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# A procedure for the analysis by mass spectrometry of the structure of oligosaccharides from high-mannose glycoproteins

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ABSTRACT

A strategy, based on mass spectrometry, for analysis of the structure of oligosaccharides obtained from high-mannose glycoproteins, is described. The oligosaccharides are analysed first by f.a.b.-m.s. as the acetylated alditols, then *O*-deacetylated, oxidised with periodate, and reduced with borodeuteride. The products are analysed further by f.a.b.-m.s. after acetylation and subsequently by both f.a.b.- and e.i.-m.s. after methylation. The entire procedure is carried out on the same sample and the data allow assignment of the positions of all of the glycosidic linkages, including those of the branched residues. Individual components in mixtures of isomeric compounds can often be identified from f.a.b.-m.s. by their molecular weights after periodate oxidation.

## INTRODUCTION

Various forms of mass spectrometry play an important role in the determination of the structure of glycoconjugates. Even though the complete structure cannot be obtained, the superior sensitivity compared to those of other spectroscopic techniques makes mass spectrometry an attractive alternative. F.a.b.-m.s. can be used for the analysis of underivatised as well as derivatised compounds. Information about the molecular weight is always obtained, and, after acetylation or methylation, information on sequence is obtained also. Ions that indicate the sequence are particularly intense in f.a.b.-m.s. of methylated compounds when internal HexNAc residues are present. These ions are formed by cleavage of the HexNAc linkages, whereas ions produced by cleavage of Hex linkages are of low intensity or absent. Therefore, the analysis of oligosaccharides that contain only Hex residues is a problem in f.a.b.-m.s. and this type of oligosaccharide is best analysed in the e.i. mode. In contrast to f.a.b.-m.s., e.i. spectra shows abundant sequence ions from both the non-reducing (A-series[1]) and the reducing (J-series[1]) terminals. In general, mass spectrometry gives no information regarding the positions of the glycosidic linkages. However, a method, based on periodate oxidation, that extends the information on structure obtainable by f.a.b.-m.s. to include determination of the positions of the linkages has been developed[2].

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We describe a further application of this method to high-mannose oligosaccharides. In order to identify the sequence ions, methylated compounds are analysed both in the e.i. and f.a.b. modes.

## EXPERIMENTAL

The high-mannose oligosaccharides, obtained from BioCarb Chemicals AB (Lund), were isolated from human mannosidosis urine and contained one GlcNAc residue as the reducing terminal. Gel filtration in acettone–chloroform (2:1) was performed on a column (1 × 20 cm) of LH-20 (Pharmacia). All reagents used were of analytical grade.

*Periodate oxidation*[3]. — A solution of each reduced oligosaccharide (50–100 μg) in 0.1 M acetate buffer (5 mL, pH 5.5) that contained 8 mM sodium periodate was stored at 4° in the dark for 48 h. The pH of the solution was adjusted to 7.0 with 0.1 M NaOH, $NaBD_4$ (25 mg) was added, and the mixture was stored at 4° overnight. After the addition of acetic acid to pH 4.5, the sample was concentrated to dryness. Boric acid was removed by evaporation of methanol (3 × 3 mL) from the residue.

*Acetylation and methylation.* — Reduced compounds were acetylated with acetic anhydride–pyridine (2:1, 2 mL) at 100° for 30 min. Ethanol (2 mL) was added and, after concentration to dryness, the acetylated product was extracted from water with chloroform (3 × 1 mL). The combined extracts were washed with water (3 × 5 mL) and then concentrated to dryness. Pyridine was removed by evaporation of toluene from the residue. *O*-Deacetylation was carried out in methanol–aqueous 25% $NH_4OH$ (4:1,5 mL) at room temperature overnight. Acetylated compounds were methylated as described[4]. Methylated samples were purified on an LH-20 column. Fractions were assayed for hexose by t.l.c. (chloroform–methanol, 9:1) and staining with an anisaldehyde reagents[5].

*Mass spectrometry.* — F.a.b.-m.s. (positive ion mode) was performed with a VG ZAB SE instrument. Samples in thioglycerol (1-thio-2,3-propanediol) were loaded on the stainless-steel target, which was bombarded with Xe atoms with a kinetic energy of 8 keV. Ions were accelerated using a potential of 10 kV. The molecular weight (M) was determined from the $[M + 23]^+$ ion in separate high-mass scans after the addition of sodium iodide. Direct-inlet-probe e.i.-m.s. was carried out using the above instrument. All e.i.-mass spectra were recorded at 70 eV with an ion-source temperature of 300°. The mass annotations are the nearest integer of the measured masses expressed in mass units (m.u.). Assuming singly charged ions ($z = 1$) of carbohydrate origin, for $m/z < \sim 10^3$ m.u., these data correspond to the mass numbers of the ions (defined as the sum of atomic mass numbers of all the atoms), whereas, for the higher-mass ranges studied, a mass annotation 1 unit higher will result. Mass annotations for isotopic clusters refer to the most abundant ion therein. The clusters will be dominated by the $^{13}C$ contribution for ions with $> \sim 90$ carbons (based on the standard $^{13}C$ content of 1.108%).

RESULTS

*Strategy.* — A flow chart that summarises the series of reactions designed for analysis of the structure of high-mannose oligosaccharides is shown in Fig. 1. F.a.b.-m.s. of each reduced and acetylated compound gives information about the molecular weight, the sequence of monosaccharides, and the presence of branched residues. After *O*-deacetylation, the compound is subjected to periodate oxidation and borodeuteride reduction. F.a.b.-m.s. of the acetylated product shows if complete oxidation has been achieved. In addition, from the sequence ions, the positions of some of the glycosidic linkages can be deduced. If the oxidation is incomplete, the compound can be *O*-deacetylated and subjected to further periode oxidation. The completely oxidised and acetylated compound is methylated and analysed by f.a.b.-m.s. and e.i.-m.s. to give additional information on structure in terms of the positions of substitution of the branched residues. All of these reactions are carried out on the same portion of the sample. The resulting data allow assignment of all glycosidic linkages as discussed below.

*Mass spectrometry of periodate-oxidised compounds.* — The procedure is demonstrated with the heptasaccharide **7**.

An f.a.b.-mass spectrum of the reduced and acetylated compound is shown in Fig. 2A. A disaccharide sequence is determined from the primary sequence ions with *m/z* 331 and 619, and a trisaccharide sequence from the ion with *m/z* 907. The ion with *m/z* 374 is

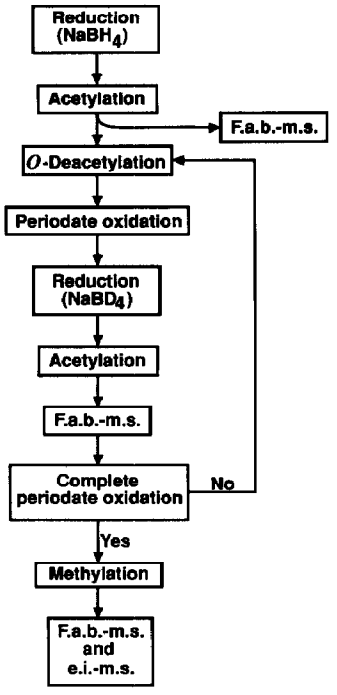



Fig. 1. Strategy for analysis of the structure of high-mannose oligosaccharides by mass spectrometry.

