Carbohydrate Research, 221 (1991) 209–217
Elsevier Science Publishers B.V., Amsterdam

Collisional-activation tandem mass spectrometry of sodium adduct ions of methylated oligosaccharides: sequence analysis and discrimination between α-NeuAc-(2→3) and α-NeuAc-(2→6) linkages

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(Received May 4th, 1991; accepted for publication July 17th, 1991)

ABSTRACT

Collision-activated dissociation (c.a.d.) of sodium adducts of molecular ion species have been carried out on methylated β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-Glc*p* (**1**), β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-Glc*p* (**2**), α-D-NeuAc-(2→3)-β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-Glc*p* (**3**), α-D-NeuAc-(2→6)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-Glc*p* (**4**), and α-D-NeuAc-(2→6)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→2)-α-D-Man*p*-(1→3)-β-D-Man*p*-(1→4)-D-Glc*p*NAc (**5**). The numerous daughter ions reflect the sequences, clearly differentiate (1→3) and (1→4) linkages, and discriminate between α-NeuAc-(2→3) and α-NeuAc-(2→6) linkages.

INTRODUCTION

Although determination of the amino acid sequences of proteins is relatively easy to achieve by the Edman procedure and, more recently, by tandem mass spectrometry (m.s.–m.s.), determination of the structure of complex oligosaccharides remains a challenge. N.m.r. and mass spectrometry are the most common and convenient techniques for the determination of the anomeric configuration of the sugar moieties, the position of the linkages and the sequences and branching patterns. With the latter technique, the results are based on methylation analysis by g.l.c.–m.s.[1], f.a.b.-m.s.[2,3], and, more recently, m.s.–m.s.[4–6]. F.a.b.-m.s.–m.s. can be helpful not only for the sequence determination of oligosaccharides but also for linkage analysis of the deprotonated molecular ions of disaccharides[7] and of the alkali metal adducts of the molecular

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0008-6215/91/$03.50

ions of larger structures[8–9]. For instance, carbon–carbon ring cleavages of $(M + 2Li - H)^+$ or $(M + Na)^+$ ions are more extensive than for $(M + H)^+$ ions and consequently yield more information on structure[10,11].

Methylation also results in increased sensitivity in f.a.b.-m.s. and l.s.i.-m.s. by enhancing the hydrophobicity and surface activity. Moreover, methylated derivatives undergo specific fragmentations from the non-reducing termini at HexNAc linkages[12]. Likewise, acetylated derivatives give c.a.d.-mass spectra from $(M + H)^+$ ions which allow (1→3) and (1→4) linkages to be differentiated[13].

Since parent signals may be enhanced greatly by adding an alkali salt to the matrix, we have investigated the c.a.d.-mass spectra of several cationised and methylated oligosaccharides.

EXPERIMENTAL

A high-resolution Kratos Concept II HH ($E_1B_1E_2B_2$) tandem mass spectrometer was used at an accelerating voltage of 8 kV. The f.a.b. gun was operated at 7 kV with xenon. Each positive-ion mass spectrum was the sum of ten scans. Precursor ions were fragmented at a collision energy of 6 kV with He at a pressure sufficient to reduce the parent signal by 75%. Daughter ions were analysed by linked scanning at a constant B/E ratio, using a DS90 (DG DG/30) data system. A 1:1000 resolution was selected in both MS1 and MS2.

β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-Glc*p* (**1**), β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-Glc*p* (**2**), α-D-NeuAc-(2→3)-β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-Glc*p* (**3**), and α-D-NeuAc-(2→6)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-Glc*p* (**4**) were isolated from human milk[15], and α-D-NeuAc-(2→6)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→2)-α-D-Man*p*-(1→3)-β-D-Man*p*-(1→4)-D-Glc*p*NAc (**5**) was prepared from the urine of patients suffering from sialosidosis[16].

The oligosaccharides were methylated according to Ciucanu and Kerek[14], and a solution of each product (5 μg) in methanol was dried on the probe tip and then mixed with NaI-saturated thioglycerol (2 μL).

