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Identification and structural analysis of synthetic oligosaccharides of *Shigella sonnei* using MALDI-TOF MS

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Abstract

MALDI-TOF mass spectroscopy was used for the molecular weight determination of protected synthetic oligosaccharides related to a cell surface bacterial polysaccharide. By-products containing chlorinated protecting groups caused isotopic patterns characteristic of the natural isotopic distribution of chlorine, were identified on the basis of isotopic distribution. 2,4,6-Trihydroxyacetophenone (THAP) as a matrix was better than 2,5-dihydroxybenzoic acid (DHB) for compounds containing chlorine, since monoisotopic resolution and no fragmentation were observed. In the post source decay (PSD) mode the identification of the oligosaccharide sequence through cleavage of the interglycosidic linkages was also possible, thus providing a sensitive and accurate tool for the structural verification of synthetic oligosaccharide intermediates. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: MALDI-TOF MS; Post source decay (PSD); Oligosaccharide, *Shigella sonnei*

1. Introduction

In the past few years we have been studying chemical synthetic approaches[1–3] to well-defined oligosaccharides related to the O-specific polysaccharide (Fig. 1) of the Gram-negative bacterium *Shigella sonnei*.[4–6] The eventual purpose of these studies is the development of a semi-synthetic conjugate vaccine for the prevention of diseases caused by this bacterium in both industrialised and developing countries. The complexity of the chemical approach is highlighted by the fact that the targeted oligosaccharides contain a 2-acetamido group in each residue as well as free amino and carboxyl groups in alternating residues.

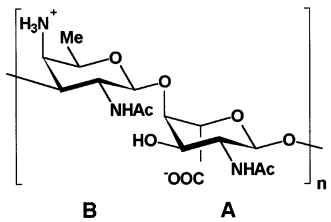

Fig. 1. The chemical repeating unit of the O-specific polysaccharide of *S. sonnei*.

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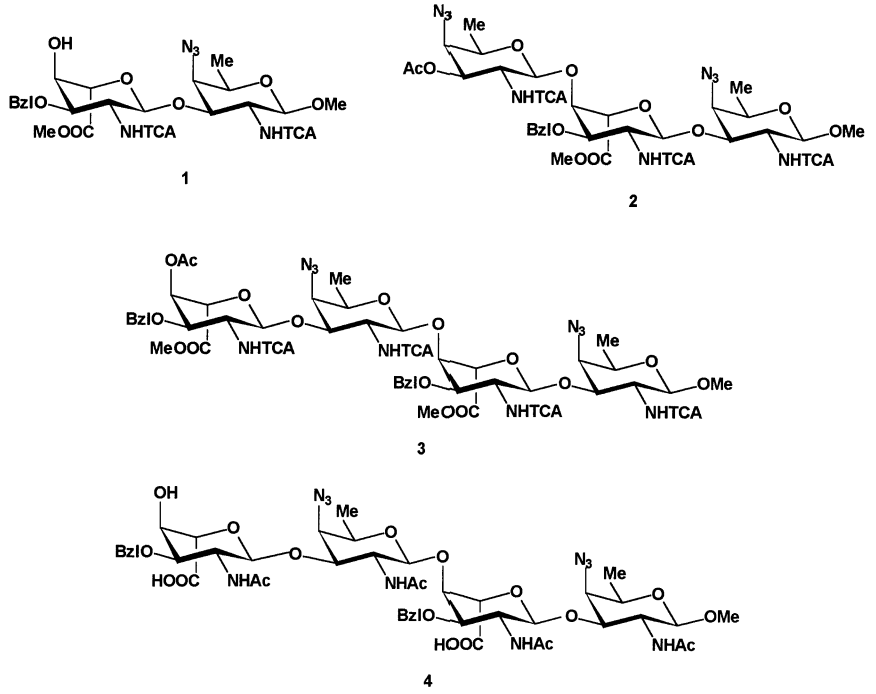


Fig. 2. Synthetic oligosaccharide intermediates with AB sequences of *S. sonnei* polysaccharide.

