ELSEVIER

International Journal of Mass Spectrometry 226 (2003) 1–35

International Journal of Mass Spectrometry

www.elsevier.com/locate/ijms

Review

Matrix-assisted laser desorption/ionization mass spectrometry of carbohydrates and glycoconjugates

David J. Harvey

Department of Biochemistry, Oxford Glycobiology Institute, South Parks Road, Oxford OX1 3QU, UK

Received 7 August 2001; accepted 8 July 2002

Abstract

The application of matrix-assisted laser desorption/ionization (MALDI) mass spectrometry to the analysis of carbohydrates and their conjugates with proteins and lipids is reviewed. Among the topics discussed are instrumentation, MALDI matrices, derivatization, fragmentation and application of MALDI to various structural types.

Keywords: Carbohydrates; Matrix-assisted laser desorption/ionization (MALDI); Matrix; Fragmentation; Glycoproteins

1. Introduction

Unlike proteins and nucleic acids that are linear polymers of amino acids and nucleotides respectively, with linkages at only one position, carbohydrates can adopt complex branched structures with individual monomeric units linked at one of several sites. Furthermore, each monomer can adopt different ring sizes and conformation or can sometimes appear in a linear

Abbreviations: 2-AA, 2-aminobenzoic acid; AA-Ac, 3-(acetylamino)-6-aminoacridine; 2-AB, 2-aminobenzamide; AB-DEAE, 4-aminobenzoic acid 2-(diethylamino)ethyl ester; ABEE, 4-aminobenzoic acid ethyl ester; AEC, anion-exchange chromatography; AMAC, 2-aminoacridone; 2-AP, 2-aminopyridine; APCI, atmospheric pressure chemical ionization; APTS, 1-aminopyrene-3,6,8-trisulfonate; 3-AQ, 3-aminoquinoline; Arg, arginine; BOC, butoxycarbonyl; CAM, cell adhesion molecule; CHO, Chinese hamster ovary; CID, collision-induced decomposition; CMBT, 5-chloro-2-mercaptobenzithiazole; Da, Dalton; DHA, dihydroxyacetophenone; DHB, dihydroxybenzoic acid; EI, electron impact; Endo-H, endoglycosidase-H; EPS, extracellular polysaccharide; ESI, electrospray ionization; FAB, fast atom bombardment; Frc, fructose; FT, Fourier transform; Fuc, fucose; FWHM, full-width half-maximum; Gal, galactose; GalNAc, *N*-acetylgalactosamine; GC/MS, gas chromatography/mass spectrometry; Glc, glucose; GlcNAc, *N*-acetylglucosamine; Gly, glycine; HABA, 2-(*p*-hydroxyphenylazo)benzoic acid; 4-HCCA, α-cyano-4-hydroxycinnamic acid; Hex, hexose; HexNAc, *N*-acetylaminohexose; HIQ, 1-hydroxyisoquinoline; HPAE, high pH anion exchange; HPLC, high performance liquid chromatography; ICR, ion cyclotron resonance; IgG, immunoglobulin G; ISD, in-source decay; LC/MS, liquid chromatography/mass spectrometry; LPS, lipopolysaccharide; MALDI, matrix-assisted laser desorption/ionization; Man, mannose; PAGE, polyacrylamide gel electrophoresis; PD, plasma desorption; PGC, porous graphitised carbon; PMP, 1-phenyl-3-methyl-5-pyrazolone; PNGase, protein-*N*-glycosidase; PSD, post-source decay; PVDF, polyvinylidine difluoride; Q, quadrupole; SDS, sodium dodecylsulphate; TFA, trifluoroacetic acid; THAP, 2,4,6-trihydroxyacetophenone; TIMP, tissue inhibitor of metalloproteinases; TLC, thin-layer chromatography; TMAPA, trimethyl-(*p*-aminophenyl)amino; TMR, tetramethylrhodamine; TOF, time-of-flight; TPA, tissue plasminogen activator.

E-mail address: dh@glycob.ox.ac.uk (D.J. Harvey).

1387-3806/02/$ – see front matter
PII S1387-3806(02)00968-5

form. Thus, for a carbohydrate of a given mass, it is possible to produce very large numbers of isomeric structures. For example, it has been calculated that, for a simple hexasaccharide, there are approximately 1.05×10^{12} possible isomers [1]. Such structural complexities present major problems for the analyst, particularly as many compounds are available in only trace quantities. Fortunately, however, only a few of the possible structures are found in nature because of the limit in the number of glycosyltransferases involved in their biosynthesis. Knowledge of the activity of such enzymes often plays an important role in structural determination. Mass spectrometry is used extensively in this work particularly as the techniques

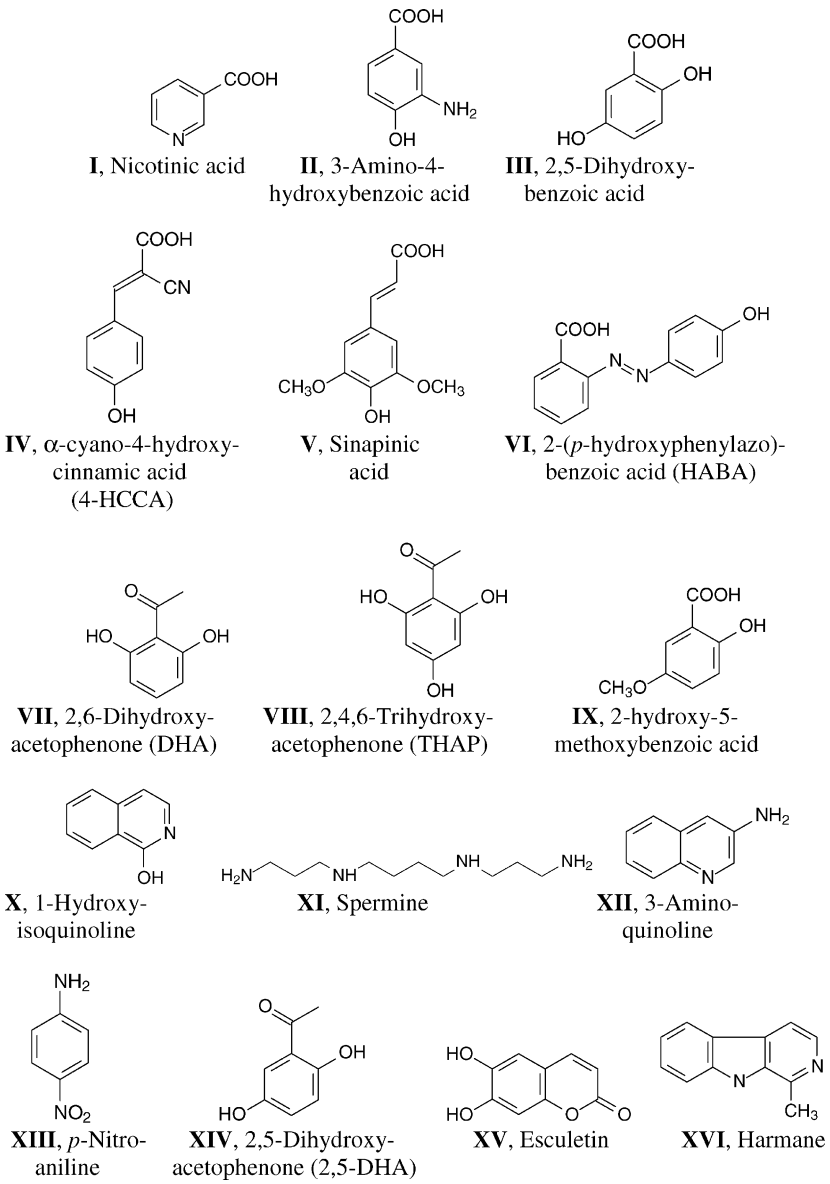


Fig. 1. Structures of common matrices used to record MALDI mass spectra from carbohydrates and their conjugates.

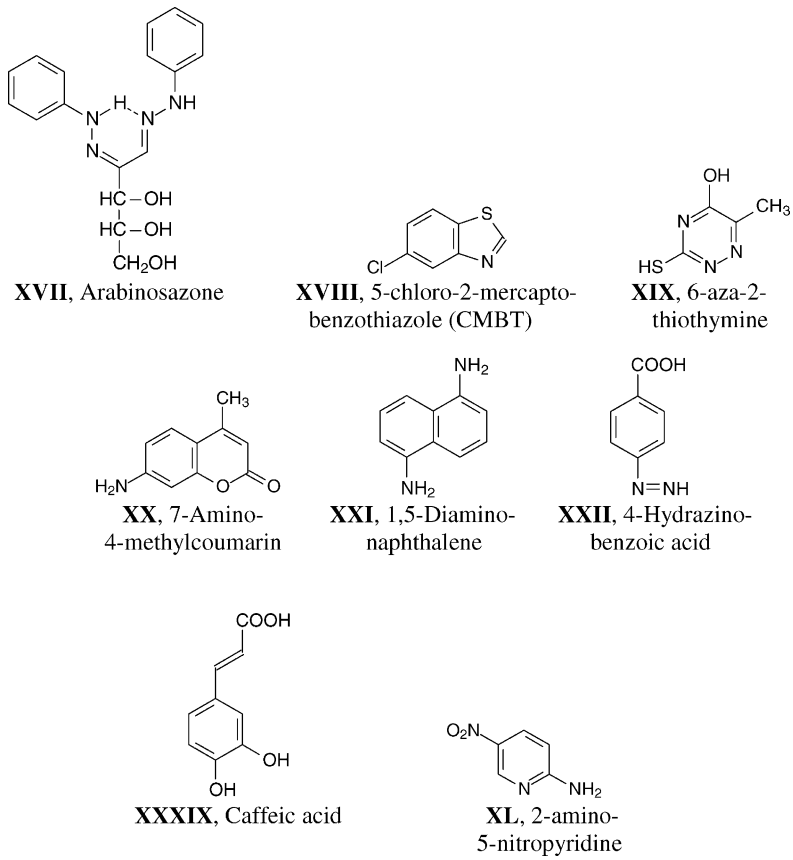


Fig. 1. (*Continued*).

of fast atom bombardment (FAB) [2] and, more recently, matrix-assisted laser desorption/ionization (MALDI) [3–5] and electrospray ionization (ESI) have enabled spectra of the larger and more polar molecules to be examined directly. This paper will describe the role of MALDI to this work and, in particular, to the structural determination of *N*- and *O*-linked glycans found attached to proteins, and to the glycan portions of glycolipids. Earlier reviews include those of Bahr et al. [6], Harvey and co-workers [7–9], and Garozzo [10].