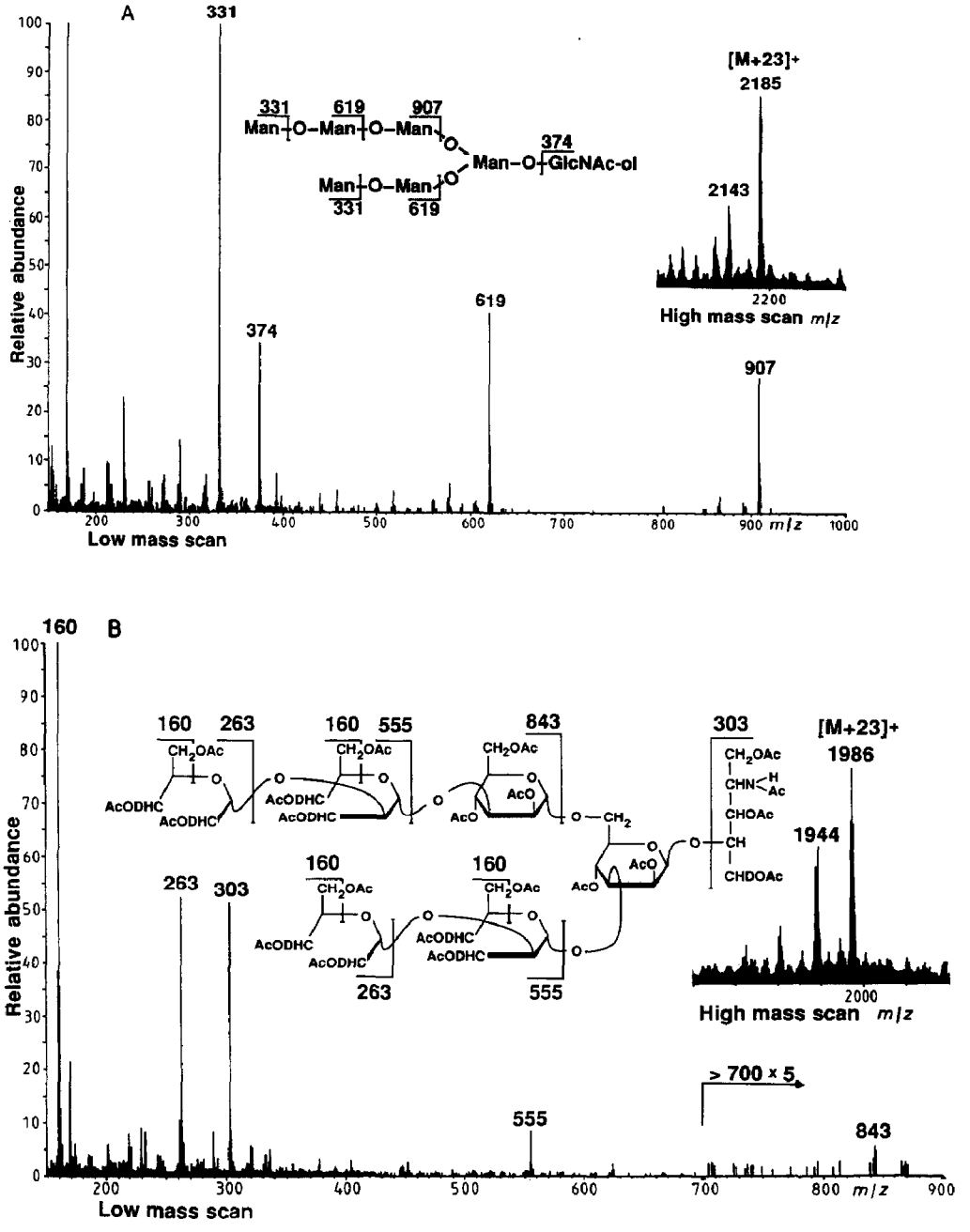
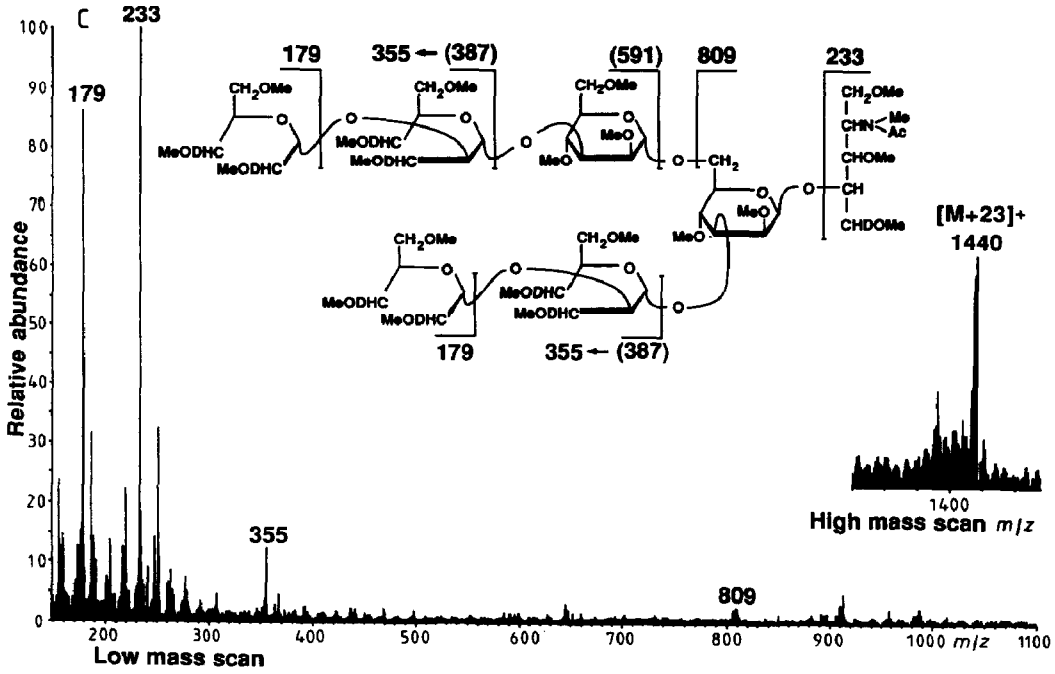
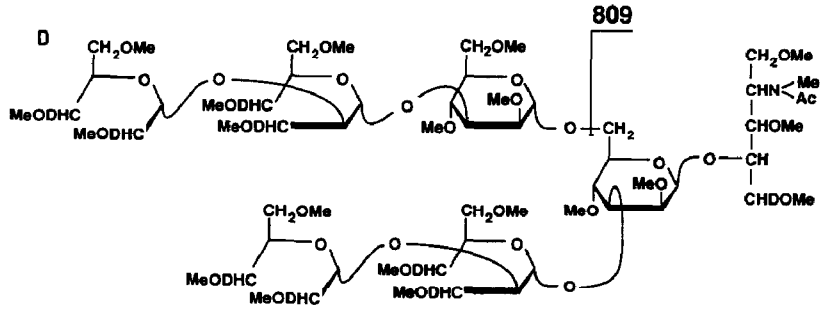
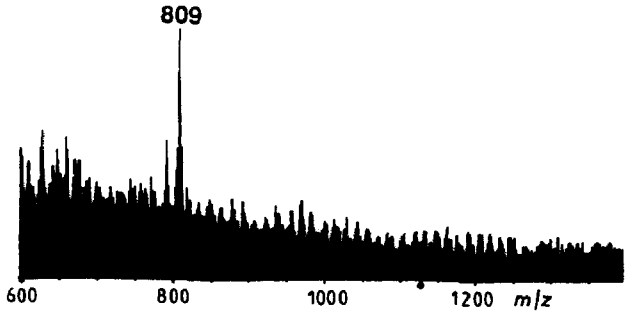



Fig. 2. F.a.b. mass spectra of the compounds obtained from **7** (Table I) by A, acetylation; B, periodate oxidation, borodeuteride reduction, and acetylation; C, periodate oxidation, borodeuteride reduction, and methylation; D, part of an e.i.-mass spectrum of the product in C.