The chemical synthesis of these oligosaccharides requires numerous protecting groups and synthetic steps. The structure of the reaction products and by-products is usually determined by NMR spectroscopy after purification by column chromatography or HPLC separation. Because of the inherently low sensitivity of NMR spectroscopy and thus the need for relatively large amounts even in the exploratory phase of synthetic studies, we have been studying the feasibility of using MALDI-TOF mass spectroscopy for the structural identification of protected oligosaccharides.

MALDI-TOF MS is a relatively new method of mass analysis, first applied to carbohydrates in 1991 by Mock et al.[7] The method, combined with TLC, has been used recently for the analysis of organic reactions[8] and for the structural analysis of free oligosaccharides.[9–12] Harvey reported a very comprehensive review on MALDI-TOF of carbohydrates which presents the MALDI MS of oligosaccharide derivatives and application of MALDI to monitoring of the products of chemical synthesis. Derivatisation methods for oligosaccharides have been developed with the goal of increasing the sensitivity of the MS analysis. Reductive amination and other reducing terminal derivatisation increase the signal strength, permethylation and peracetylation help the transfer of oligosaccharides to the vapour phase. Only a few examples are listed on MALDI analysis of synthetic oligosaccharides.[13]

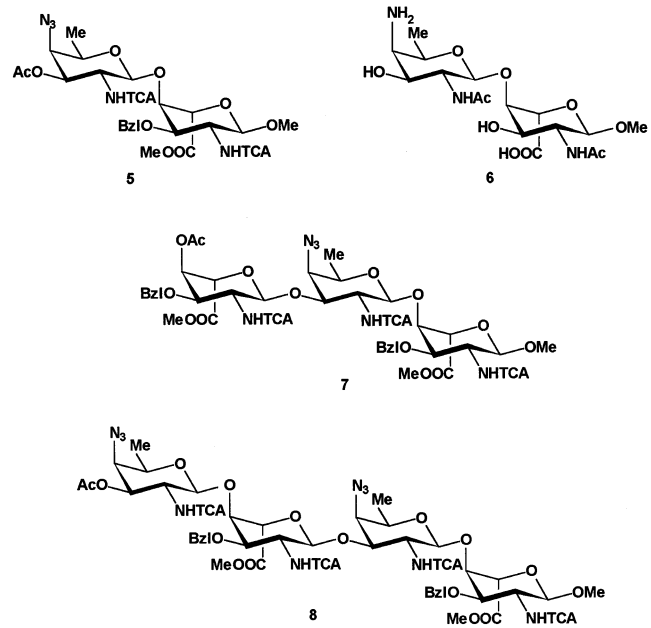


Fig. 3. Synthetic oligosaccharide intermediates with BA sequences of *S. sonnei* polysaccharide.

Table 1
Mass spectrometric data for sodium cationised species of synthesized oligosaccharides

No.	Formula	*m/z*		Accuracy (%)	Resolution (*m/z*)/FWHM	Matrix
		Calculated	Measured			
1	$C_{25}H_{29}Cl_6N_5NaO_{10}$	791.9938	792.33	0.04	1277	DHB
			792.17	0.02	3491	THAP
2	$C_{35}H_{40}Cl_9N_9NaO_{14}$	1147.9789	1148.84	0.07	1225	DHB
			1148.35	0.08	2041	THAP
3	$C_{51}H_{56}Cl_{12}N_{10}NaO_{20}$	1577.49	1576.23	0.08	766	THAP
4	$C_{47}H_{62}N_{10}NaO_{19}$	1093.4090	1093.20	0.02	2050	DHB
5	$C_{25}H_{29}Cl_6N_5NaO_{10}$	791.9938	792.23	0.03	1440	THAP
6	$C_{17}H_{28}N_5NaO_{10}$	458.1745	458.03	0.03	2831	DHB
			458.39	0.05	3537	THAP
7	$C_{43}H_{47}Cl_9N_6NaO_{17}$	1257.0087	1258.03	0.08	1664	DHB
			1257.51	0.04	3545	THAP
8	$C_{51}H_{56}Cl_{12}N_{10}NaO_{20}$	1577.49	1578.74	0.08	1452	THAP

The PSD method, an extension of MALDI-MS, was introduced by Kaufmann in 1992.[14] Fragment ions from glycopeptides were first reported by Huberty et al.,[15] and from native sugars by Spengler et al.[16] Fragments, observed in the PSD spectra of free oligosaccharides, originate mainly from the cleavage of glycosidic bonds.[9,12] Permethylation resulted in reduced fragmentation with cleavage of glycosidic bonds,[17] peracetylated glycans fragmented better than permethylated ones, but other ions formed by loss of acetic acid, as well. There are no examples of PSD analysis of partially protected oligosaccharides bearing different protecting groups.