MALDI has been applied to the analysis of carbohydrates since the earliest reports of the technique when stachyose, a phytochemical tetrasaccharide, was shown to give an enhanced signal to that of the isolated compound when nicotinic acid (**I**, Fig. 1) was used as the matrix [3]. Ionization was achieved with a frequency-quadrupled Nd-YAG laser (266 nm). The major ion was $[M + Na]^+$, in contrast to the more usual $[M + H]^+$ ion produced by proteins. Mock et al. [11] were the first to apply the technique to *N*-linked carbohydrates and, in contrast to FAB mass spectrometry, showed that strong signals could be obtained from underivatised compounds using 3-amino-4-hydroxybenzoic acid (**II**) as the matrix. MALDI has since been found to be from 10 to 100 times more sensitive than FAB [12–14] or ^{252}Cf plasma desorption (PD) [15] mass spectrometry for carbohydrate and glycoprotein analysis and to be capable of ionization of carbohydrates of higher mass than was possible by FAB [10].

2. Instrumentation

The mass spectrometer used by Mock et al. was the first Finnigan LaserMat [16], a linear MALDI mass spectrometer that we later acquired as a β-test instrument and which is still in use today. These early linear MALDI-time-of-flight (TOF) instruments suffered from low resolution but, by using a magnetic sector instrument fitted with an array detector we were able to record MALDI spectra of carbohydrates with resolutions of up to 2000 (FWHM) [17,18]. An alternative method of using a sector instrument with a MALDI ion source for carbohydrate analysis was later investigated by Orlando and co-worker [19,20]. This method involved slow scanning with a liquid matrix (see below) to produce a long-lasting ion beam. Multiple scans were acquired so that all of the spectrum could eventually be captured and resolutions of over 8000 were achieved. A resolution of about 20,000 was later attained by our group with a magnetic sector instrument by using slow scanning and using separate laser pulses to trace the ion peaks in a similar way to the method used for peak digitisation [21,22]. For ultimate resolution, Fourier transform (FT) ion-cyclotron resonance (ICR) spectrometers are necessary and using such an instrument, combined with a MALDI ion source, Carroll et al. [23] have recorded spectra of methylated β-cyclodextrins at a resolution of 210,000. Most carbohydrate studies are now performed with reflectron-TOF instruments fitted with time-lag focusing (delayed extraction) [24], a combination that produces resolutions of up to 10,000. MALDI ion sources have also been interfaced to ion-trap mass spectrometers [25–34, Qin, 1996 #274,35] although only recently has this combination been used for carbohydrate analysis [35,36]. MALDI has also been interfaced with hybrid quadrupole-TOF (Q-TOF) mass spectrometers [37–41] and used for CID fragmentation studies of carbohydrates [42] and glycolipids [43]. Atmospheric pressure MALDI has also been achieved with the ions being examined with either an orthogonal-TOF instrument originally designed for electrospray [44] or with an ion trap [45]. This ionization technique again produces $[M + Na]^+$ ions but these are often accompanied cleavage fragments not seen with a vacuum source.

3. Matrices

3.1. Glycopeptides and glycoproteins

Glycopeptides and glycoproteins contain free amino groups that can be protonated efficiently and these compounds frequently give stronger spectra than sugars that are ionized by sodium addition. For glycopeptides, the ratio of the $[M + H]^+$ ion to that of $[M + Na]^+$ tends to rise with the percent of peptide in the molecule. 2,5-Dihydroxybenzoic acid (DHB, **III**) will ionize both glycans and glycopeptides with masses below about 5000 but not as effectively as α-cyano-4-hydroxycinnamic acid (4-HCCA, **IV**) [46]. Above about 5000 Da, signals from these matrices tend to fall and it is usually more appropriate to use sinapinic acid (**V**) [47] or 2-(*p*-hydroxyphenylazo)benzoic acid (HABA, **VI**) [48]. Pitt and Gormon [49] have found that 2,6-dihydroxyacetophenone (DHA, **VII**) is a useful matrix for glycoproteins, particularly when mixed with diammonium hydrogen citrate (10%, 1 M), as it tends to give less metastable fragmentation from sialylated glycoproteins than the more commonly used matrices.

For larger glycoproteins, resolution of salts, adducts, fragment ions, and other components such as glycoforms becomes increasingly difficult with increasing mass and can result in problems with accurate mass measurements. Fragmentation will give peak broadening on the low mass side of the peak, and adduct formation (from matrix or alkali metals) will cause the peak to broaden towards high mass. Thus, matrices should be chosen to reduce the production of these unresolved components. For example, glycoforms of ribonuclease B, a glycoprotein with a mass of about 15 kDa with five neutral glycans, can be resolved easily on a linear TOF instrument from 2,4,6-trihydroxyacetophenone (THAP, **VIII**) and 4-HCCA (Fig. 2) but only with more difficulty from sinapinic acid [50].

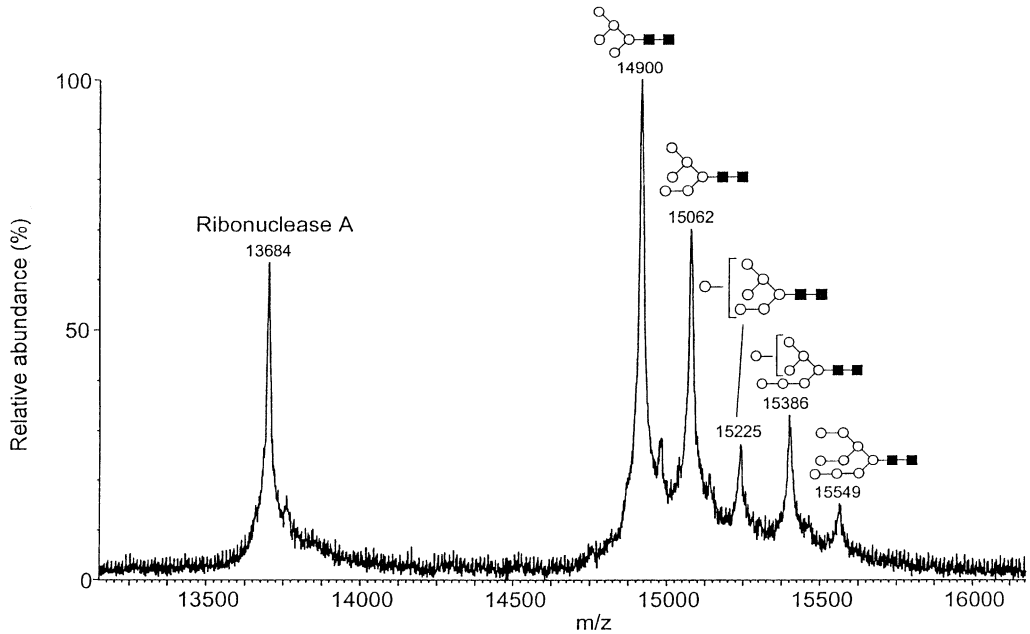


Fig. 2. Positive ion linear MALDI mass spectrum of a mixture of ribonuclease A (unglycosylated) and B (glycosylated) recorded from 4-HCCA with a Micromass TofSpec 2E mass spectrometer. The structures of the five high-mannose glycans attached to the protein to form ribonuclease B are shown. Key to symbols: (■) GlcNAc, (○) mannose.

3.2. *Free sugars*

3-Amino-4-hydroxybenzoic acid (**II**), as mentioned above, was the first matrix to be used specifically for carbohydrates [11]. It produced $[M + Na]^+$ ions from as little as 1 pmol of dextran hydrolysate and *N*-linked glycans released from glycoproteins. An analogue, 2,5-DHB, **III**), a matrix originally introduced for proteins by Strupat et al. [51] was, however, found by Hillenkamp's group to be superior [5] and is still in use today as the most popular matrix for carbohydrates. Again, $[M + Na]^+$ ions are the dominant products (Fig. 3) although $[M + DHB - H]^-$ ions have been detected from some sugars in their negative ion spectra [52]. When this matrix:sample solution evaporates, DHB tends to crystallise from the periphery of the target spot in the form of long needles that point towards the centre of the target. In many cases the centre is devoid of crystals. Several methods have been devised to overcome this problem. Karas et al. [53] have added a small (10%) amount of 2-hydroxy-5-methoxybenzoic acid (**IX**) to the DHB to produce a mixture known as "super-DHB". This matrix crystallises more evenly than DHB and has been reported to enhance signal strength and to improve resolution of carbohydrates and glycoproteins [15]. Addition of 1% 1-hydroxyisoquinoline (HIQ, **X**) also improves signal quality and produces a matrix that is remarkably tolerant to the presence of buffers and other contaminants [54]. Tolerance was such that a strong signal from maltose oligomers up to the 35-mer was obtained from the confectionery "Gummy Bears" by simply dissolving it in water, filtering through a 22 μm Millipore filter, and mixing with the matrix. Mechref and Novotny [55] have used the base spermine (**XI**) as a co-matrix with 2,5-DHB for the examination of sialylated glycans in the negative ion mode and report much reduced sodium salt formation. Detection limits of about 50 fmol were attained. Gusev et al. [56] have obtained improved reproducibility and resolution with the addition of α-L(−)-fucose to 2,5-DHB, and to super-DHB. The fucose was thought

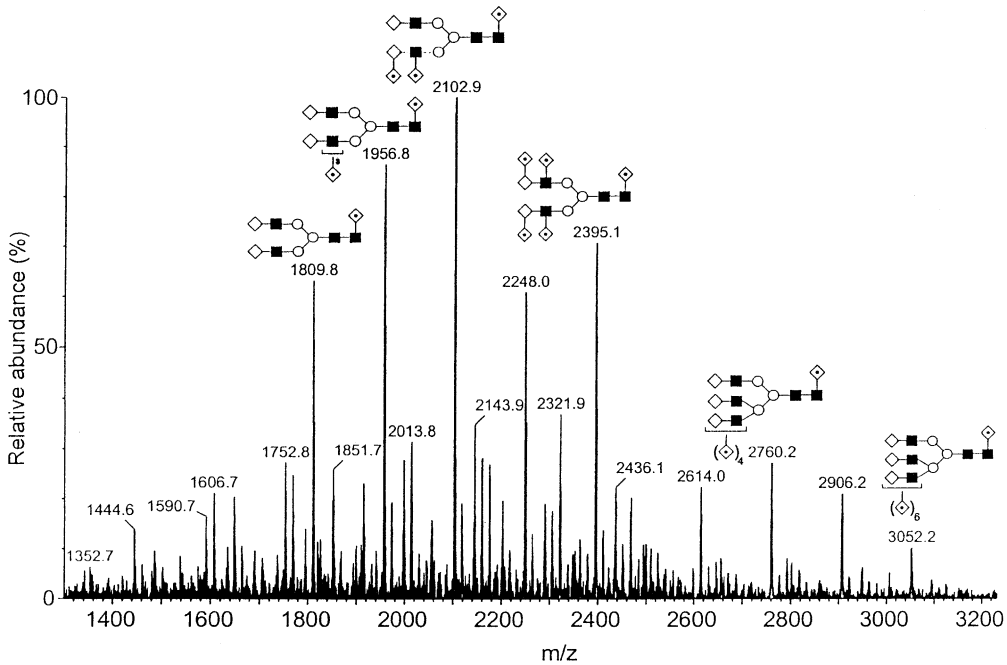


Fig. 3. Positive ion reflectron MALDI mass spectrum of *N*-linked glycans from human parotid gland glycoproteins recorded from DHB with a Micromass TofSpec 2E mass spectrometer. Structures of some of the major glycans are shown, others can be found in the paper by Guile et al. [370]. Key to symbols: as in the footnote to Fig. 2 and (◇) galactose, (◈) fucose.

to decompose to yield gaseous products such as carbon dioxide and water during the desorption process, creating a dense environment that could cool the analyte molecules [57]. We have improved signal quality from 2,5-DHB by recrystallising the dried sample spot from a small amount of ethanol [58].