C
233
179
355
809
Relative abundance
Low mass scan
High mass scan m/z
[M+23]+
1440
179
355 ← (387)
(591)
809
233
MeODHC
MeO
CHOMe
CHDOMe
CHN
Ac


D
809
MeODHC
MeO
CHOMe
CHDOMe
CHN
Ac


809
600
800
1000
1200 m/z

characteristic for a GlcNAc-ol residue. No ions which indicate longer linear sequences are seen, which suggests a branched structure. An $[M + 23]^+$ ion with *m/z* 2185 and a secondary ion with *m/z* 2143, formed by elimination of ketene, are consistent with a heptasaccharide-alditol.

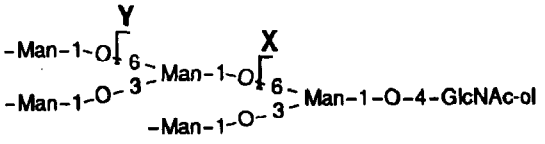
The compound obtained after periodate oxidation, reduction, and acetylation gave the f.a.b.-mass spectrum shown in Fig. 2B. A non-reducing terminal Hex residue is recognised by the sequence ion with *m/z* 263, and an ion with *m/z* 160 which is formed by cleavage of a C–O bond in the periodate-degraded terminal and 2-substituted Man residues. The sequence ion with *m/z* 555 determines a sequence Man-(1→2)-Man or Man-(1→4)-Man, since 2- and 4-substituted Hex residues give the same mass increment to the sequence ions[2]. However, these sequences can be discriminated as discussed below. A sequence ion with *m/z* 843 is deduced by the addition of 288 m.u. to *m/z* 555 and represents a periodate-resistant residue, which, therefore, must be 3-substituted. The sequence ions with *m/z* 263 and 555 determine the disaccharide sequence to be Man-(1→2)-Man and the ions with *m/z* 263, 555, and 843 indicate the linear trisaccharide sequence to be Man-(1→2)-Man-(1→3)-Man. The positions of attachment of these sequences to the disubstituted residue cannot be inferred from the spectrum. The ion that represents the GlcNAc-ol residue has shifted from *m/z* 374 in the untreated compound to *m/z* 303, showing that it is 4-substituted. An $[M + 23]^+$ ion with *m/z* 1986 supports the positions of the linkages, and the absence of ions with higher *m/z* values shows complete periodate oxidation. A secondary fragment with *m/z* 1944 is formed from the $[M + 23]^+$ ion by elimination of ketene.

The periodate-oxidised and acetylated compound was methylated and f.a.b.-m.s. then gave the spectrum shown in Fig. 2C. The sequence ions are of low intensity, and a Man-(1→2)-Man sequence is recognised from the primary fragment with *m/z* 179 and a secondary fragment with *m/z* 355 formed by elimination of methanol, from *m/z* 387, which is not seen. In a previous study of periodate-oxidised and methylated oligosaccharides, it was shown that a secondary fragment (elimination of methanol) which originated from a 2-substituted Hex residue differentiates this residue from a 4-substituted residue[2]. A sequence ion that determines the Man-(1→2)-Man-(1→3)-Man sequence for the trisaccharide (*m/z* 591) is too weak, in the methylated sample, to be identified unambigously.

In all of the oligosaccharides investigated that contained a 3,6-disubstituted Man residue adjacent to the GlcNAc-ol residue, a specific X-ion of the J-type is formed by cleavage of the (1→6)-linkage with retention of the positive charge on C-6 of the 3,6-disubstituted residue (Table I). The ion with *m/z* 809 is such a linkage-specific ion that locates the Man-(1→2)-Man sequence to the 3-position of the branched Man residue. The 6-linkages in the branched residues are calculated from an $[M + 23]^+$ ion with *m/z* 1440 and the ion with *m/z* 809, that correspond to nominal masses of 1439 and 809, respectively (see Experimental). The nominal mass for the branch in the 6-position is given by $1439 - 39 - 809 = 591$, where 39 is the sum of sodium and the glycosidic oxygen. The remainder determines the trisaccharide sequence Man-(1→2)-Man-(1→3)-Man. This sequence was also determined in the acetylated sample. 4-Sub-

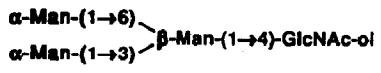
TABLE I

Summary of f.a.b.- and e.i.-m.s. data on branched high-mannose oligosaccharide-alditols



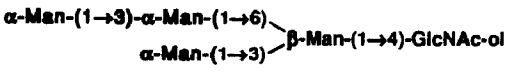

| *Structure* | *Periodate oxidised and methylated* (m/z) | | *[M + 23]⁺ ion* (m/z) *Intact acetylated* | *Periodate oxidised* | |
|---|---|---|---|---|---|
| | *X* | *Y* | | *Acetylated* | *Methylated* |
| *Tetrasaccharide* | | | | | |
| **1** | 601 | – | 1320 | 1113 | 819 |
| *Pentasaccharide* | | | | | |
| **2** | 601 | – | 1608 | 1401 | 1024 |
| *Hexasaccharides* | | | | | |
| **3** | 809 | – | 1897 | 1694 | 1232 |
| **4** | 601 | 970 | 1897 | 1622 | 1188 |
| *Heptasaccharides* | | | | | |
| **5** | 601 | 970 | 2185 | 1914 | 1396 |
| **6** | 809 | 1178 | 2185 | 1914 | 1396 |
| **7** | 809 | – | 2185 | 1986 | 1440 |
| **8** | 809 | – | 2185 | 1918 | 1400 |
| **9** | 601 | 1178 | 2185 | 1914 | 1396 |
| *Octasaccharides* | | | | | |
| **10** | 809 | 1178 | 2474 | 2206 | 1604 |
| **11** | 809 | 1386 | 2474 | 2206 | 1604 |
| **12** | 1018 | 1386 | 2474 | 2206 | 1604 |
| *Nonasaccharides* | | | | | |
| **13** | 1018 | 1594 | 2762 | 2499 | 1812 |
| **14** | 809 | 1386 | 2762 | 2499 | 1812 |
| **15** | 1018 | 1386 | 2762 | 2499 | 1812 |
| *Decasaccharide* | | | | | |
| **16** | 1018 | 1594 | 3050 | 2791 | 2020 |

*Tetrasaccharide*



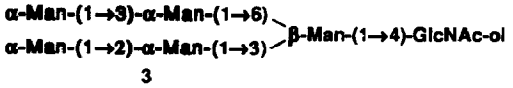

1

*Pentasaccharide*




2

*Hexasaccharides*

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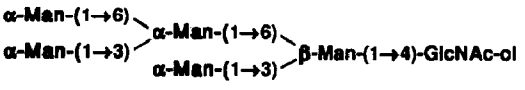
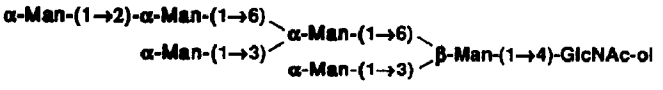



4

*Heptasaccharides*

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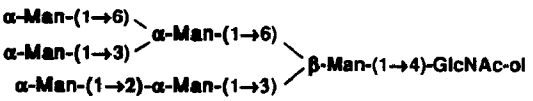


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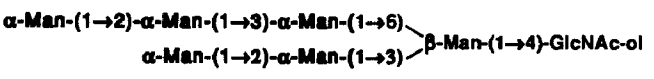