In this paper we report the use of the MALDI-TOF and MALDI-PSD techniques for the identification and the structural analysis of the synthetic oligosaccharides fully or partially protected with different blocking groups, e.g. methyl (Me), benzyl (Bzl), acetyl (Ac), phthalimido (NPhth), *tert*-butyldiphenylsilyl (TBDPS) and trichloroacetyl (TCA).

2. Results and discussion

Di-, tri-, and tetrasaccharides **1**–**4** (Fig. 2) and **5**–**8** having different sequences (Fig. 3) were prepared as described.[1–3] The MALDI-TOF spectra were recorded and the quasi-molecular ions corresponding to sodium and

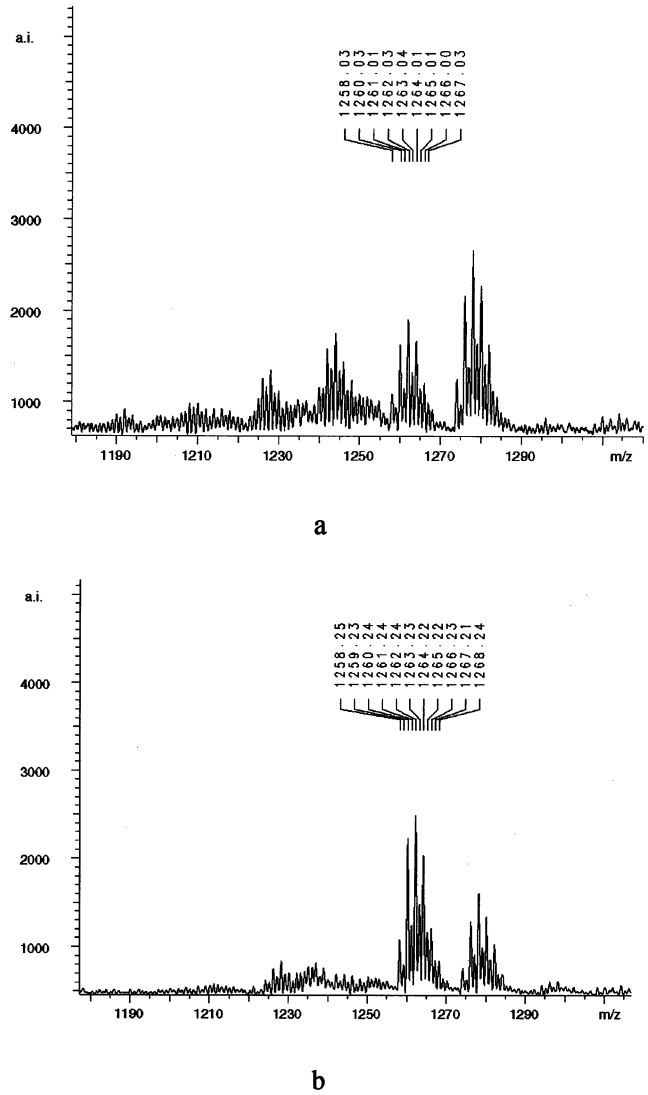
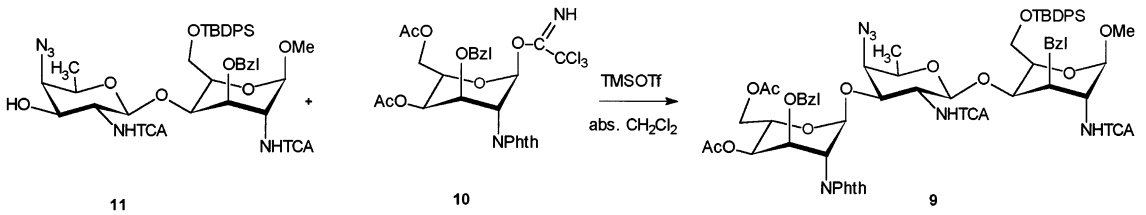


Fig. 4. MALDI-TOF MS analysis of protected trisaccharide **7** using (a) DHB matrix. (b) THAP matrix.