RESULTS AND DISCUSSION

Only cationised fragments were present in the c.a.d.-mass spectra of the $(M + Na)^+$ ions of the methylated **1–5**. This finding constrasts with the results[11] for natriated native oligosaccharides, the c.a.d.-mass spectra of which still contain oxonium-type fragments. These observations suggest that the alkali metal strongly interacts with the polar functional groups to give a stable adduct. For instance, the $^{1,5}X_i$, Y_i, B_i, and C_i ions may reflect local decomposition induced by the interaction of Na^+ and the heterocyclic and glycosidic oxygens or the acetamido group of GlcNAc. However, "charge-remote fragmentation" (*i.e.*, cleavage of a glycosidic bond other than at the site of Na^+ attachment) is a more likely explanation for the Z_i cleavage.

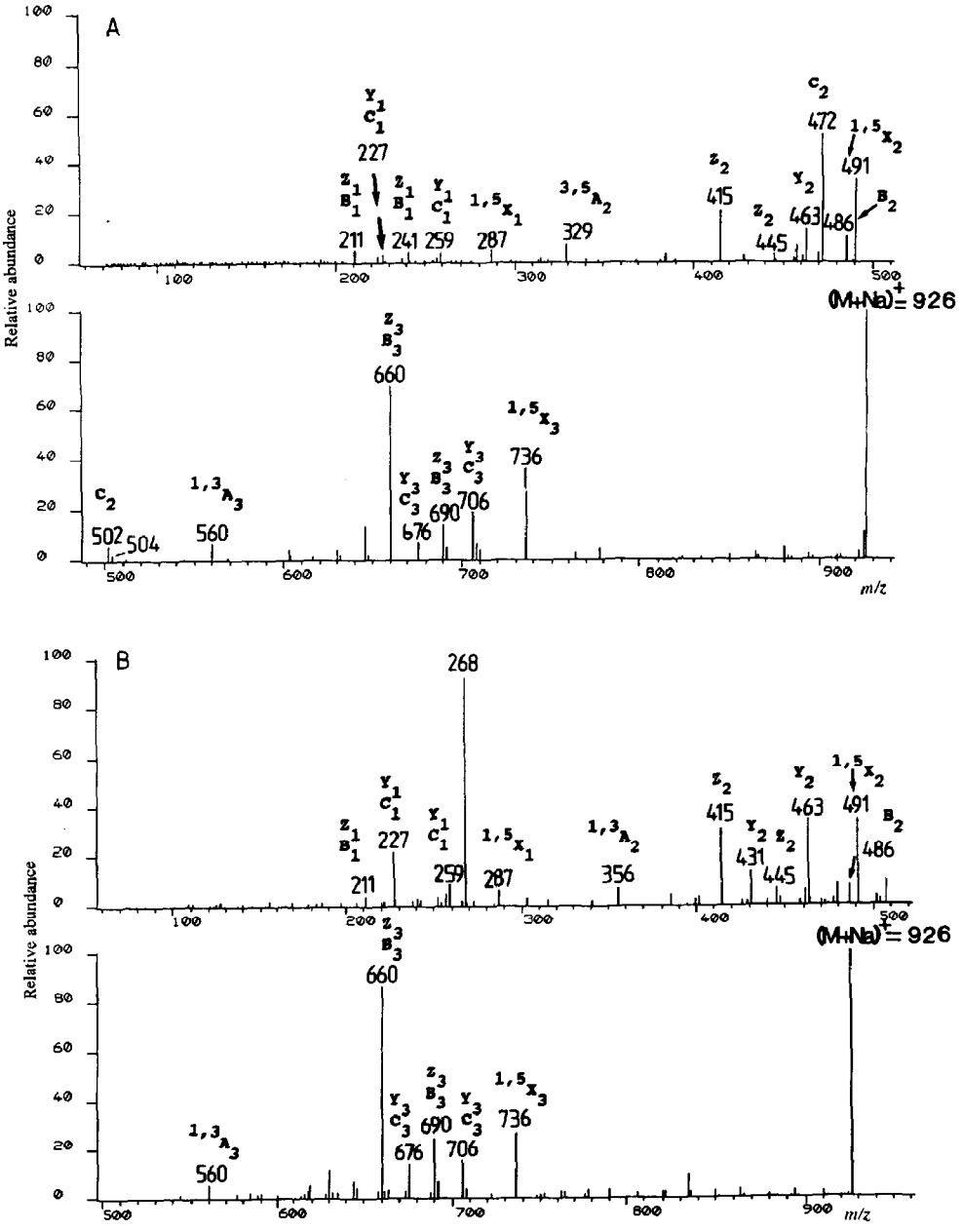

Fig. 1. C.a.d.-mass spectra (6 kV) of the $(M + Na)^+$ ion of A, methylated β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-Glc*p* (**1**); and B, methylated β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-Glc*p* (**2**).

The major ions that reflect the sequences of **1–5** are listed in Table I according to the nomenclature of Domon and Costello[5]. Double designations are used for the ions produced by isomeric fragmentations from the reducing and non-reducing ends of the oligosaccharides, *e.g.*, the ion at *m/z* 211 (Fig. 1A) is attributed to both Z_1 and B_1 cleavages. This double designation is necessary because c.a.d.-m.s. of Hex–Hex–Hex-NAc sequences produces Z_1 and B_1 ions with a mass shift of 41 (difference in mass between Hex and HexNAc).

Fig. 1 gives the $(M + Na)^+$ c.a.d.-mass spectra of the methylated isomeric tetrasaccharides **1** and **2**. Their sequences may be deduced, for instance, from the $^{1,5}X_i$

TABLE I

Major ions obtained on f.a.b.-m.s.–m.s. of $(M + Na)^+$ ions from the methylated oligosaccharides **1–5**

	1	**2**	**3**	**4**	**5**
Mol. wt. of $(M + Na)^+$	926	926	1287	1287	1532
Daughter ions					