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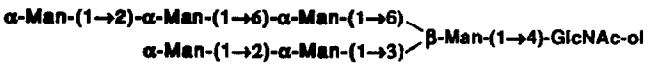


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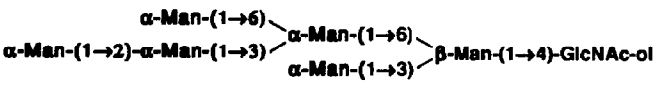
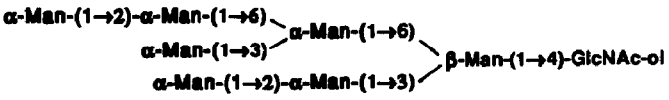



9

*Octasaccharides*

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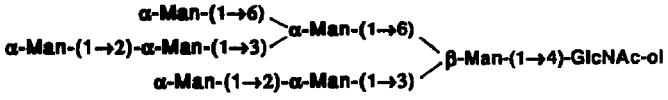


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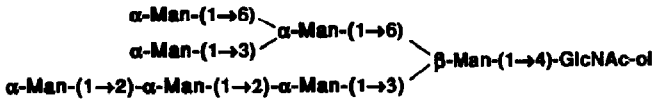
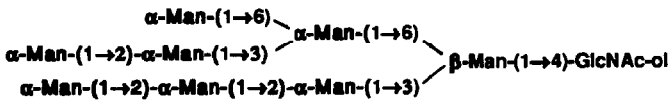



12

*Nonasaccharides*

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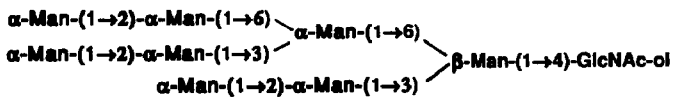


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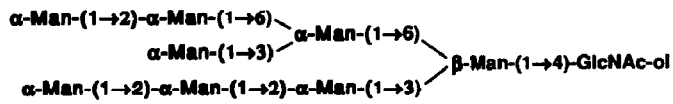
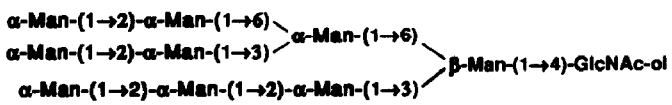



15

*Decasaccharide*




16

stitution of the GlcNAc-ol residue is confirmed in the methylated sample by the ion with *m/z* 233.

As mentioned above, ions formed by cleavage of Hex linkages are of low intensity in f.a.b.-m.s., but more intense in e.i.-m.s.; therefore, the periodate-oxidised and methylated compound was analysed by direct-probe e.i.-m.s. and a part of the spectrum is shown in Fig. 2D. In general, the fragmentation pattern, including the X-ion with *m/z* 809, is similar to that obtained by f.a.b. with the exception of the molecular ion species. Consideration of all of the mass spectrometric data allows assignment of the positions of all of the linkages.

Another fifteen linear and branched high-mannose oligosaccharides were subjected to the procedure described above, but only a few representative examples are discussed. The data obtained from the branched structures are summarised in Table I.

The first example is a linear pentasaccharide with the following structure.

α-Man-(1→2)-α-Man-(1→2)-α-Man-(1→3)-β-Man-(1→4)-GlcNAc

An e.i.-mass spectrum of the methylated product is shown in Fig. 3. The position of the linkages are determined best from the J-series of ions, which in e.i.-m.s. are more intense than those of the A-series. Ions with *m/z* 233 and 437 show a sequence from the reduced terminal of →3)-Man-(1→4)-GlcNAc-ol. Addition of 208 m.u. to *m/z* 437 gives *m/z* 645 and determines the sequence →2)-Man-(1→3)-Man-(1→4)-GlcNAc-ol. Further addition of 208 m.u. gives *m/z* 853 and shows another 2-substituted Man residue in

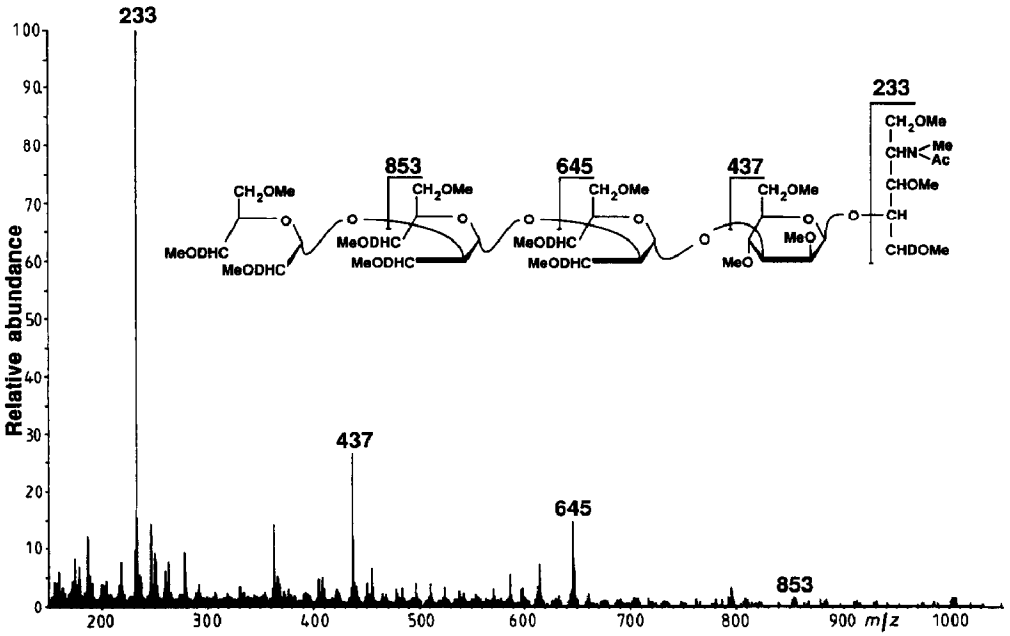



Fig. 3. E.i. mass spectrum of α-Man-(1→2)-α-Man-(1→2)-α-Man-(1→3)-β-Man-(1→4)-GlcNAc-ol, after periodate oxidation, borodeuteride reduction, and methylation.

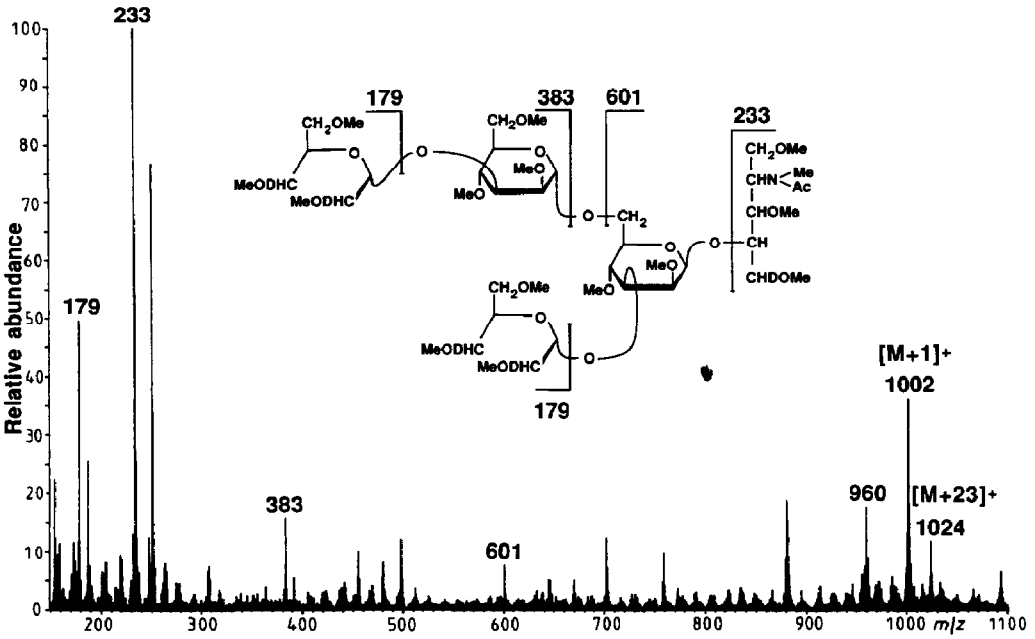



Fig. 4. F.a.b. mass spectrum of **2** (Table I) after periodate oxidation, borodeuteride reduction, and methylation.