Scheme 1.

potassium cationised species were observed in monoisotopic resolution. The results of the sodium cationised species in 2,5-dihydroxybenzoic acid (DHB) as well as in 2,4,6-trihydroxyacetophenone (THAP) are summarised in Table 1.

Fragmentation was observed in the presence of TCA protecting group using DHB as the matrix. An example is shown in Fig. 4(a), the spectrum of trisaccharide **7** recorded in a DHB matrix. THAP does act as a matrix for neutral carbohydrates, it does not appear to offer any significant advantages over DHB according to Harvey.[13] In our experiments better resolution and no fragmentation were observed for chlorine containing compounds using a THAP matrix (Fig. 4(b)).

The XMASS software gives an opportunity to calculate the isotopic distribution of compounds with a known formula. This method is very useful for the identification of peaks, especially in the presence of chlorine containing protecting groups, which caused a characteristic isotopic pattern corresponding to the natural isotopic distribution of chlorine. During the preparation of trisaccharide **9** (Scheme 1) a very complex reaction mixture was obtained. The spectrum of the reaction mixture, shown in Fig. 5, contains a peak of **9** at m/z 1467.9 and other peaks at m/z 971.6, 834.4, 767.3, 649.3 and 614.3. The value m/z 649.3 is equal to the molecular mass of donor **10** ($C_{27}H_{25}O_9N_2Cl_3Na^+$). Since the TLC did not

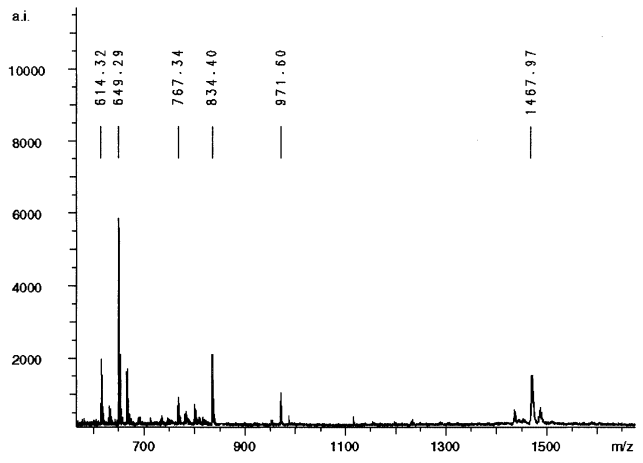


Fig. 5. MALDI-TOF MS analysis of the glycosylation reaction shown in Scheme 1.