$B_i + Na - H$ or $+H$	241 486 690	486 690	604	604	
$B_i + Na + H - CH_3OH$	211 660	211 660	572 1021	572 1021	572 817 1225
$C_i + Na + H - CH_3OH$	227 472 676	227 676	833	588 833	588 1037 833 1241
$C_i + Na - H$ or $+H$	259 502 706	259 706	618 1067	618 1067	618 1271
$^{1,5}X_i + Na$	287 491 736	287 491 736	491 736	491 736	532 736 981
$Y_i + Na + H$ or $-H$	259 463 706	259 463 706	463 910	463 910	708
$Y_i + Na + H - CH_3OH$	227 676	227 431 676			921
$Z_i + Na - H$	241 445 690	445 690	690	690	
$Z_i + Na - OCH_3$	211 415 660	211 415 660	415 660 864	415 660	660 905

series of ions (*m/z* 287, 491, and 736) that correspond to the reducing-end sequence HexNAc–Hex–Hex, and from the B_i series of ions (*m/z* 241 or 211, 486, and 690) that correspond to Hex–HexNAc–Hex.

Methylated isomers **1** and **2** can be discriminated by comparison of the C_2 fragments. For **1** (Fig. 1A), the daughter ion at *m/z* 472 corresponds to the elimination of methanol from a 4-substituted HexNAc and, for **2** (Fig. 1B), the fragment at *m/z* 268 is due to the elimination of Hex (loss of 236 m.u. from the C_2 ion at *m/z* 504) characteristic of a 3-substituted HexNAc. Similar results have been reported[2] for classical f.a.b.-m.s. of methylated oligosaccharides. However, in contrast to f.a.b.-mass spectra, where ions from both cleavage of linkages and loss of methanol are present, the present results indicate that the C_2 ion at *m/z* 504 is either weak or absent. This result may be explained by a decomposition of this C_2 ion simultaneously after its formation in the collision cell by loss of a neutral fragment (Hex or MeOH).

Other specific fragmentations were observed. Thus, for **2**, the $^{1,3}A_2$ ion at *m/z* 356 and the $^{1,3}A_3$ ion at *m/z* 560 are attributed to the sequences Hex-(1→3)-HexNAc and Hex-(1→3)-HexNAc-(1→3)-Hex, respectively. For **1**, the Hex-(1→4)-HexNAc sequence produces an $^{3,5}A_2$ ion at *m/z* 329 and the expected $^{1,3}A_3$ fragment is also present.