Other isomers of DHB produce relatively poor signals from carbohydrates unless they contain an *ortho*-hydroxy group that is able to undergo an intramolecular hydrogen transfer between their phenolic and carboxyl groups [59]. Of these compounds, the 2,5-isomer gives the strongest signals, possibly as the result of photochemical decarboxylation and oxidation to the relatively stable *p*-benzoquinone [58]. However, the strong signals may simply reflect the extent to which the analyte is taken up by the crystals. In a recent study with five DHB isomers, cytochrome C was only incorporated extensively into the 2,5-isomer [60]. HABA produces very small crystals but weaker signals from carbohydrates than those from 2,5-DHB. It tends to be a hotter matrix, catalysing extensive post-source fragmentation. Unfortunately, it produces relatively abundant matrix ions up to about *m*/*z* 1000 [54] that can have an adverse effect on the analysis of smaller glycans in this mass range.

3-Aminoquinoline (3-AQ, **XII**) has been used for ionization of plant inulins with masses of up to 6 kDa [61]. Although the matrix gives a comparatively low background and appears better than DHB for sialylated glycans [62], it sublimes too rapidly to be of general use [63] and appears to be more sensitive to the presence of contaminants than DHB [64]. When mixed with 4-HCCA it produces a viscous liquid that is capable of giving long-lasting signals from a single laser spot [19]. A mixture of potassium hexacyanoferrate and glycerol also produces a liquid matrix with similar properties, and gives strong signals from hydrophobic compounds such as glycolipids [65,66]. *p*-Nitroaniline (**XIII**):glycerol mixtures also act as matrices and have been found to produce mainly $[M + H]^+$ rather than

$[M + Na]^+$ ions from carbohydrates [67]. However, none of these liquid matrices have found general acceptance for carbohydrate analysis.

Several substituted acetophenones bearing *ortho*-hydroxy groups act as matrices for carbohydrates, particularly in the negative ion mode. 2,4,6-THAP (**VIII**) effectively ionizes sialylated glycans, particularly when mixed with ammonium citrate [63], a compound that was thought to prevent salt formation from carbohydrates [68]. Less loss of sialic acid is produced by fragmentation than when DHB is used as the matrix. In positive ion mode 2,5-dihydroxyacetophenone (2,5-DHA, **XIV**) produces relatively strong signals from neutral carbohydrates but does not appear to offer any significant advantages over 2,5-DHB. Another neutral matrix is esculetin (6,7-dihydroxycoumarin, **XV**). This compound efficiently ionizes neutral glycans and gives better resolution than DHB (unpublished observations). It also appears to perform better than DHB with atmospheric pressure MALDI, but not as efficiently as HABA (unpublished observations). A number of β-carbolines, such as harmane (**XVI**) have, unusually, been reported to yield $[M + H]^+$ rather than $[M + Na]^+$ ions in the positive ion mode from cyclodextrins [69]. However, they give the usual $[M + Na]^+$ ions from maltooligosaccharides and *N*-linked glycans, accompanied by a prominent fragment ion due to loss of water. In the negative ion mode, these matrices have been reported to produce $[M - H]^-$ ions from small, neutral glycans, again an unusual observation although Hao et al. [70] have reported that DHB was able to produce negative ions from dextrans with masses of around 7500 Da. The failure of this matrix to produce ions from smaller carbohydrates was thought to indicate a occurrence of a different ionization mechanism at these masses.

Arabinosazone (**XVII**), a highly crystalline compound, has been found to be particularly effective as a matrix [71]. It has been reported to give better resolution and sensitivity than DHB for neutral sugars and we have found it to be particularly valuable for sialylated [72] or sulphated [73] glycans in negative ion mode. This matrix would also appear to be valuable for ionization of larger glycans as illustrated by laminarin, a linear D-glucan from brown algae that contains 1,3- and 1,6-linkages. Its MALDI spectrum showed larger peaks from the higher mass constituents when recorded from arabinosazone than from 2,5-DHB, an effect that was attributed to less fragmentation as the result of the lower laser energies needed to produce signals.

Other matrices that have proved useful for carbohydrate analysis include 5-chloro-2-mercaptobenzithiazole (CMBT, **XVIII**) for high-mannose *N*-linked glycans [74], 6-aza-2-thiothymine (**XIX**) for gangliosides [75] and sialylated biantennary *N*-linked glycans [63], and 7-amino-4-methylcoumarin (**XX**) for monosulphated disaccharides [76].

Although $[M + Na]^+$ ions are the normal products of MALDI analysis of carbohydrates, other adducts may be formed by addition of the appropriate inorganic salts [5], preferably to sodium-depleted samples. A Nafion-117 membrane can be used to exchange sodium for another metal if it is saturated with the metal salt and used to separate the sample from a 100 mM solution of the salt [77]. Mohr et al. [54], have found that the affinity of different alkali metal ions for carbohydrates is Cs > K > Na > Li > H. Caesium, however, although the most efficient at producing ions, is unable to ionize small carbohydrates [78]. Di- and trivalent metals are also capable of ionizing carbohydrates, but only singly charged molecular ions are formed [79]. Sulphate addition has also been observed to yield negative ions of the type $[M + HSO_4]^-$ from neutral carbohydrates [80].

3.3. Glycolipids

The first report of the analysis of gangliosides by MALDI mass spectrometry found that 2,5-DHB, 1,5-diaminonaphthalene (**XXI**), 4-hydrazinobenzoic acid (**XXII**) and 6-aza-2-thiothymine could be used as matrices [75]. Best results were obtained in the negative ion mode but considerable metastable loss of sialic acid was seen with a linear TOF instrument. Better resolution was reported by our group, using a magnetic sector instrument [81]; neutral glycosphingolipids gave the strongest signals from 2,5-DHB,

4-HCCA, and esculetin, whereas acidic compounds were best examined from 2,5-DHB to reduce fragmentation [81–83]. Both HABA [48] and CMBT [74] have also proved to be useful for ionization of gangliosides.

4. Quantitative aspects of MALDI mass spectrometry

The signal strength given by *N*-linked glycans, ionized by MALDI appears to reflect accurately the amount of material on the target, providing that the correct matrix is chosen [58]. Thus, DHB produces a linear response from samples over several decades of concentration while 3-amino-4-hydroxybenzoic acid does not. Using DHB, we have been able to quantify a biantennary glycan with its fucosylated analogue as the internal standard. A linear relationship has also been found for *N*-linked glycans derivatized with 1-phenyl-3-methyl-5-pyrazolone (PMP, **XXII**) when measured against PMP-$(Glc)_6$ with 2,6-DHA as the matrix [84] and similar results have been obtained with cyclodextrins, using maltohexaose as the internal standard and 4-HCCA as the matrix [85]. With all of these studies, it has been found that shot to shot variation in signal strength varies widely. However, by averaging spectra from a sufficiently large number of shots taken from different areas of the target, reasonably accurate results can be obtained albeit with precisions of around 10%.

Although, for polymer mixtures with high molecular weights, there appears to be a small drop in sensitivity with increasing molecular weight [54,86], Stahl et al. [5,64] have found no significant effect below about 10 kDa. Profiles for *N*-linked glycans (mass range about 1000–3000 Da) closely match those obtained by high-performance liquid chromatographic (HPLC) analysis of fluorescently-tagged compounds [87] and there appears to be no significant effect of structure on the signal produced by these compounds [5,88], providing that their mass is above about 1 kDa. Below that mass, there appears to be a fall in sensitivity [88,89], particularly with linear TOF instruments without low mass ion suppression, an effect that is probably attributable to transient saturation of the detector by the high matrix signal or to loss of the smaller, more volatile carbohydrates as the laser-generated plume is pumped away. The drop in sensitivity at higher mass observed with the higher homologues of large polymers has been attributed to differences in solubility rather than to ionization effects [90].

5. Sample preparation

When analysing mixtures, it is important to ensure that isolation and purification techniques do not cause any fractionation of the sample with loss of quantitative information. Sialic acids, for example, are often lost from glycoproteins if the pH becomes too low or the sample temperature is too high. Care should also be taken to ensure that there are no glycosidases present in the sample as exemplified by Field et al. [91] who have reported the co-purification of a β-galactosidase with a sample of *N*-linked glycans with the result that the glycans underwent extensive degalactosylation over a period of 65 days.

5.1. Contaminant removal

Salts and buffers, generally have an adverse effect on crystal formation and ion yield and should be removed in order to obtain high quality MALDI spectra. Fortunately, however, MALDI analysis of proteins, glycoproteins and carbohydrates appears to be less affected by the presence of these contaminants than do most other forms of ionization such as electrospray. However, metal salts have recently been found to cause clustering between the matrix and sample, with adverse effects on resolution and the formation of matrix multimers, frequently seen with DHB in the region of *m*/*z* 200–1000 [92].

Many methods for removing salts and buffers have been reported. Whereas glycoproteins may be cleaned on the MALDI target by washing with water [93,94] or dilute trifluoroacetic acid (TFA) solution [95], native

carbohydrates are generally too soluble for the technique to succeed although derivatised carbohydrates have been treated successfully [96]. Loss of sample by washing in this manner may reflect the extent to which the sample is entrained within the crystal or simply deposited on the surface. The general unsuccessful outcome of this washing approach, together with the observation that, in many cases, the strength of the MALDI signal from DHB rapidly fades with successive laser shots suggests that sample deposition on the crystal surface may be more common than generally realised.

A better procedure for sample clean-up is drop dialysis on a membrane with a reasonably low, e.g.