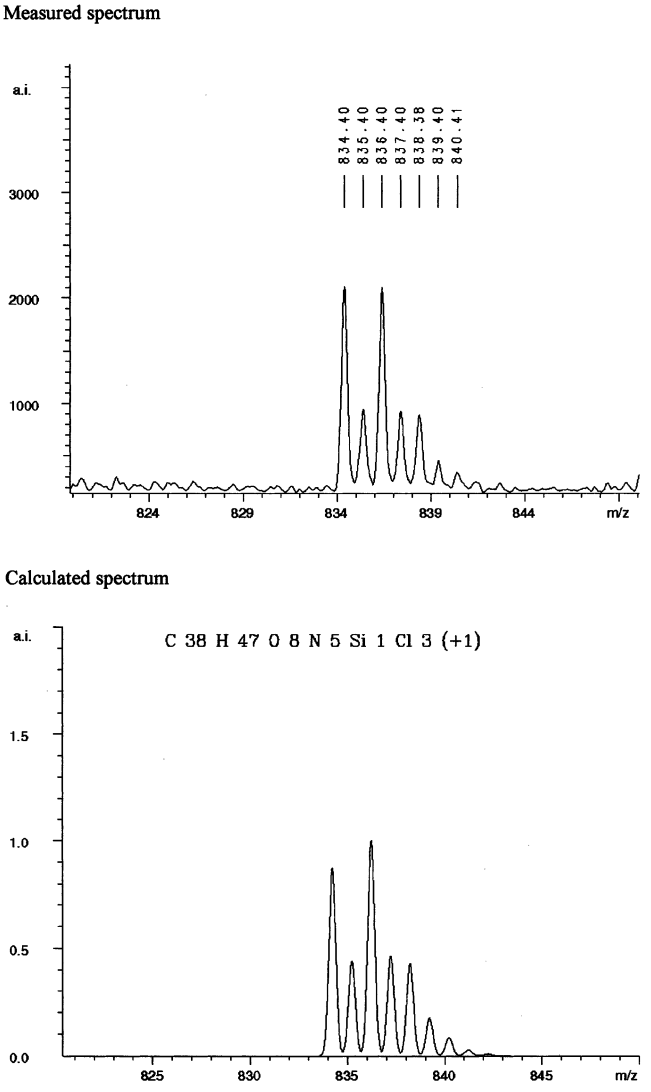


Fig. 6. Measured and calculated isotopic distribution of a by-product (MW 834.4) from the reaction shown in Scheme 1.

show the presence of donor, probably a rearrangement occurred[18] and *N*-trichloroacetyl-4,6-di-*O*-acetyl-3-*O*-benzyl-2-deoxy-2-phthalimido-α-L-altropyranosylamine of the same molecular weight formed. The peak at *m/z* 834.4 was identified as a hydrolysis product of the acceptor. The loss of one TCA group resulted in an $-NH_2$ group formation, and the ionisation occurred by the protonation of the amino group. The measured and calculated spectra of the side product ($C_{38}H_{47}Cl_3N_5O_8Si$) are shown in Fig. 6. The peak at *m/z* 767.4 was thought to be a compound formed in the reaction mixture from the **11** acceptor by the hydrolysis of benzyl and trichloroacetyl groups. The formula of this hypothetical compound is $C_{31}H_{44}Cl_3N_5NaO_8Si$, and the calculated molecular weight is 767.17. Fig. 7 shows the measured (a) and calculated (b) spectrum of the compound. Not only the measured molecular mass, but the isotopic distributions are in very good agreement. In the presence of two TCA groups (Fig. 7(c)), or in the lack of both (Fig. 7(d)) the isotopic distributions differ significantly. The peak at *m/z* 971.6 corresponds to a compound not containing Cl atoms, on the evidence of isotopic distribution (Fig. 8). The compound could form exclusively from the trisaccharide **9** by the loss of both trichloroacetyl groups and other unidentified protecting groups. The identification of by-products, formed under different conditions by the loss of protecting groups can be useful information for the optimisation of reactions.

The PSD-MALDI spectrum of tetrasaccharide **4** *m/z* 1093 is shown in Fig. 9. In