the sequence. The mass increment of 208 m.u. to the J-series of sequence ions is also consistent with a 4-substituted Hex residue. However, the f.a.b.-m.s. data on the acetylated and methylated product showed sequence ions from the non-reducing terminal and confirmed the positions of the linkages (data not shown).

An f.a.b.-mass spectrum of the methylated product obtained from the following branched pentasaccharide **2** is shown in Fig. 4.

One of the branches contains the disaccharide sequence Man-(1→3)-Man, determined from the primary sequence ions with *m/z* 179 and 383, and the other branch contains a monosaccharide residue, *m/z* 179. The pattern of substitution of the 3,6-disubstituted residue is determined by the X-ion with *m/z* 601, which shows that the 3-position is occupied by a single Man residue. Therefore, the 6-position must be substituted by the Man-(1→3)-Man sequence, which can be verified by a calculation similiar to that discussed above. As discussed above, the GlcNAc-ol residue is 4-substituted as shown by the ion with *m/z* 233. The interpretation is confirmed by the $[M + 1]^+$ and $[M + 23]^+$ ions with *m/z* 1002 and 1024, respectively.

The hexasaccharide **3** is an example of a branched oligosaccharide with two disaccharide branches.

The f.a.b.-m.s. spectrum of the periodate-oxidised, borodeuteride-reduced, and acetylated compound shows sequence ions with *m/z* 263 and *551*, which determine the sequence Man-(1→3)-Man (Fig. 5A). The sequence Man-(1→2)-Man is determined by the ions with *m/z* 263 and 555. The ion with *m/z* 303 shows, as discussed above, a

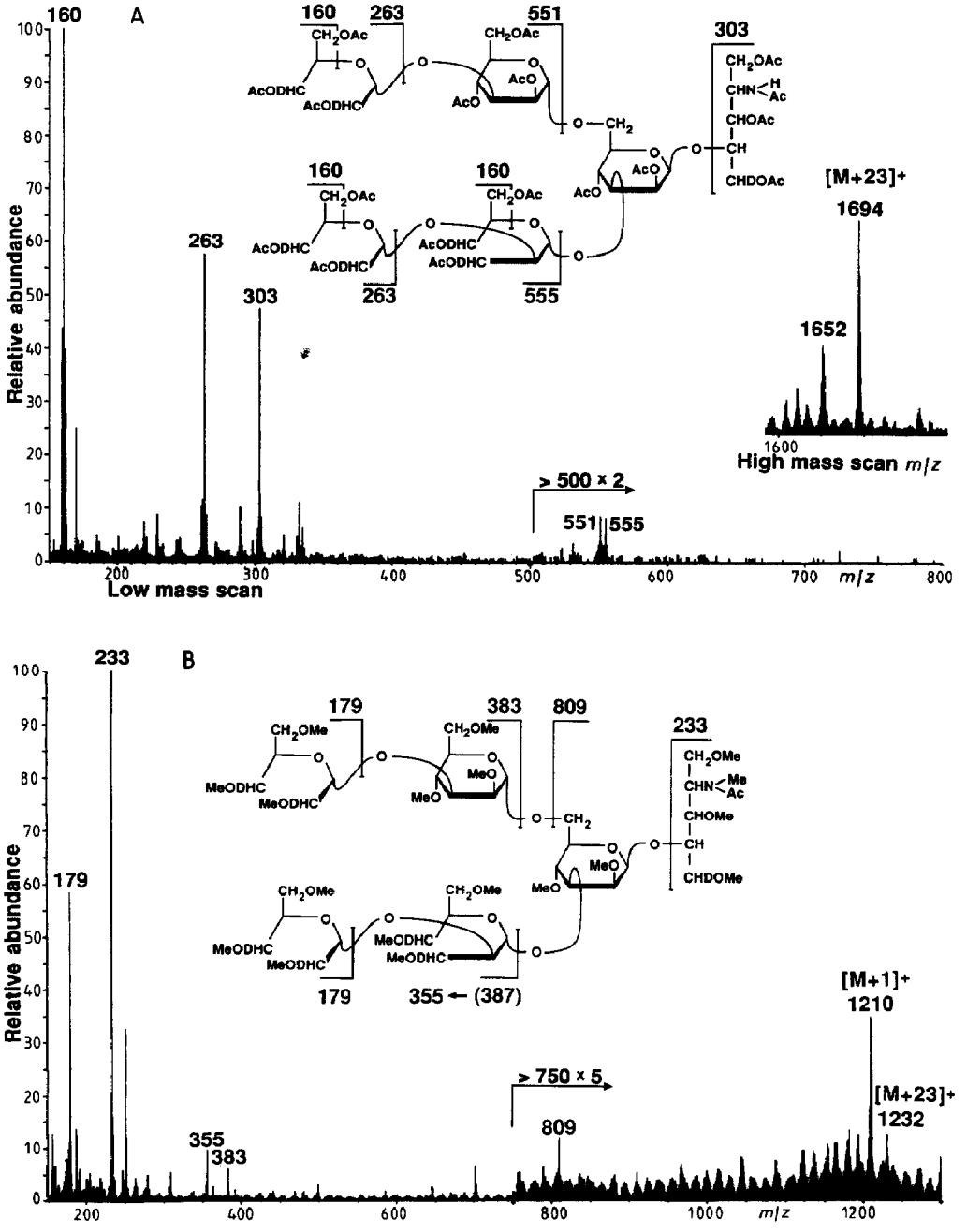



Fig. 5. F.a.b. mass spectra of 3 (Table I) after periodate oxidation, borodeuteride reduction, and acetylation (A), or methylation (B).

4-substituted GlcNAc-ol residue. An $[M + 23]^+$ ion with $m/z$ 1694, together with a secondary ion with $m/z$ 1652, formed by elimination of ketene, gives additional support for the linkages. F.a.b.-m.s. of the methylated compound shows the position of attachment of these disaccharide sequences to the 3,6-disubstituted Man residue (Fig. 5B). The X-ion with $m/z$ 809 shows that the Man-(1→2)-Man sequence is 3-linked to the 3,6-disubstituted Man residue, and consequently the 6-position must be occupied by the Man-(1→3)-Man sequence. The sequence ions with $m/z$ 355 and 383 confirm the positions of the linkages in the disaccharide branches and an $[M + 1]^+$ ion with $m/z$ 1210 is consistent with the expected product.