The specific elimination of the 3-linked substituent also occurs with **3** (Fig. 3A): loss of the 3-linked sequence NeuAc–Hex from the HexNAc produces the ion at *m/z* 268.

The results also allow the α-NeuAc-(2→3) linkage in **3** (Figs. 2A and 3A) and the α-NeuAc-(2→6) linkage in **4** (Figs. 2B and 3B) to be discriminated on the basis of their $(M + Na)^+$ c.a.d.-mass spectra. Except for an intense $^{1,5}X_3$ ion at *m/z* 736, ions containing the non-reducing end of the molecule preponderate for the α-(2→6) linkage (C_i ions at *m/z* 588, 618, 833, 1061: B_4 ion at *m/z* 1021). In contrast, reducing-end fragments preponderate for the α-(2→3) linkage (Z_i at *m/z* 660, 690, and 864; Y_i fragments at *m/z* 463 and 910).

These observations suggest that the α-NeuAc-(2→6)-Hex sequence further stabilises the sodium adduct and induces a charge-remote fragmentation. This process, which has been described also for lithiated fatty acids[17], may reflect enhanced chelation caused by folding of the NeuAc–Hex moiety. In contrast, α-(2→3) linkages do not enhance the chelation and, in fact, the alkali metal interacts at the α-(2→3) linkage and promotes the formation of the C_1 ion at *m/z* 356.

The same fragmentation pattern was seen in the c.a.d.-mass spectra of methylated **5** (Fig. 4), where the NeuAc residue is α-(2→6)-linked to Hex (C_i daughter ions at *m/z* 588, 618, 833, 1037, 1241, and 1271; lack of a C_1 ion at *m/z* 356, and no intense Z_5 cleavage ions at *m/z* 1109 and 1141). The sequence of the hexasaccharide **5** is deduced from C_i ions (*m/z* 588, 833, 1037, and 1241), which correspond to the sequence NeuAc–Hex–HexNAc–Hex–Hex, and the $^{1,5}X_i$ ions (*m/z* 532, 736, and 981), which correspond to the reducing-end sequence HexNAc–Hex–Hex–HexNAc.

Thus, c.a.d.-mass spectrometry of sodium adducts of methylated oligosaccharides is an alternative to that of $(M + H)^+$ ions when additional sensitivity is necessary. The fragmentation mechanisms proposed are hypothetical at present and clarification