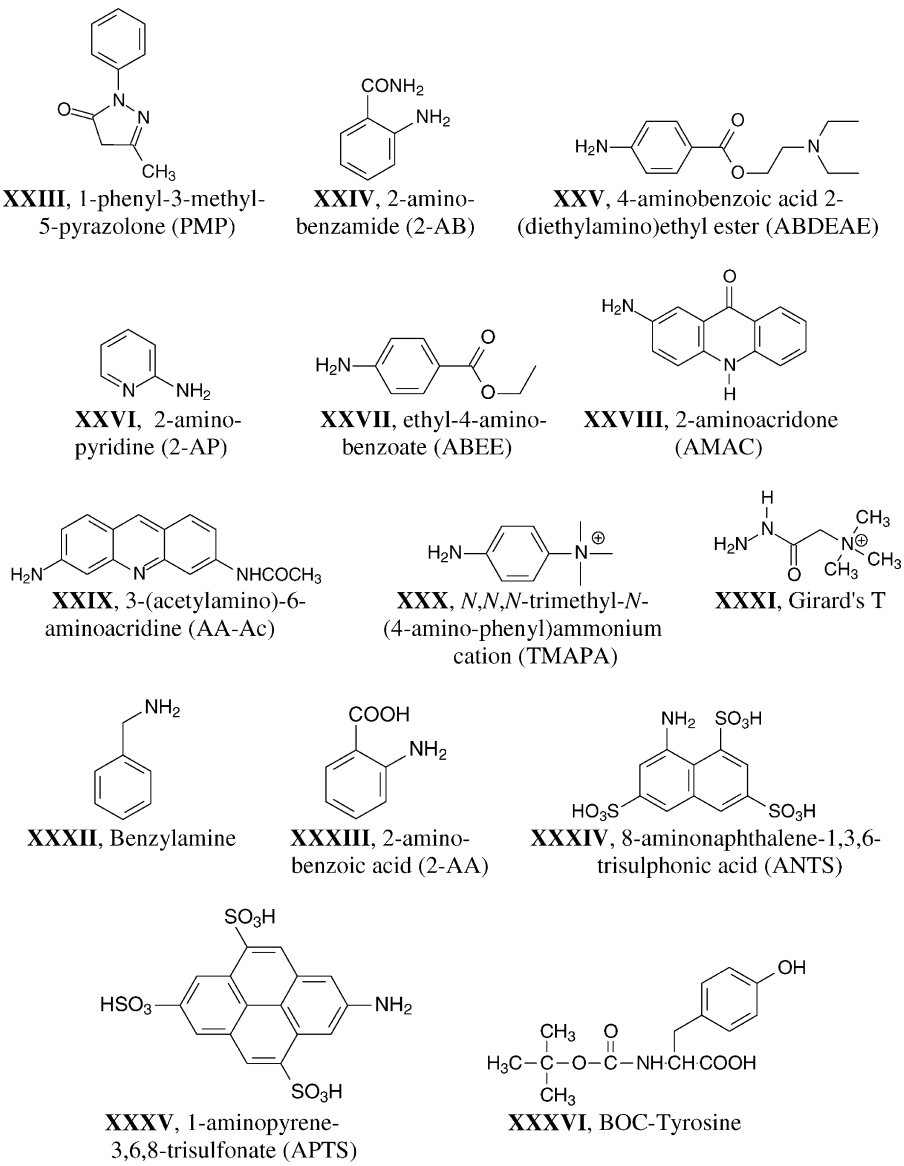
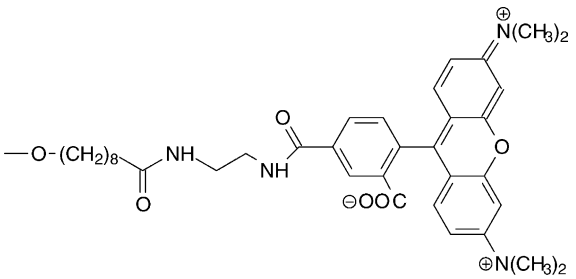
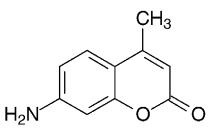


Fig. 4. Structures of the amines and other compounds used to prepare reducing-terminal derivatives.



XXXVII, tetramethylrhodamine ion with amide spacer



XXXVIII, 7-Amino-4-methylcoumarin

Fig. 4. (*Continued*).

500 Da, molecular weight cut-off [97,98] prior to deposition of the sample onto the target. Börnsen et al. [77], have used a Nafion-117 membrane in a similar fashion and we find this method very satisfactory for a range of *N*-linked glycans and their 2-aminobenzamide (2-AB, **XXIV**, Fig. 4) derivatives both after [99] and, more recently, without pre-treatment by drop dialysis. Ion-exchange or hydrophobic resins packed into microcolumns or disposable pipette tips are also popular. For example, Kussmann et al. [46] used micro-columns of Poros 50 R1 (about 1 μL) packed into Eppendorf GELoader tips and we [100] have used a three-resin column of AG50, AG3 and C-18 for removing cations, anions and organic material, respectively. Charlwood et al. [101] prefer GlycoClean-H from Oxford GlycoSciences packed into 1 mL polythene filtration tubes. Porous graphitised carbon (PGC) also appears to be very effective at removing contaminants [73,102] and for fractionation of carbohydrates into neutral and acidic fractions.

Several investigators have removed contaminants directly from the MALDI probe. Thus, for example, Rouse and Vath [103] have added resin beads to the sample/matrix mixture deposited onto the MALDI probe tip and later removed them mechanically prior to analysis. Huang et al. [104] prefer to add resin (C-18 or SP20SS) to the solution immediately following enzymatic release of glycans from proteins. Bioaffinity clean-up was employed by Wang et al. [105] in a technique that involved derivatization of the glycans with EZ-Link biotin hydrazide from Pierce using the reductive amination procedure. These derivatised compounds were then extracted by binding to avidin that had been immobilised on a small piece of Cannon NP transparency film type E attached directly onto the MALDI probe. Bundy and Fenselau [106] have immobilised the lectin, concanavalin A to gold foil attached to the MALDI target and used this to extract high-mannose sugars from solution. Contaminants could then be washed from the surface with water. Lipid A samples from *Escherichia coli* have been purified in a similar manner except that, in this case, the molecules were adsorbed onto polyethylene or polypropylene films and washed with 70% methanol:water after addition of 4-HCCA [107]. Nafion films have also been used for the desalting of acidic carbohydrates on the probe [108] without the need for additional washing.

5.2. Derivatization

Derivatization, although not essential for MALDI analysis of carbohydrates can, nevertheless, be advantageous under certain circumstances, particularly for improving sensitivity and simplifying fragmentation. Permethylation with methyl iodide, catalysed either by the methylsulphenyl carbanion [109] or by sodium hydroxide [110], can improve the sensitivity by about an order of magnitude, but at the expense of a considerable increase in the molecular weight and problems with sample clean-up, particularly with trace amounts of sample. Peracetylation is also effective and can be conveniently accomplished cleanly in the gas phase [111]. It has been used to determine the linkage, α2 → 3 or α2 → 6, of sialic acids by making use of the observation that sialic acids with α2 → 3 linkage form lactones and thus, incorporate fewer acetyl groups than the α2 → 6 isomers that do not; the resulting mass difference allows ready identification of the linkage

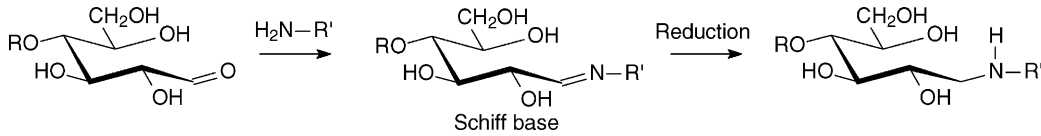


Fig. 5. Preparation of derivatives of carbohydrates by reductive amination. R = remainder of sugar. Amines, incorporating the R' group are listed in Fig. 4.

[112]. Similar results have been obtained following perbenzoylation [113]. Although this latter procedure leads to an increase in sensitivity, the benzoyl groups produce an undesirable increase in mass.

Reducing sugars are conveniently derivatised at their reducing terminus, normally by reductive amination with aromatic amines (Fig. 5). The procedure is commonly used for introducing chromophores and fluorophores [114] to aid detection and many samples presented to analytical laboratories for MALDI analysis will have been derivatised in this manner. A number of investigators have used reductive amination to add a site with a constitutive charge or high proton affinity in order to aid detection. Derivatives imparting increased proton affinity include those prepared from 4-aminobenzoic acid 2-(diethylamino)ethyl ester (ABDEAE, **XXV**) [115], a derivative reported to improve sensitivity by up to three orders of magnitude following formation of the $[M + H]^+$ ion. Somewhat less dramatic increases have been reported for 2-aminopyridine (2-AP, **XXVI**) [116] and ethyl-4-aminobenzoate (ABEE, **XXVII**) [116]. Other derivatives of this type include those prepared from 2-aminoacridone (AMAC, **XXVIII**) [117–120] and 3-(acetylamino)-6-aminoacridine (AA-Ac, **XXIX**) [121]. Most derivatives incorporating amino groups, in addition to the linking amine in those derivatives produced by reductive amination, yield $[M + H]^+$ as well as $[M + Na]^+$ ions and conditions have to be adjusted carefully in order not to split the signal between these two ion types if highest sensitivity is a priority. Derivatives such as those prepared from 2-AB, (**XXIV**) [122], as used in our laboratory for fluorescent detection, do not appear to have a significant effect on sensitivity as ionization is predominantly to give the $[M + Na]^+$ ion.

Derivatives incorporating a constitutive cationic charge, such as the trimethyl-(4-aminophenyl)amino- (TMAPA, **XXX**) [116] and Girard's T (**XXXI**) [123] derivatives, usually increase sensitivity about 10-fold. A related cationic derivative has been prepared by dimethylation of the reductive amination product of sugars with benzylamine (**XXXII**) [124]; again a sensitivity increase of about an order of magnitude was found. Several derivatives capable of forming negative charges, such as those from 2-aminobenzoic acid (2-AA, **XXXIII**) [125], 8-aminonaphthalene-1,3,6-trisulphonic acid (ANTS, **XXXIV**) [126] and 1-aminopyrene-3,6,8-trisulfonate (APTS, **XXXV**) [127] allow carbohydrates to be examined as negative ions with good sensitivity.

Other miscellaneous derivatives, not prepared by reductive amination, that have proved useful for MALDI analysis include those prepared from basic peptides [128], *tert*-butoxycarbonyl-L-tyrosine (BOC-tyrosine, **XXXVI**) [129], 1-phenyl-3-methyl-5-pyrazolone (P-MP, **XXIII**) under basic conditions [84], tetramethylrhodamine (TMR, **XXXVII**) [96] and malononitrile [130]. The latter three derivatives gave considerable increases in sensitivity whereas the BOC-tyrosine derivative could be hydrolysed back to the parent glycan.