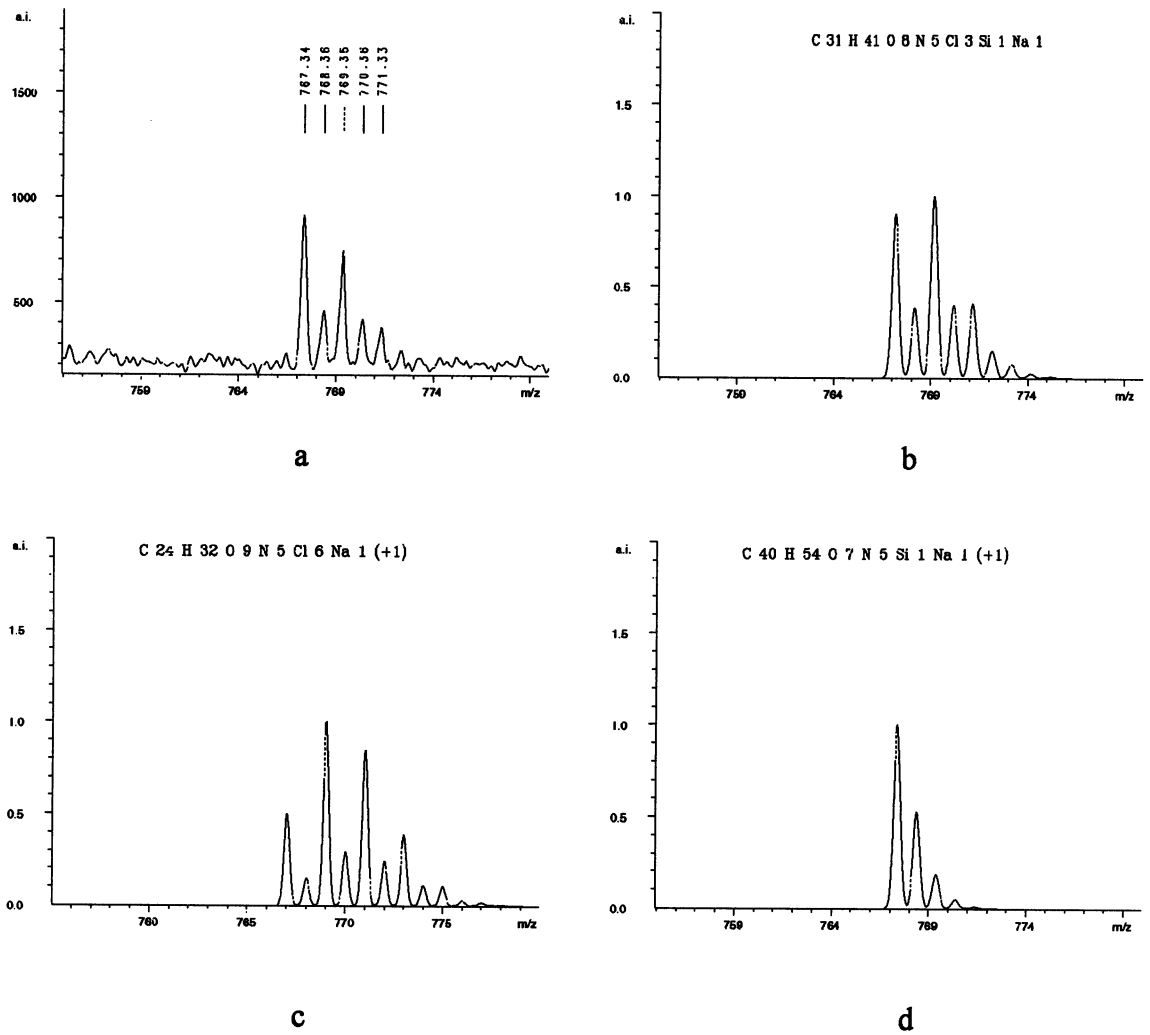


Fig. 7. Measured and calculated isotopic distributions of a by-product (MW 767.17): (a) Measured isotopic distribution. (b) Loss of one TCA. (c) In the presence of both TCA. (d) Loss of both TCA.

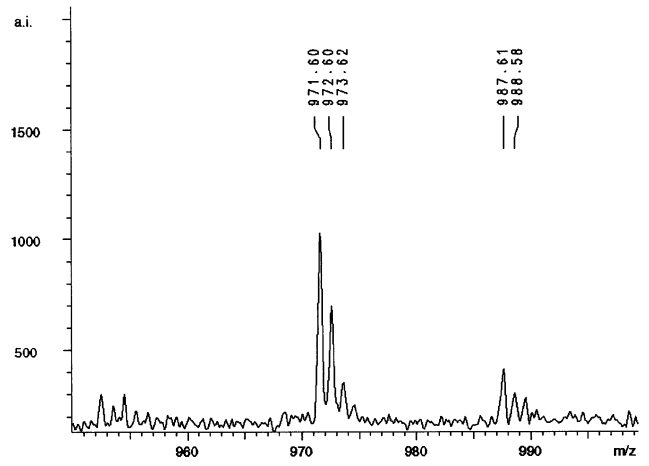


Fig. 8. Isotopic distribution of a by-product (MW 971.6).