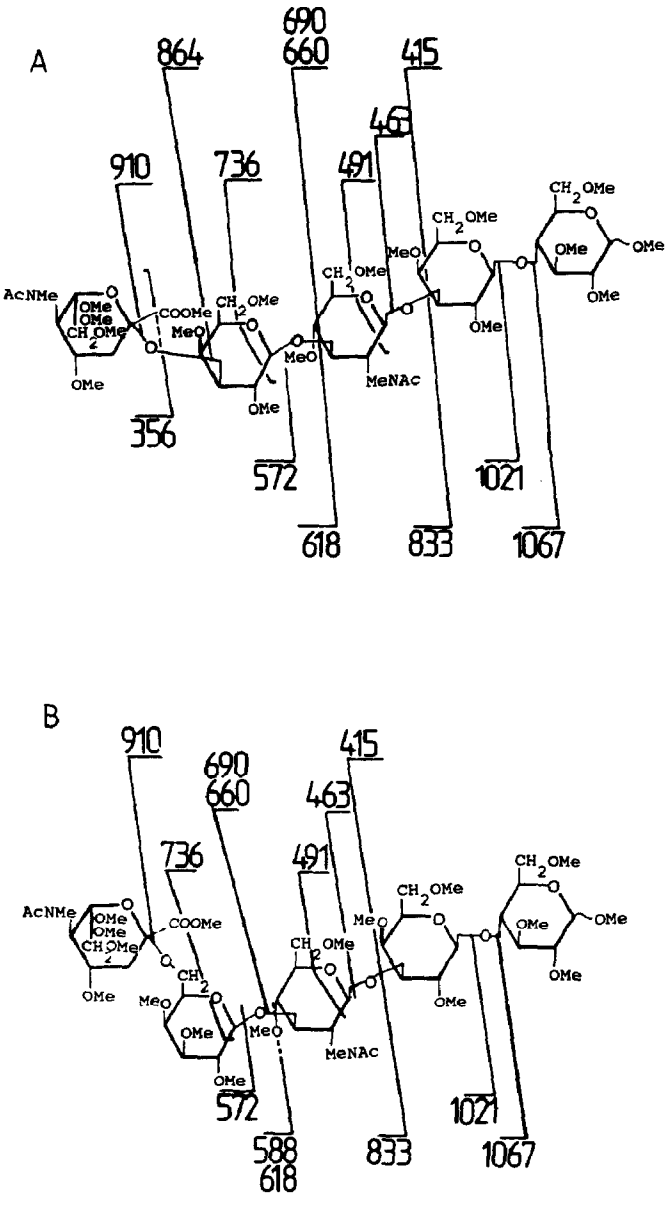

Fig. 2. Fragmentation pattern of methylated α-D-NeuAc-(2→3)-β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-Glc*p* (**3**) (A) and methylated α-D-NeuAc-(2→6)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-Glc*p* (**4**) (B) obtained by c.a.d.-m.s. (6 kV) of the $(M + Na)^+$ ions.