6. Fragmentation

A number of options are available for obtaining fragmentation spectra from carbohydrates ionised by MALDI depending on the type of instrument fitted with the MALDI ion source. However, all of these methods produce similar spectra with respect to the position of the main cleavage sites. The major

differences arise from the type of ion, ($[M + H]^+$, $[M + Na]^+$, etc.), formed from the carbohydrate, the energy imparted during the ionization process and the length of time allowed for fragmentation to occur. In general, fragmentation patterns are similar to those obtained with other methods of ionization [131]. The major cleavages, termed glycosidic cleavages, involve breaking a single bond between adjacent sugar rings and are thought to be mainly charge-induced [132]. They provide much information on sequence and branching but little on linkage. The less significant cross-ring cleavages, on the other hand, which appear to be formed by charge-remote processes [132], involve the cleavage of two bonds and are much more useful for linkage determination.

The nomenclature used by most investigators to describe fragmentation is that introduced by Domon and Costello [133] (Fig. 6). Ions retaining charge on the reducing terminus are labelled X (cross-ring), Y ($C_1 \rightarrow$ O glycosidic) and Z (O $\rightarrow C_x$ glycosidic) or A, B and C if the charge is located at the non-reducing end. Subscript numerals denote the bond or sugar ring broken, starting from the reducing end for the X, Y and Z fragments and the non-reducing terminus for the others. The heaviest chains from branched compounds are additionally given α, β, etc. suffixes with α assigned to the heaviest chain. Bonds involved in cross-ring cleavage fragments are denoted by a superscript prefix consisting of the lowest numbered carbon (or oxygen) atom of the two bonds cleaved. In the spectra of most carbohydrates, the B and Y ions are usually the most abundant.

The relative proportions of the different types of fragment ion vary with the type of parent ion produced from the carbohydrate. Protonated species decompose much more readily than metal-cationized species, but produce fewer cross-ring cleavage products [134]. Of the alkali metal-adducted ions, those involving lithium decompose most readily with the extent of fragmentation falling through the series $Li^+ > Na^+ > K^+ > Rb^+ > Cs^+$ [78,135].

6.1. Types of fragmentation encountered in MALDI spectra

6.1.1. In-source decay (ISD) or prompt fragmentation

Ions that decompose very rapidly will do so in the ion source and produce focused fragments. They can be observed with TOF instruments, particularly those fitted with delayed extraction that are operated with relatively long delay times [136]. They have also been observed with magnetic sector instruments [137]. ISD ions formed by Y-type cleavages are indistinguishable from molecular ions and their presence can distort carbohydrate profiles when MALDI is used to examine mixtures. Fortunately, these Y-type cleavage reactions are normally too slow and the products too weak to pose a significant problem [138] except when sialic acids are present [12,139]. Loss of sialic acid from

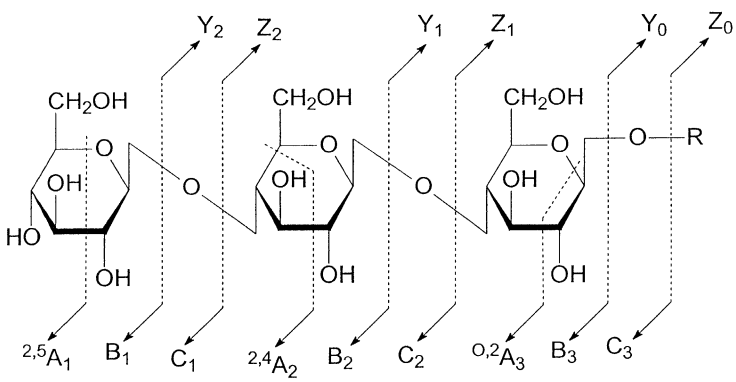


Fig. 6. Nomenclature for describing the fragmentation of carbohydrates, after Domon and Costello [133].

underivatised carbohydrates by fragmentation is a major problem in MALDI spectra, particularly at higher laser powers [140], but can largely be overcome by derivative formation such as the preparation of methyl ester [141] or by use of higher pressures, under which conditions the ions are stabilised by collisional cooling [142,143]. As these fragment ions are only resolved on TOF instruments when used in reflectron mode, many investigators record the spectra of sialylated glycans in linear mode [144] where separation of the fragments from the molecular ions does not occur.

6.1.2. Post-source decay (PSD) fragmentation

Ions whose lifetime is such that they decompose between the ion source and reflectron in a reflectron-TOF mass spectrometer yield unfocused or metastable ions appearing as broad peaks at higher apparent mass than their focused counterparts (Fig. 7). Although they are not observed with linear TOF instruments, metastable fragmentation can, nevertheless, cause broadening of the molecular ion peaks [145] and have adverse effects on mass accuracy. Even though the apparent mass of these metastable ions is dependent on the type of instrument used to record the spectra and on the focusing conditions, a second order relationship has been found that enables this mass to be calculated [146] and for the presence of the peaks to be used diagnostically. The relative abundance of these metastable ions is inversely related to the time that the ions spend in the ion source; thus, for efficient observation of PSD ions, the ISD should be reduced to a minimum. The abundance of the PSD ions is also dependent on accelerating voltage, a parameter that can affect both ion lifetime and collision energy for ions in the ion source [147]. Kaufmann et al. [148] have proposed that loss of PSD fragments can also be due to a reduced collisional activation in delayed-extraction sources.

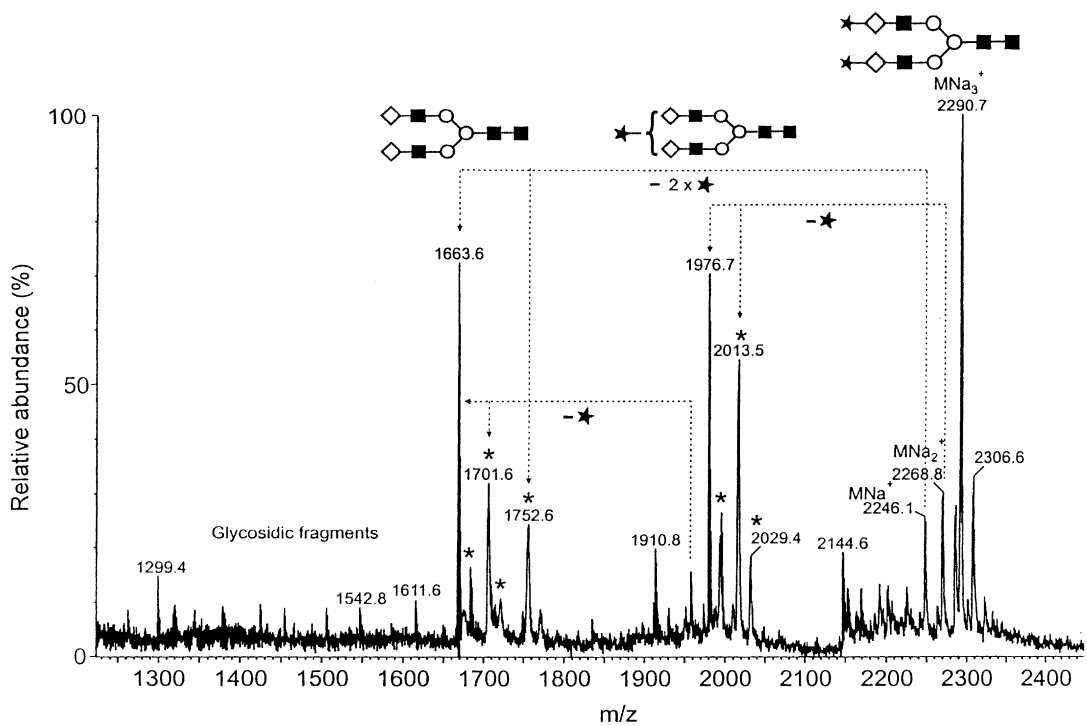


Fig. 7. Positive ion reflectron MALDI mass spectrum of a disialylated biantennary *N*-linked glycan glycoproteins recorded from DHB with a Micromass TofSpec 2E mass spectrometer. The broad peaks are metastable (PSD) ions and the horizontal lines show the fragmentations resulting from losses of sialic acids.

Although the true mass of the unfocused metastable ions can be calculated [146], PSD ions are used diagnostically either by focusing them with a curved-field reflectron [149–151] or, more commonly, by stepping the reflectron voltage and combining the resulting partial spectra. The latter method has the disadvantage that the relative abundances of ions may be distorted if they are recorded in different segments. Such methods are used extensively for structural determination [152–155] but generally suffer from relatively low sensitivity and only moderate resolution. In a further development in PSD analysis, fragment ions produced in the ion source have been selected by an ion gate and found to possess enough internal energy to fragment further [156]. The method was applied, for example, to a B-ion generated by cleavage of the GlcNAc1 → 3Gal bond from a branched pentasaccharide Gal1 → 3(Fuc1 → 4)GlcNAc1 → 3Gal1 → 4Glc and clearly showed the presence of fucose in the selected fragment.

PSD fragmentation is influenced, not only by time, but also by the matrix employed. Matrices such as 4-HCCA usually catalyse considerable fragmentation such as sialic acid loss from carbohydrates whereas "cooler" matrices such as DHB [81,157,158], 6-aza-2-thiothymine [63] or 1,3,5-THAP [159] do not. Variation in the composition of two-layer matrices also has a considerable effect on the extent of fragmentation. Pfenninger et al. [160] have shown that much more extensive fragmentation was achieved with a matrix of 3-AQ overlying CMBT than with DHB overlying the same matrix.

Fragmentation can also be influenced if the molecules are derivatised. Thus, it has been noted that, if the carbohydrate can be protonated, such as when derivatised by reductive amination, matrices such as 4-HCCA tend to produce $[M + H]^+$ ions whereas those such as DHB still produce $[M + Na]^+$ ions. Because the charge is localised at the reducing terminus of the former ions, their PSD spectra tend to contain only Y-type fragments whereas the $[M + Na]^+$ ions fragment to give both B and Y ions [116]. The addition of malononitrile to the reducing terminus of maltoheptaose has been shown to produce a much more complicated PSD spectrum than that of the underivatised compound; B-, C-, Y-, Z- and A-type cross-ring fragments were abundant in the spectrum of the derivatised compound whereas, in the absence of the derivative, only B and C ions were found [130]. Differences have also been noted in the fragmentation of derivatised *N*-linked glycans when recorded by PSD from singly-charged ions or by ISD in an electrospray source; the latter spectra contained more low mass ions. The reason was attributed to the presence of two charges on the electrosprayed ions (one on the derivative) that yielded two fragments on decomposition [161].

PSD spectra of the $[M + Na]^+$ ions from neutral carbohydrates (Fig. 8) tend to be dominated by glycosidic cleavage reactions with only weak contributions from cross-ring products [137,162]. However, the relative abundance of these cross-ring fragments has been increased in a TOF instrument fitted with a collision cell [163,164]. When helium was used as the collision gas, prominent X-type ions were produced [164]. Losses of fragments from different regions of the molecules give rise to abundant "internal fragments" that make spectral interpretation particularly difficult, especially if the reducing terminus is not derivatised. Derivatization by permethylation can also be used to aid spectral interpretation because glycosidic cleavages leave hydroxy rather than methyl groups at the cleavage site. However, the extent of fragmentation has been noted as sometimes being less than that from underivatised carbohydrates [165], probably due to the shortage of labile hydrogen atoms. All glycosidic cleavages of the even-electron ions generated by MALDI involve a hydrogen migration. With underivatised carbohydrates, the hydrogen usually originates from one of the labile hydroxyl groups [166]. Thus, the presence of the methyl groups in the permethylated glycans prevents this reaction and presumably contributes to the low ion yield.