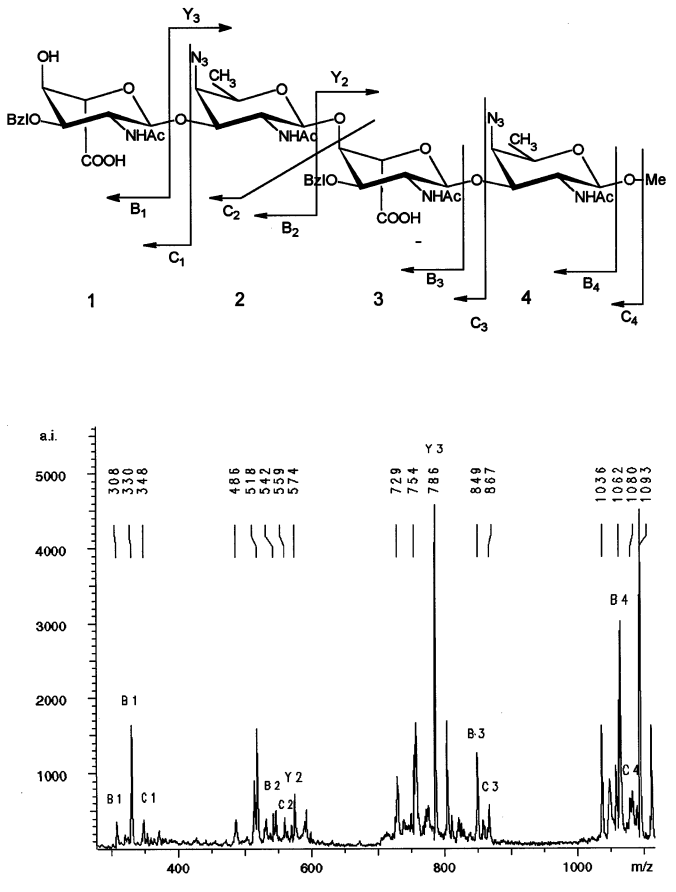


Fig. 9. Positive MALDI-TOF PSD spectrum of **4** tetrasaccharide and assignment of product ions. Parent ion $[M + Na]^+$ m/z 1093.3.

addition to the molecular ion, several fragment ions were present corresponding to successive losses of glycosyl residues through cleavage at glycosidic linkages. Cleavage of the glycosidic bond between 1 and 2 monosaccharide units is favoured and yields the base peak of the product (Y_3^+) ion at m/z 786, but the complementary (B_1^+) fragment is also observed. Fragmentation of underivatised glycans is facilitated by a H transfer that involves one of the labile OH groups.[19] Only one OH group being free in compound **4** could explain why Y_3^+ was the most intense fragment ion. The other ions observed in the PSD spectrum are mainly sodium cationised B and Y fragments. The signals of C ions appeared with lower intensities. The proton ionised species (B_1^+) and internal fragment ions ($M + Na^+ - OMe - 1$), ($M + H^+ - 1 - 4$) are also present at m/z 308, and 754 and 518, respectively. Cleavage of one –NAc group from the parent ion ($M + Na^+$), (Y_3^+) and (B_2^+) yields peaks at m/z 1036, 729 and 486, respectively. Fragment ions of peracetylated glycans usually contained ions produced by loss of acetic acid[13]

The observed peaks and the corresponding species for compound **4** are presented in Table 2. using the nomenclature of the fragmentation proposed by Donan and Costello.[20] If the glycosidic bond is cleaved and the charge is retained on the carbohydrate portion ions containing the aglycon or the reducing sugar unit, they are labelled Y_j, Z_j depending on whether the cleavage results in the retention or the loss of the linking oxygen atom respectively, where j is the number of the interglycosidic bonds counted from the aglycon numbered. Fragments, retaining charge of the non-reducing terminus are designated as B_i and C_i where i represents the number of the glycosidic bond cleaved, counted from the nonreducing end.

In summary, we have shown that MALDI-TOF MS is a very useful tool for the molecular weight measurement and structural analysis of oligosaccharides containing a variety of protecting groups. DHB and THAP were found to be good matrices, although THAP was superior for compounds with trichloroacetamido groups. Complete sequence analysis and structural verification of highly complex, protected oligosaccharides are possible using a single mass spectrum recorded in the MALDI PSD mode.

