the only unambiguous interpretation of the B/E spectra results from the approach involving the search for B-type ions, with subsequent verification of the masses of the A-type ions. Compounds **26** and **27** are representatives of branched oligosaccharides. Their branching on cycle **b** is the reason that the fragmentation according to the B-pathway does not take place on the neighbouring **c** unit (Table I). The lack of B-type ions indicates the presence and position of the branching point of the oligosaccharide.

In conclusion, B/E scanning of $[M + H]^+$ ions is very informative regarding the sequence of underivatiyed oligosaccharides. Due to its simplicity and the relatively low cost of the instrumentation, the technique can compete effectively with methods requiring more expensive tandem MS–MS instrumentation[15].

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