Although linkage-revealing cross-ring cleavages are usually weak in PSD spectra, it is sometimes possible to extract linkage information from the relative abundance of glycosidic cleavage ions, particularly if pure samples of the relevant isomeric compounds are

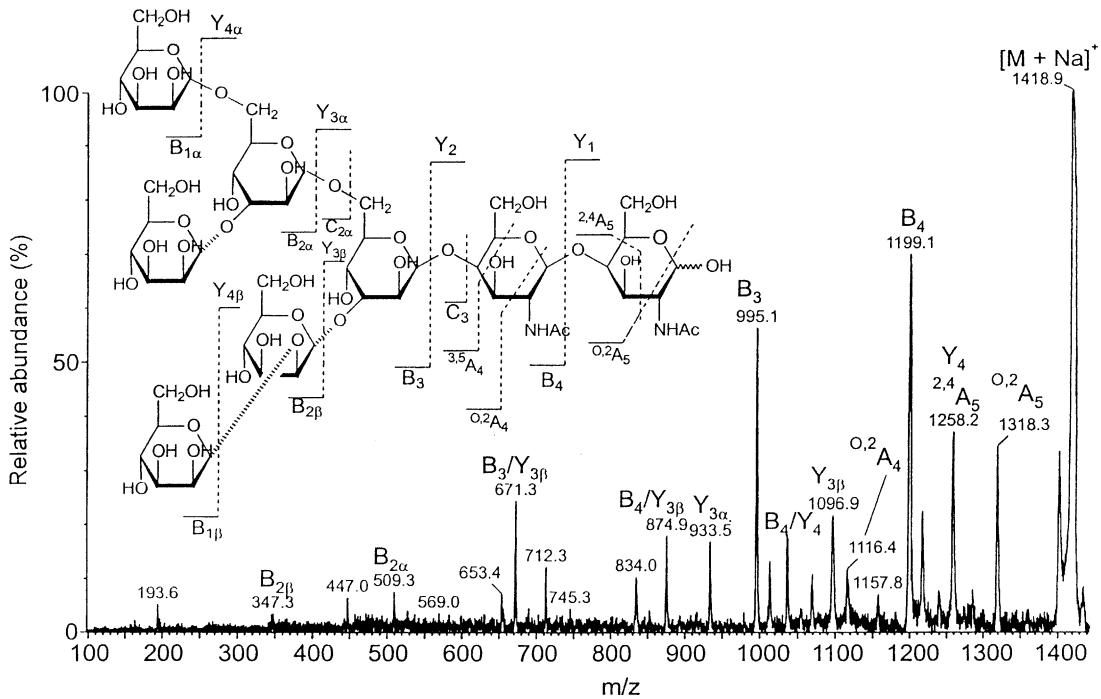


Fig. 8. Positive ion MALDI-PSD spectrum of the high-mannose *N*-linked glycan $(Man)_6(GlcNAc)_2$ from chicken ovalbumin recorded from DHB. The major fragmentations are shown on the structural formula. Several other pathways are possible for some of the ions shown.

available. Thus, for example, Spengler et al. [162] have found that for the α1 → 3- or α1 → 6 linkage between two mannose units of isomeric pentasaccharides, the C-ion was the more abundant from the α1 → 6 isomer, whereas in the α1 → 3-isomer the position was reversed. Viseux et al. [167] have noted preferential elimination of groups attached to the 3-position of GlcNAc residues, in common with earlier observations from FAB spectra [168–172]. Yamagaki et al., using PSD, have utilised this property to distinguish between Lewis x and Lewis a type carbohydrates that have, respectively, fucose or galactose substituted to C-3 of the GlcNAc residue. We have found that an ion formed by loss of the chitobiose core and 3-antenna from *N*-linked glycans reflects the differential composition of the antenna in many cases [173,174], such as with high-mannose *N*-linked glycans. On the other hand, Rouse et al. [154] failed to find the presence of the ion useful for differentiating between triantennary isomers although they did find that the ratios of B-ions formed by loss of the terminal GlcNAc residue together with one or more *N*-acetyllactosamine units was diagnostic (Fig. 9). Subsequent loss of water from the ion formed by loss of the chitobiose core and 3-antenna, however, appears to be diagnostic for the presence of a "bisecting" GlcNAc (4-linked to the core branching mannose) residue in the spectra of these compounds [173]. PSD spectra from two triantennary glycans from bovine fetuin that differ only in that one of the terminal galactose residues is in β1 → 3 rather than β1 → 4 linkage, show characteristic differences. Thus, the two fragments ascribed to loss of galactose and Galβ1 → 3GlcNAc in the spectrum of the β1 → 3 isomer have been reported to be considerably more abundant that in the spectrum of the other [175].

Although interpretation of the spectra of *N*-linked glycans is complex, a computer program has been

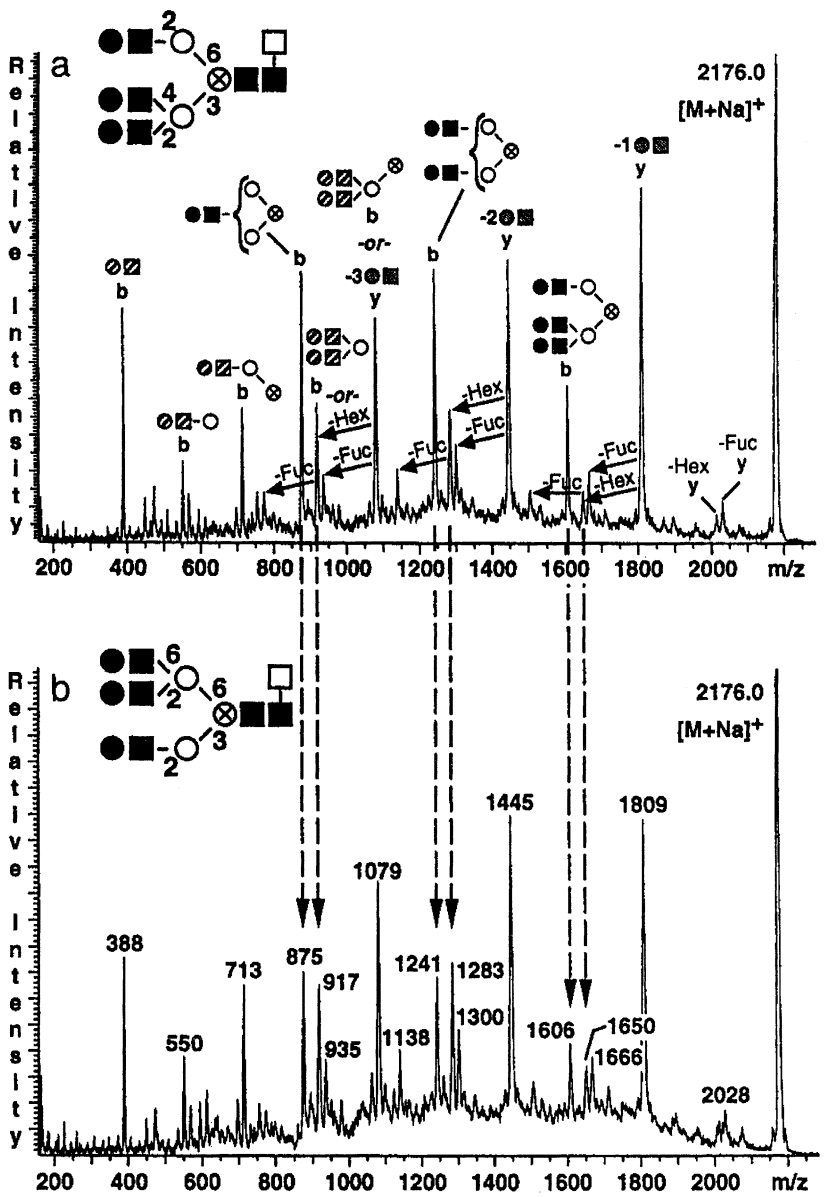


Fig. 9. Positive ion MALDI-PSD spectra of isomeric triantennary *N*-linked glycans showing ions (*m/z* 1606, 1241, and 875) whose relative intensity enables the isomers to be differentiated. Symbols for the monosaccharide units are: (■) GlcNAc, (○) and (⊗) mannose, (●) galactose, (□) fucose. Reproduced from [154] with permission.

developed that works by assigning compositions to the fragment ions and by constructing predicted spectra from known structures to compare with the experimental data. The program was claimed to be able to interpret spectra in only a few seconds [176].

6.1.3. Fragmentation produced by collision-induced decomposition

This technique yields well-focused ions, is controllable and overcomes many of the problems associated with PSD spectra. The recent interfacing of MALDI

ion sources with Q-TOF mass spectrometers [37] provides high quality fragmentation spectra from carbohydrates [42] (Figs. 10 and 11) in which the extent of fragmentation can be varied by changes in the collision energy. Spectra were found to be virtually identical to those recorded on the same instrument following electrospray ionization [177–179], thus demonstrating efficient decoupling of the ionization and fragmentation mechanisms. High-energy (800 eV) collision-induced decomposition (CID) spectra of MALDI-generated ions recorded with a magnetic sector mass spectrometer fitted with an orthogonal-TOF