Table 2
Results of PSD fragmentation analysis of **4**, partially deprotected tetrasaccharide

m/z		Species		Type of fragment ion
Measured	Calculated			
1093	1093.4	$C_{47}H_{62}N_{10}NaO_{19}$	$M+Na^+$	parent ion
1062	1062.4	$C_{46}H_{59}N_{10}NaO_{18}$	$M+Na^+-OMe$	B_4
849	850.3	$C_{38}H_{47}N_6NaO_{15}$	$M+Na^+-4$	B_3
542	542.2	$C_{23}H_{30}N_5NaO_9$	$M+Na^+-(3—4)$	B_2
308	308.0	$C_{15}H_{18}NO_6$	$M+H^+-(2—3—4)$	B_1
330	330.1	$C_{15}H_{17}NNaO_6$	$M+Na^+-(2—3—4)$	B_1
1080	1080.4	B_4+H_2O	$M+Na^+-OMe$	C_4
867	868.3	B_3+H_2O	$M+Na^+-1$	C_3
559	561.2	B_2+H_2O	$M+Na^+-(1—2)$	C_2
348	348.1	B_1+H_2O	$M+Na^+-(1—2—3)$	C_1
786	785.3	$C_{32}H_{44}N_9NaO_{13}$	$M+Na^+-1$	Y_3
574	573.2	$C_{24}H_{32}N_5NaO_{10}$	$M+Na^+-(1—2)$	Y_2
518	520.2	$C_{23}H_{30}N_5O_9$	$M+H^+-1—4$	
754	754.	$C_{31}H_{41}N_9NaO_{12}$	$M+Na^+-OMe$-1	
486	485.2	B_2–NAc	$M+Na^+-(3—4)$-NAc	
729	728.3	Y_3–NAc	$M+Na^+-1$-NAc	
1036	1036.3	$C_{45}H_{59}N_9NaO_{18}$	$M+Na^+-NAc$	

3. Experimental

Materials.—Matrix compounds 2,5-dihydroxybenzoic acid (DHB) and 2,4,6-trihydroxyacetophenone (THAP) were purchased from Sigma–Aldrich (Germany). Solvents (MeCN, EtOH, MeOH, EtOAc) were HPLC grade from Scharlau (Germany). Aqueous solutions were prepared using deionized (MilliQ) water. The standard maltooligosaccharides were synthesized from β-cyclodextrin according to the procedure previously reported.[21]

Mass spectrometric analysis.—MALDI-TOF MS analyses of the compounds were carried out in the positive reflectron mode using a BIFLEX III mass spectrometer (Bruker, Germany) equipped with delayed-ion extraction. Spectra from multiple (at least 100) laser shots (N_2 laser, 337 nm) were summarised using 19 kV accelerating and 20 kV reflectron voltage. External calibration was applied using the $[M+Na]^+$ peaks of maltooligosaccharides DP 3–7 (m/z: 527.158, 689.211, 851.263, 1013.316 and 1175.369, respectively) as calibrants. DHB matrix solution was prepared by dissolving DHB (10 mg) in a mixture (0.5 mL) of 1:1 EtOH–water. The THAP matrix solution was satd THAP solution in MeCN. The protected oligosaccharides (1 mg) were dissolved in EtOAc or CH_2Cl_2 (1 mL). A 10 μL aliquot of this solution was diluted to 0.5 mL with EtOH. 10 μL sample and 10 μL matrix solution were mixed, then 0.5 μL was applied to the sample target and allowed to dry at rt. The fully and partially deprotected samples **6**, **4** were dissolved in MeOH.

The quasi-molecular ions corresponding to sodium and potassium cationised species were observed in monoisotopic resolution. The calculated values used for comparison were derived from IUPAC exact isotopic weights using XMASS 5.0 software from Bruker.[22] The resolution was lower for two protected tetrasaccharides **3** and **8**, and therefore the average molecular mass was used for the comparison. The accuracy and resolution value (R) were also calculated: $R=(m/z)/\mathrm{FWHM}$ when m/z: mass/charge of the ion, FWHM: full width at half maximum.

Acknowledgements

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