The general rule discussed above, concerning substitution of 3,6-disubstituted residues, is also applicable to structures that contain two branched residues. By analogy with structures with one 3,6-disubstituted residue that give the X-ion, structures which contain two 3,6-disubstituted residues give, in addition, another linkage-specific Y-ion

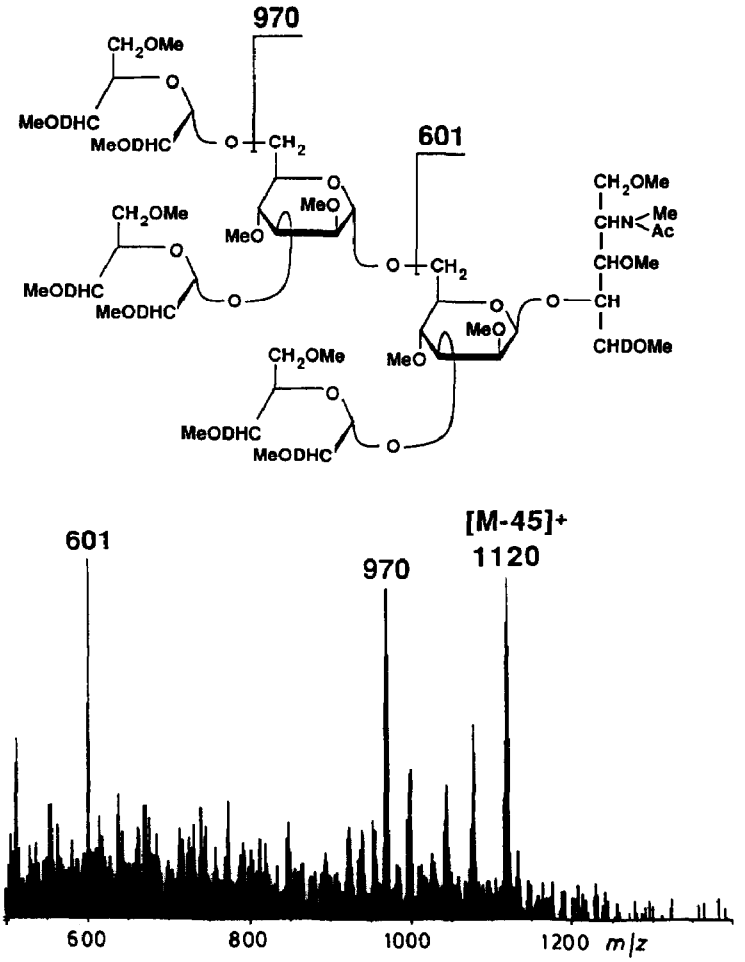



Fig. 6. Part of an e.i. mass spectrum of **4** (Table I) after periodate oxidation, borodeuteride reduction, and methylation. The $[M - 45]^+$ ion is formed by cleavage of the C-1–C-2 bond in the alditol moiety.

(Table I). An e.i.-mass spectrum, displayed in the range of the X- and Y-ions, of the following dibranched structure *4* is shown in Fig. 6.

The positions of substitution of the branched residue adjacent to the GlcNAc-ol residue are determined by the X-ion with *m/z* 601, as discussed above. Substitution of the other 3,6-disubstituted residue is determined from the Y-ion with *m/z* 970. An $[M-45]^+$ ion with *m/z* 1120 provides supporting evidence for the structure. By a calculation similar to that discussed above using the $[M + 23]^+$ ion with *m/z* 1188 (determined by f.a.b.-m.s.) and the *m/z* 970 ion, it can be concluded that the outer branched residue is 6-substituted by another monosaccharide residue.

Another demonstration of this method is shown by the branched heptasaccharide **9** that contains two 3,6-disubstituted residues.

The f.a.b.-mass spectrum, obtained after periodate oxidation, borodeuteride reduction, and acetylation, is shown in Fig. 7A. The only sequence ions seen from the non-reducing terminal have *m/z* 263 and 555, which determine the disaccharide sequence Man-(1→2)-Man as discussed above. The molecular weight of the product is provided by an $[M + 23]^+$ ion with *m/z* 1914. The ion with *m/z* 1872 is formed by elimination of ketene from the $[M + 23]^+$ ion. Information about substitution of the branched residues was furnished by e.i.-m.s. of the methylated product. The mass

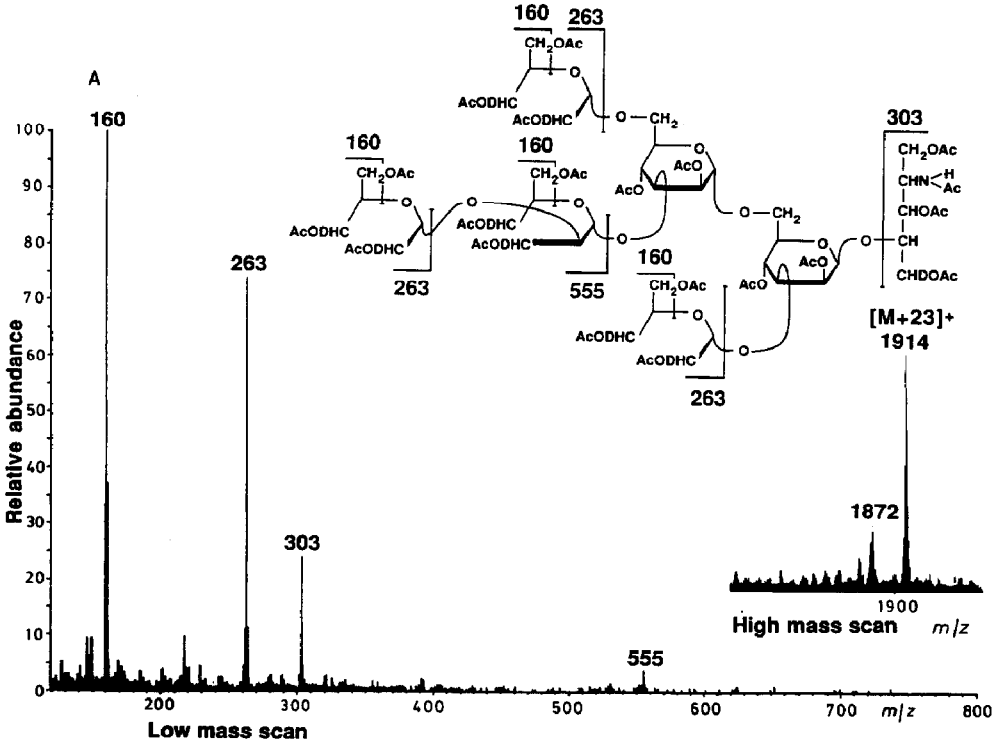
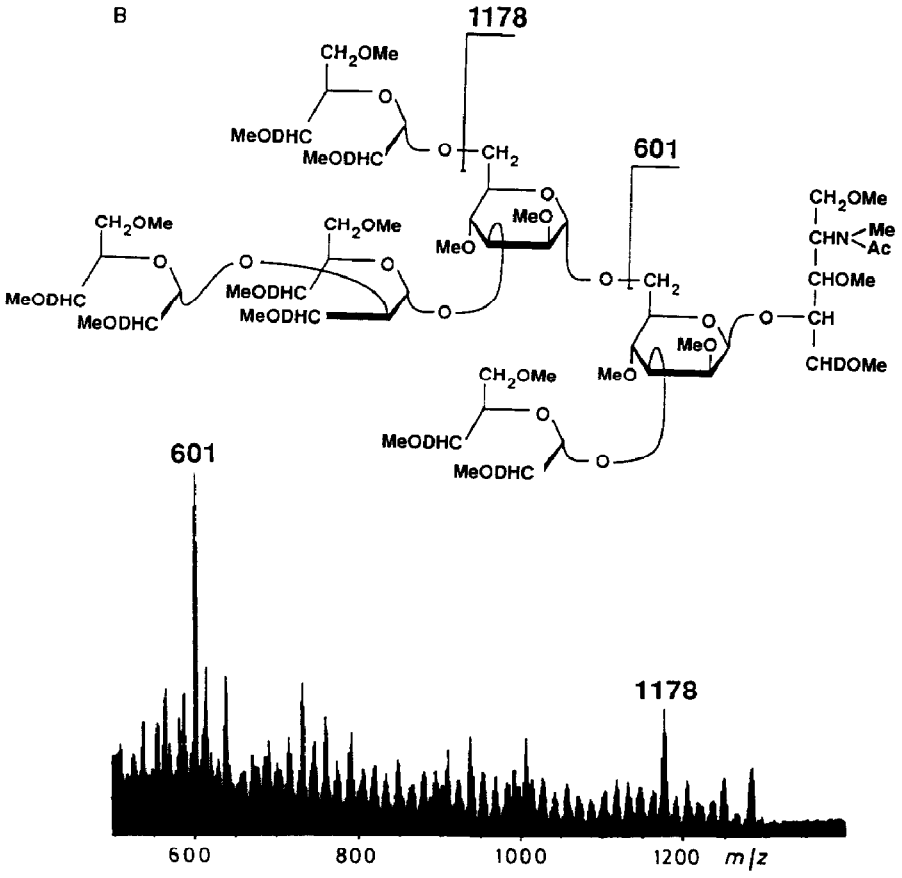