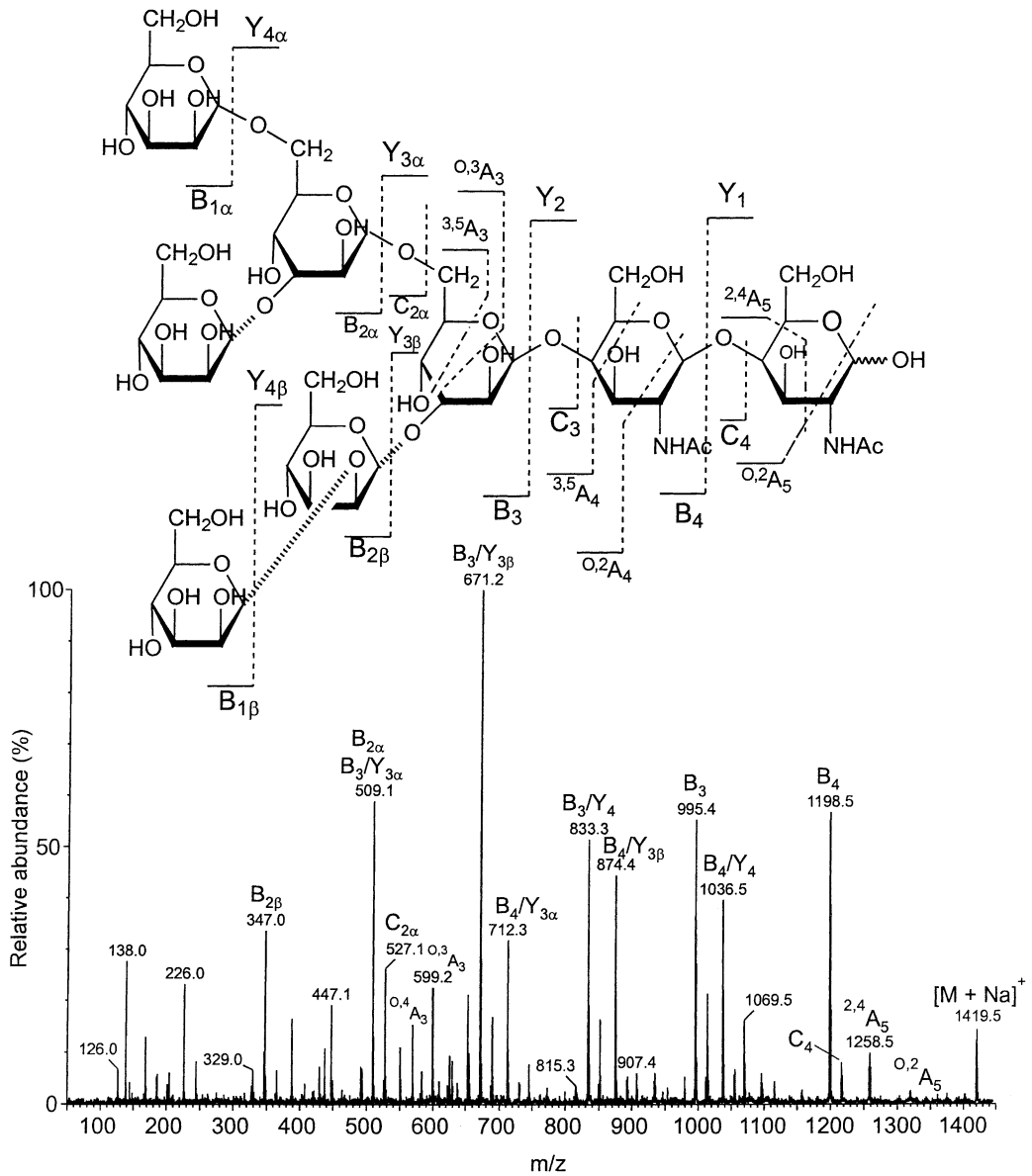


Fig. 10. Positive ion MALDI-CID spectrum of the high-mannose *N*-linked glycan $(Man)_6(GlcNAc)_2$ from ribonuclease B recorded from DHB. The major fragmentations are shown on the structural formula.

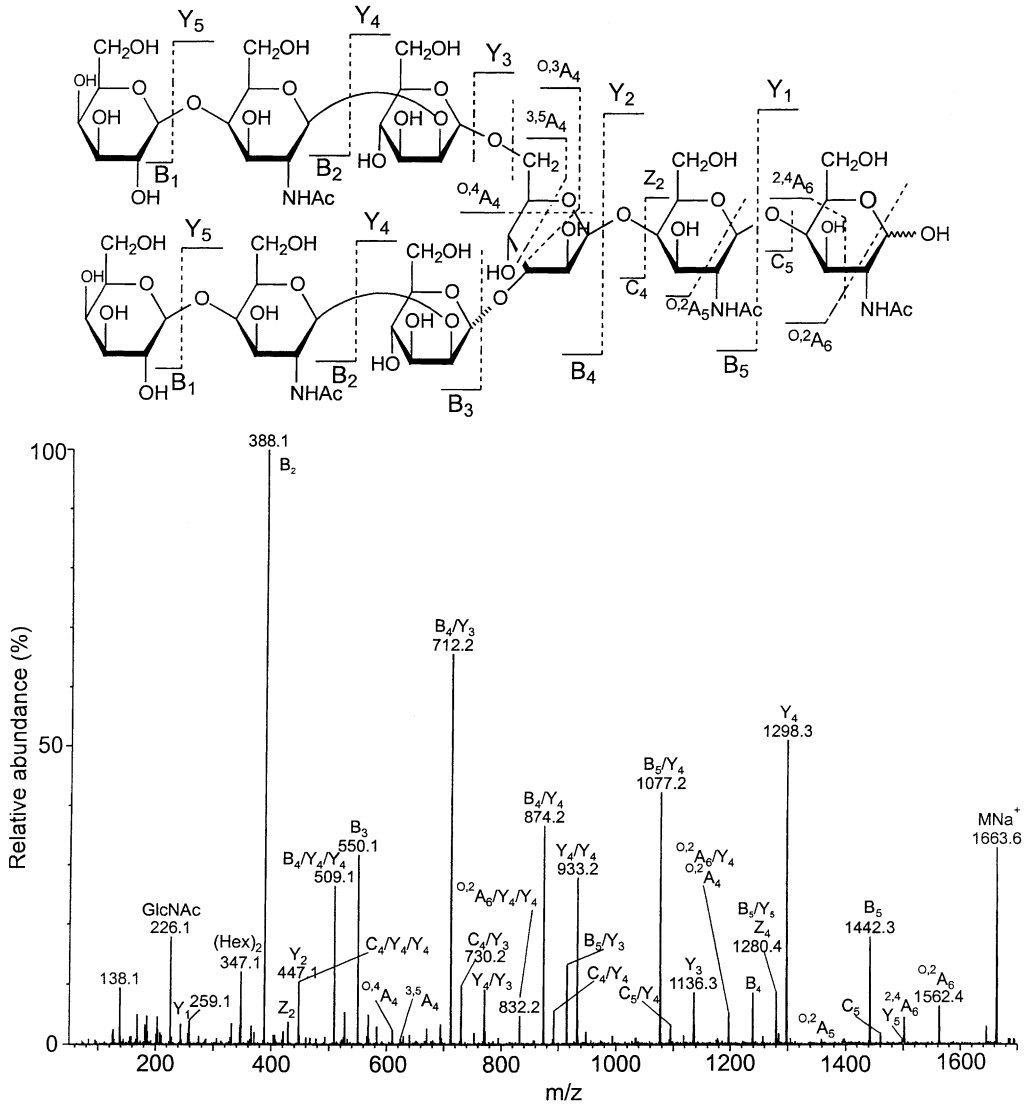


Fig. 11. Positive ion MALDI-CID spectrum of a complex *N*-linked glycan recorded from DHB. The major fragmentations are shown on the structural formula. Symbols are defined in the legends to Figs. 2 and 3.

analyser [180] showed similar fragmentation patterns with the exception that the relative abundances of the cross-ring fragments were higher [173]. In particular, abundant ^{1,5}X-type fragments were present; these ions are normally absent from spectra recorded at lower energies. A-type fragments were abundant in the high-energy spectra from many sugars derivatised at the reducing terminus [181].

Rearrangement ions are rare in the spectra of carbohydrates although they do appear in the form of "internal residue losses" from $[M + H]^+$ [182–185], but not from $[M + Na]^+$ ions [186] in FAB and CID spectra. We have noted that they are particularly abundant in the MALDI spectra of fucosylated carbohydrates derivatised at the reducing terminus by reductive amination where there is an apparent

migration of fucose towards the derivatised end of the molecule [187]. Such ions can lead to ambiguous structural conclusions from this type of compound.

7. Applications of MALDI mass spectrometry to the analysis of specific structural types

7.1. Free carbohydrates

MALDI is ideally suited to examination of carbohydrate mixtures with varying numbers of residues, many of which originate from algae and plants. Thus, for example, xylans from the seaweed *Caulerpa brachypus* have been shown to consist of linear polymers with at least 25 xylose residues in β1 → 3-linkage [188] and laminarans from eight species of brown algae have been observed to give ions up to about *m*/*z* 6000, corresponding to polymer chains in the region of 40 residues. In the latter study, MALDI was found to give superior results to FAB because it is not subject to the same degree of signal loss at higher mass values. Fructans of general structure [Glcβ(1 → 2)(Frcβ(1 → 2(or 6)))$_n$ → 2Frc], from *Dahlia variabilis* with masses up to 10,000 Da have been examined with MALDI by Stahl et al. [64] using 3-aminoquinoline as the matrix on a reflectron-TOF instrument. Components were resolved to about 7000 Da. These authors also examined fructans directly from onion (*Allium cepa* L.) skin using DHB and found that the major ions were due to $[M + K]^+$ rather than the more normal $[M + Na]^+$, reflecting the high content of potassium in the onion. Large polymers are commonly digested with enzymes to smaller units prior to MALDI analysis. Thus, xyloglucans from solanaceous plants were cleaved with fungal endo-β(1 → 4)-D-glucanase into oligosaccharides with masses of about 1 kDa by York et al. [189] and the endoxylanase from *Trichoderma reesei* has been used to cleave carbohydrate chains from birch [190] and oak (*Acer pseudoplatanus*) [191] to oligosaccharides in the 400–1000 Da region. Pectins, which contain partially methylated uronic acids have received a similar treatment [192,193] prior to analysis from several matrices of which 2,4,6-THAP gave the best results [193]; oligomers with masses to 8000 were found. PSD from 2,4,6-THAP and nitrocellulose was able to define the positions of the methyl groups [194]. Pullulanase was used by Vinogradov and Bock [195] to hydrolyse *Iso*maltooligosaccharides from beer; oligosaccharide chains were labelled with 7-amino-4-methylcoumarin (**XXXVIII**) by reductive amination and chains with up to 10 α1 → 4-linked glucose residues were found.

Milk is also a rich source of carbohydrates. In a study by Stahl et al. [62] with human milk, neutral and acidic fucosylated lactose polymers with molecular weights of up to 8000 were identified. 2,5-DHB and super-DHB gave the best signals from the neutral sugars, whereas 3-AQ was found to be the most appropriate matrix for the acidic glycans. In further studies by high-pH anion-exchange chromatography (HPAEC) [196] and preparative anion-exchange chromatography (AEC) [197] it was found that many of the peaks detected by MALDI consisted of several isomers. This observation emphasises the need to combine mass spectrometric techniques with some form of chromatography when performing detailed analysis of complex mixtures.

Free carbohydrates have been examined in urine after derivatization with ANTS and purification with porous graphatized carbon [198]. The matrix was a mixture of 3-AQ and ammonium acetate and the resulting carbohydrate profiles reflected several disease states such as α-mannosidosis and gangliosidosis.