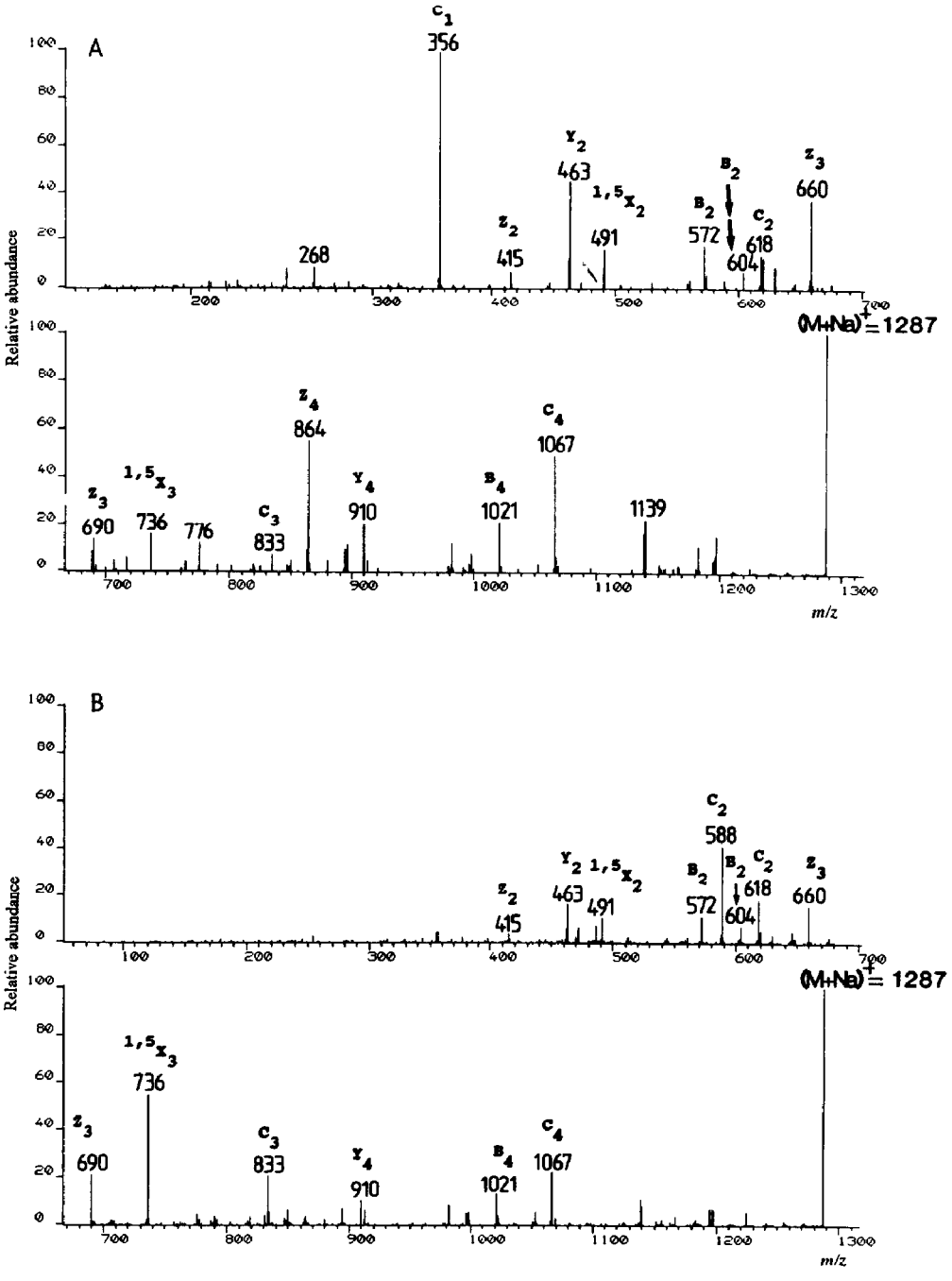

Fig. 3. C.a.d.-mass spectra (6 kV) of the $(M + Na)^+$ ion of A, methylated α-D-NeuAc-(2→3)-β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-Glc*p* (**3**); and B, methylated α-D-NeuAc-(2→6)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-D-Glc*p* (**4**).

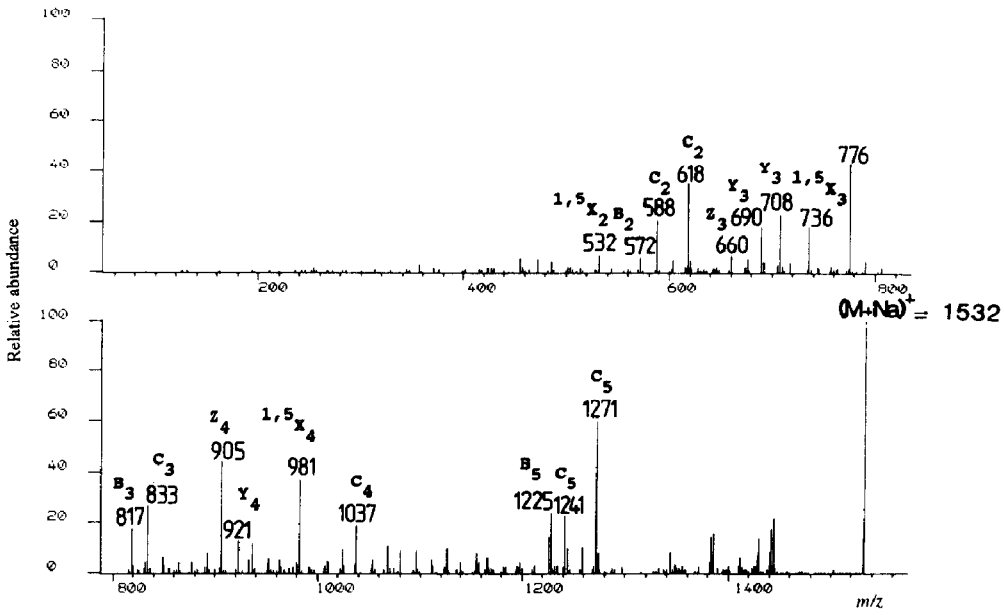

Fig. 4. C.a.d.-mass spectra (6 kV) of the $(M + Na)^+$ ion of methylated α-D-NeuAc-(2→6)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→2)-α-D-Man*p*-(1→3)-β-D-Man*p*-(1→4)-D-Glc*p*NAc (**5**).

will require further studies in order to determine the scope and limitations for the sequencing of oligosaccharides.

ACKNOWLEDGMENTS

These investigations were supported by the Centre National de la Recherche Scientifique (Unité Mixte de Recherche No. 111; Directeur Professeur A. Verbert) and by the Université des Sciences et Techniques de Lille Flandres-Artois.

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