Fig. 7. A, F.a.b. mass spectrum of **9** (Table I) after periodate oxidation, borodeuteride reduction, and acetylation; B, part of an e.i. mass spectrum of **9** after periodate oxidation, borodeuteride reduction, and methylation.

spectrum, displayed in the range of the linkage specific X- and Y-ions, is shown in Fig. 7B. 3-Substitution of the 3,6-disubstituted residue, adjacent to the GlcNA-ol residue, by a monosaccharide residue is determined from the X-ion with *m/z* 601. The other disubstituted residue is 3-substituted by the Man-(1→2)-Man sequence, as deduced from the Y-ion with *m/z* 1178. By calculations similar to those for the previous compound, using the Y-ion and an $[M + 23]^+$ ion with *m/z* 1396 (determined by f.a.b.-m.s.), it can be concluded that the outer branched residue is 6-substituted by a monosaccharide residue.

A final example involves the branched octasaccharide **10**.

F.a.b.-m.s. of the acetylated product obtained after periodate oxidation and borodeuteride reduction gave the spectrum shown in Fig. 8A. The primary sequence ions with *m/z* 263 and 555 show that linear sequences no longer than Man-(1→2)-Man are present. An $[M + 23]^+$ ion with *m/z* 2206 and a secondary with *m/z* 2164, formed by elimination of ketene, are in agreement with the product after complete periodate oxidation. Analysis of the methylated compound by e.i.-m.s. gave a spectrum, which shows the linkage-specific X- and Y-ions, and is presented in Fig. 8B. By calculation, using the ions with *m/z* 809, 1178, and an $[M + 23]^+$ ion with *m/z* 1604 (determined by



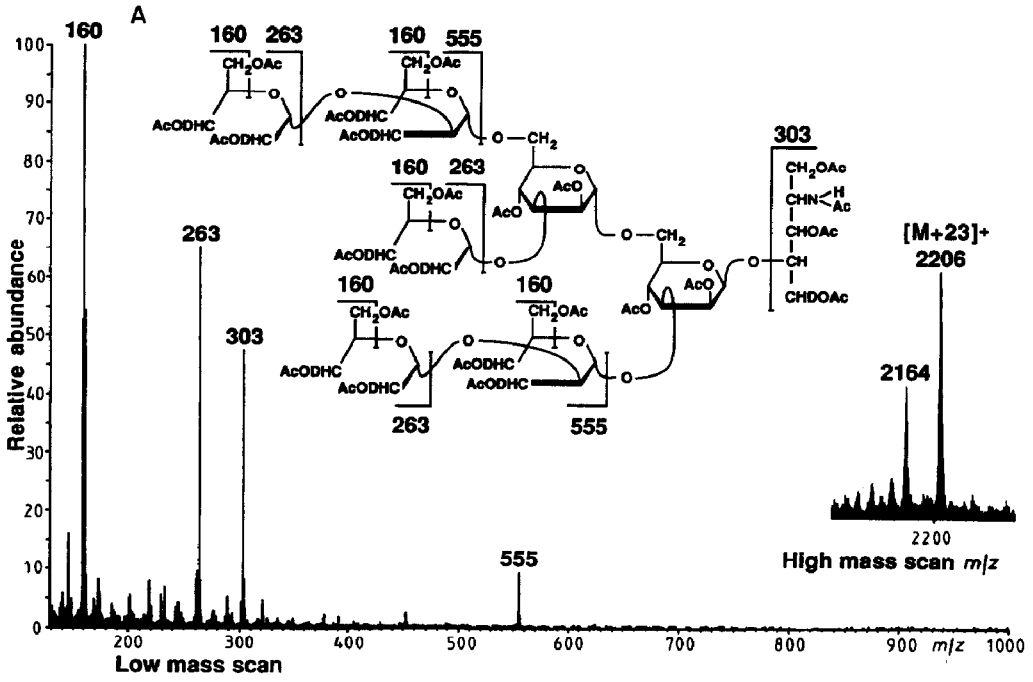
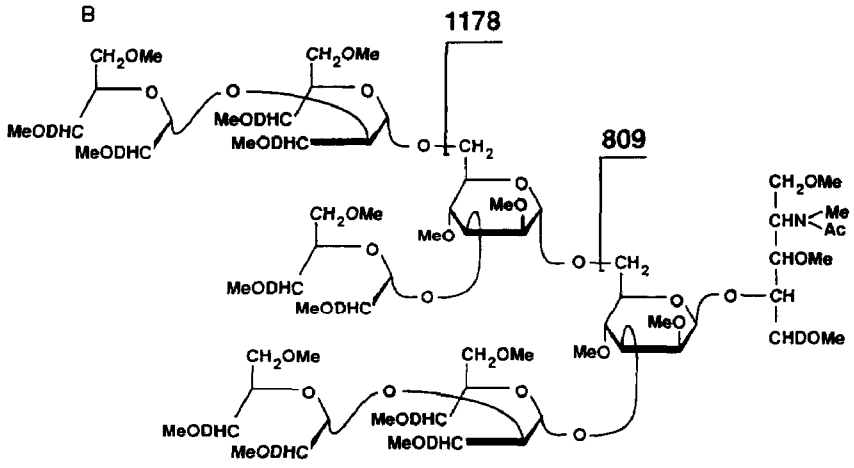
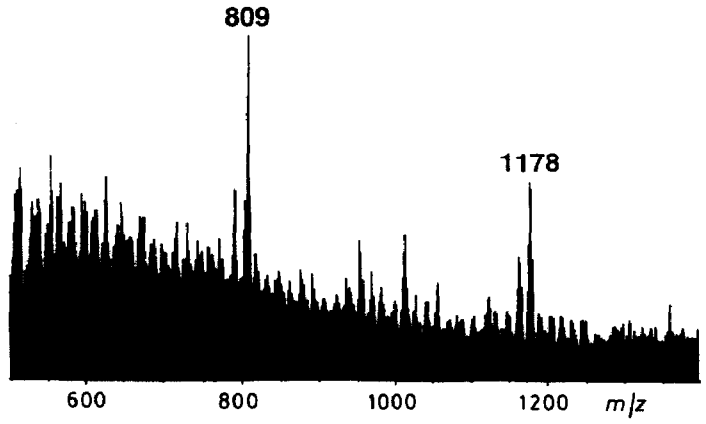



Fig. 8. A, F.a.b.-mass spectrum of **10** (Table I) after periodate oxidation, borodeuteride reduction, and acetylation; B, part of an e.i.-mass spectrum of **10** after periodate oxidation, borodeuteride reduction, and methylation.

f.a.b.-m.s.), substitution of both 3,6-disubstituted residues can be obtained by analogy with the previous branched structures.

*Mapping of high-mannose structures in glycoproteins.* — Several high-mannose structures have been studied and the data are listed in Table I. Due to periodate oxidation, the combination of X-, Y-, and $[M + 23]^+$ ions gives a unique set of numbers, which can be used to identify the compounds. However, it is necessary to analyse the oligosaccharides in both the f.a.b. and e.i. modes in order to identify all three ions. Structures smaller than octasaccharides can usually be identified by the products of periodate oxidation, solely from the $[M + 23]^+$ ions. This approach can be used as a mapping method to identify high-mannose oligosaccharides enzymically released from small amounts of glycoproteins. Glycoproteins produced by recombinant DNA techniques often exhibit variable patterns of glycosylation. Therefore, a simple method for monitoring the high-mannose glycosylation will be useful. Selective mass-spectrometric analysis for $[M + 23]^+$ ions is an extremely sensitive method for the analysis of high-mannose structures. The molecular weight range in an f.a.b.-mass spectrum of a mixture of hexa-, hepta-, and octa-saccharides analysed after periodate oxidation, borodeuteride reduction, and acetylation is shown in Fig. 9.




DISCUSSION

Nowadays, there are recombinant techniques available for production of biologically important glycoproteins for use in therapy and diagnosis. The glycosylation of these proteins may be vital for the biological function and the pattern depends on the type of cells and their growth conditions. Analysis of the structure of glycoprotein oligosaccharides has become important, especially for glycoproteins intended for use in therapy, and there is a need for sensitive methods. Mass spectrometry offers the sensitivity and, in combination with specific chemical modifications which should give quantitative yields of products, the information on structure can be increased considerably. Periodate oxidation is a reaction which meets these requirements. The combination of periodate oxidation and f.a.b.-m.s. has been applied successfully to sialylated *O*- and *N*-linked glycoprotein oligosaccharides[6-8]. These oligosaccharides have internal HexNAc residues, which give rise to intense ions formed by cleavage of their glycosidic

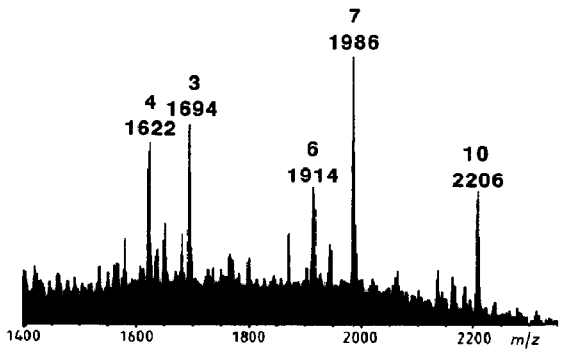



Fig. 9. Part of an f.a.b.-mass spectrum of a mixture of high-mannose structures after periodate oxidation, borodeuteride reduction, and acetylation (~ 1 ng of the mixture was loaded onto the probe). The $[M + 23]^+$ ions correspond to **3**, **4**, **6**, **7**, and **10** (Table I).

linkages. High-mannose oligosaccharides lack such residues, which makes the glycosidic fragment ions less abundant. However, the combination of periodate oxidation and f.a.b.-m.s. has been used successfully, by Pappas *et al.*[9], on hexose-containing oligosaccharides as acetylated derivatives.

The high-mannose oligosaccharides used in this study were isolated from human mannosidosis urine and each contained one GlcNAc residue. Such oligosaccharides can be obtained from glycoproteins by treatment with various endo-$\beta$-*N*-acetylglucosaminidases[10]. The urinary oligosaccharides represent undigested compounds that originate from glycoproteins and are, therefore, relevant compounds for the investigation. Characteristic features of these oligosaccharides are the absence of Man-(1→4)-Man linkages and the branched residues always are 3,6-disubstituted.

All reactions used in this procedure can be carried out on the same portion of the sample (Fig. 1). The first step involves analysis by f.a.b.-m.s. of the reduced and acetylated compound in order to determine the molecular weight, the length of linear sequences, and the presence of branched residues. After periodate oxidation, reduction, and acetylation, f.a.b.-m.s. shows if complete oxidation has been achieved. Incompletely oxidised samples give one or several $[M + 23]^+$ ions. It is advantageous to analyse the compound after acetylation since the sensitivity is thereby increased considerably. F.a.b.-m.s. of the periodate-oxidised and acetylated compound also gives information on the linkages in linear sequences, but the substitution at the branched residue cannot be determined. The $[M + 23]^+$ ion gives rise to secondary ions formed by eliminations of ketene even when acetylation is complete. Analysis by f.a.b.-m.s. or e.i.-m.s. after methylation gives data which not only confirms the positions of the linkages in linear sequences but also in the branched residues. The fragmentation pattern which determines the positions of substitution of the branched residues is applicable only to 3,6-disubstituted Hex residues. Cleavage of the (1→6) linkages and localisation of the positive charge at C-6 of 3,6-disubstituted residues, giving the X- and Y-ions, is the key factor (Table I). If the other substituent of this branched residue had been linked to the 2- or 4-position, the branched residue would be oxidised by periodate. However, in

order to formulate general rules for determination of the positions of substitution of branched residues, further studies of model compounds will be necessary.

Since the sequence ions formed by cleavage of Hex residues are of low abundance in f.a.b.-m.s., it is important that they be verified by e.i.-m.s. where both the A- and J-series of ions are seen. In the linear structure (Fig. 3), however, the J-series dominate the A-series of ions. The Y-ions (Table I) are often not observed in f.a.b.-m.s. but are quite abundant in e.i.-m.s. The drawback in e.i.-m.s. is the absence of information on molecular weight, but this information is obtained from f.a.b.-m.s. The pseudo-molecular ions are important since they can be used, together with the X- and Y-ions, to compute the positions of linkages in branches 6-linked to the 3,6-disubstituted residues.

In the spectra presented, several ions are seen which have not been annotated. Most of these ions, some of which can be explained, involve oxidised residues, but they provide no further information on structure and, therefore, are not annotated.

Some points should be noted concerning the fragmentation. For example, the sequence Man-(1→2)-Man is determined in methylated samples from a secondary fragment with *m/z* 355 formed from a primary fragment with *m/z* 387, which is not seen. Previous work on complex types of glycoprotein oligosaccharides showed that 2-substituted Hex residues give both the primary and secondary sequence ions[2]. The presence of the secondary fragment (elimination of methanol) discriminates between 2- and 4-substituted Hex residues. The eventual presence of a sequence ion with *m/z* 387 for a Man-(1→4)-Man sequence, and the possible formation of a secondary ion with *m/z* 355, was not investigated since this linkage has not been found in this type of glycoprotein oligosaccharides. When the ring structure has been cleaved by periodate oxidation, other fragmentation pathways are plausible, as demonstrated by Pappas *et al.*[9] for acetylated samples, where a 4-substituted Hex residue gives a sequence ion by cleavage of the C-5–O-5 bond. This sequence ion contains the 4-substituent. The same cleavage is seen in a 2-substituted Hex residue, which gives an ion with *m/z* 160 (Figs. 2B, 5A, 7A, and 8A) that is independent of the sequence ions.

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