7.2. Cyclodextrins

Quantitatively, cyclodextrins appear to behave analogously to other carbohydrates [85]. Excellent linear correlations ($r^2 > 0.995$) have been found between concentration and response with linear dextrans as internal standards. MALDI with DHB, DHB/HIQ or 4-HCCA has been used to check the purity of commercial samples of cyclodextrins as it appears to produce more reliable data than either PD or FAB [199,200]. Even though these compounds have a cyclic structure they, nevertheless, yield glycosidic PSD fragment ions

[201]; such ions require the rupture of two glycosidic bonds. No cross-ring fragments were seen in this work. Methylated cyclodextrins appear to give stronger signals that the cyclodextrins themselves but the relative signal strength falls as the number of methyl groups increases, an observation opposite to that found by ionization using atmospheric pressure chemical ionization (APCI) [202]. The convenience of MALDI in checking the purity of cyclodextrins is reflected in other areas where MALDI is used in a commercial environment, as illustrated by its use to investigate galactosyl distribution in commercial galactomannans used in food products [203].

7.3. Glycoproteins

The analysis of the *N*- and, to a lesser extent, the *O*-glycans from glycoproteins, is the area in which MALDI mass spectrometry has had the most impact on carbohydrate analysis [204]. Compounds are sometimes examined intact or, more commonly, as released glycans or as glycopeptides produced by enzymatic digestion. Not only is it important to identify the individual glycans, but also to ascertain which of the glycosylation sites of the protein is occupied, and with which glycans.

7.3.1. Use of MALDI to examine intact glycoproteins

Glycoforms of small glycopeptides with single glycosylation sites, or sites occupied only by a very limited range of glycans, can often be resolved by MALDI mass spectrometry with TOF instruments. Examples are ribonuclease B (15 kDa) with five high-mannose glycans at a single glycosylation site [11] (Fig. 2), Sf9-derived IL-4 receptor (30 kDa) [144] and human serum transferrin (79.6 kDa) with two sites each occupied with a single glycan and which are differentially glycosylated in the form of the disease, carbohydrate-deficient glycoprotein syndrome [205], in which one or both of the biantennary glycans are missing. In the case of the latter glycoprotein, however, other investigators have reported better resolution with instruments fitted with electrospray [206]. With larger molecules, it is necessary to cleave the protein into smaller units or to remove the glycan. The measurement of protein molecular weight before and after removal of the attached glycans by either chemical or exo- and endo-glycosidase treatment provides information on the state of glycosylation, even though the individual glycoforms may not be resolved [207,208]. If the glycoprotein contains a single glycan, then the mass difference before and after deglycosylation gives its mass, and in most cases its composition because *N*-linked glycans contain a relatively small number of isobaric monosaccharides [hexose (galactose, glucose, mannose,), *N*-acetylaminohexose (GlcNAc or GalNAc), deoxyhexose (fucose), and sialic acid]. Tables 1 and 2 list the residue masses of common constituent monosaccharides. Trypsin, either in solution or within a sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) gel [46] is the most common enzyme for proteolysis but pronase has also been used in an attempt to leave just asparagine at the reducing terminus [209]. This amino acid provides a site for proton attachment but the method does not provide any information on the specific glycosylation site. The approach worked in the experiment described by Juhasz and Martin [209]. *N*-Linked glycans from ribonuclease; the single asparagine residue was protonated in the positive ion MALDI spectrum acquired from DHB. However, with the larger ovalbumin, di-, tri- and penta-peptides were retained at the reducing terminus.

7.3.2. Determination of glycosylation site occupancy

Identification of site occupancy generally depends on enzymatic or chemical cleavage of the protein chain, separation of the resulting peptides and glycopeptides by reversed-phase HPLC and detection by mass spectrometry. A variety of mass spectrometric techniques can be used, either off-line as in the case of MALDI or, more commonly, on-line as with electrospray [210–213]. Conditions can usually be found which induce cleavage between the glycosylation sites to leave a mixture of peptides and glycopeptides with each glycosylation site, or group of sites in the case of

Table 1
Residue masses of monosaccharides commonly found in oligosaccharides

Monosaccharide	Residue formula	Monoisotopic mass	Average mass
Pentose	$C_5H_8O_4$	132.0423	132.12
Deoxyhexose	$C_6H_{10}O_4$	146.0579	146.14
Hexose	$C_6H_{10}O_5$	162.0528	162.14
Hexosamine	$C_6H_{11}NO_4$	161.0688	161.16
Hexuronic acid	$C_6H_8O_6$	176.0321	176.13
Na salt	$C_6H_7O_6Na$	198.0140	198.11
Heptose	$C_7H_{12}O_6$	192.0634	192.17
N-Acetylaminohexose	$C_8H_{13}NO_5$	203.0794	203.19
KDO	$C_8H_{12}O_7$	220.0583	220.18
N-Acetylneuraminic acid	$C_{11}H_{17}NO_8$	291.0954	291.26
Na salt	$C_{11}H_{16}NO_8Na$	313.0773	313.24
N-Glycoylneuraminic acid	$C_{11}H_{17}NO_9$	307.0903	307.26
Na salt	$C_{11}H_{16}NO_9Na$	329.0734	329.24

The oligosaccharide masses are obtained by addition of the residue masses together with the mass of one molecule of water (18.0106 monoisotopic, 18.02 chemical). (Alternatively, the full masses of the monosaccharides less $n-1$ water molecules (where n is the number of monosaccharides) may be used.) For the mass of the $[M+Na]^+$ ion, an additional 22.9899 (monoisotopic) or 23.00 (chemical) mass units should be added.

O-linked glycans, localised to a single glycopeptide. Enzymatic removal of the sugars with endoglycosidases such as protein-*N*-glycosidase-F (PNGase-F) or endoglycosidase-H (endo-H) and revaluation of the residual peptide mixture by LC/MS reveals the glycopeptides by their shift in the chromatogram [214]. Both enzymes leave a modified protein and, thus, partial site occupancy can be determined. Endo-H, cleaves the sugar between the two GlcNAc residues of the core, and thus, leaves the reducing-terminal GlcNAc residue, together with any substituents, attached to the peptide. PNGase-F cleaves the sugar at the amide bond to leave an aspartic acid residue rather than asparagine and this can be detected by a mass increment of 1 Da [215]. The extent of acid formation gives an indication of site occupancy. If the incubations are performed in water containing $H_2{}^{18}O_2$, the labelled oxygen is incorporated into the aspartic acid resulting in doublet peaks separated by two mass units in the resulting mass spectra [216,217], thus, aiding detection.

Table 2
Masses of common carbohydrate modifications

Modification	Residue formula	Monoisotopic mass	Average mass
Sulphate	SO_3	79.9568	80.06
Phosphate	PO_3H	79.9663	79.98
Acetate	C_2H_2O	42.0106	42.04
Methyl	CH_2	14.0156	14.03

Site analysis of *O*-linked sugars has not received as much attention as the *N*-linked carbohydrates due, largely, to the clustered nature of many *O*-linked sites and to the absence of a general *O*-glycosidase. These glycans are usually released by β-elimination with sodium hydroxide, a procedure that leaves an unsaturated propyl substituent from threonine and dehydroalanine from serine-linked glycans [218]. However, some peptides are degraded by the base treatment necessary for β-elimination, thus, reducing the general applicability of the method. In order to avoid degradation, Rademaker et al. [219] have released sugars from serine-containing peptides with the milder reagent, ammonium hydroxide. Under these conditions, the resulting dehydroalanine reacted with the ammonia to produce an aminated amino acid that exhibited a mass increment of 17 Da. Site analysis has also been performed by partial deglycosylation with trifluoromethanesulfonic acid to the level of a single GalNAc residue at each site followed by cleavage of the protein with the Arg-C-specific endopeptidase, clostripain. The resulting glycopeptide was analysed

be MALDI mass spectrometry employing PSD fragmentation [220]. However, there appears to be a tendency for GalNAc residues to be lost under PSD conditions from some glycopeptides [221], thus, limiting the applicability of the technique. Hanisch et al. [222] have removed *O*-linked glycans by incubation with methylamine at 50 °C. Under these conditions, the alkylamine is added to the resulting unsaturated amino acids and acts as a label for the position of the original glycans. The residual protein can then be cleaved by proteolysis in the normal manner.

7.3.3. Release of glycans from glycoproteins

7.3.3.1. Chemical release. Hydrazine releases both *N*- [223,224] and *O*-linked [224] glycans by cleavage of all peptide bonds in the glycoprotein. *O*-Linked glycans are specifically released at 60 °C, whereas 95 °C is needed to release the *N*-linked sugars. Although both types of glycan can be released in this way to leave an intact reducing terminus, unlike the situation with some enzymatic release methods, the technique has several major disadvantages for mass spectral analysis. Because all peptide bonds are destroyed, all information relating to the protein, such as the site of glycan attachment, is lost. Secondly, the acyl groups are cleaved from the *N*-acetylamino sugars and sialic acids and are normally replaced chemically, with the assumption that they were originally acetyl. This is not always the case, as with *N*-glycoylneuraminic acids. The reacetylation step also adds a small amount of acetyl substitution to hydroxyl groups. Thirdly, the reducing terminus of some of the glycans contains residual hydrazide or amino groups. Acetylation of the amine group can lead to glycan masses that are indistinguishable from those of native glycans and give the false impression of additional glycans in MALDI profiles. Bendiak and Cumming [225] estimate that about 25% of glycans are affected in this way. Finally, if the hydrazinolysis conditions are too vigorous, some *N*-acetylamino group or terminal GlcNAc residues can be removed [226].

7.3.3.2. Enzymatic release. The most popular endoglycosidase for *N*-glycan release is PNGase-F [227] which cleaves the intact glycan as the glycosylamine and leaves aspartic acid in place of the asparagine at the *N*-linked site of the protein. The released glycosylamine readily hydrolyses to the glycan except if the reaction is performed in ammonium-containing buffers [228] in which case mild acid treatment completes the hydrolysis. PNGase-F releases most glycans except those that contain fucose α1–3 linked to the reducing-terminal GlcNAc [229] in which case PNGase-A, as used by Wilson et al. [230] in a comparative MALDI study of *N*-linked glycans from plants, for example, is usually effective. Endo-H, as mentioned above, hydrolyses the bond between the two GlcNAc residues of the chitobiose core, leaving the core GlcNAc with any substituent, such as fucose, attached to the protein. Information on the presence of core fucosylation is, thus, not available from the spectra of the resulting glycans. Another potential disadvantage of this enzyme is that it only releases high-mannose and hybrid, glycans although this property does yield some structural information.

7.3.4. Detailed glycan analysis by use of exoglycosidase sequencing and MALDI profiling

7.3.4.1. N-Linked glycans. Because of the potentially very large number of isomeric glycan structures for any particular combination of monosaccharides, detailed structural analysis by purely physical methods is a daunting problem. Consequently, much use is made of the highly specific ability of several enzymes to recognise various structural features. Perhaps the most important method for structural analysis of *N*- and, to a lesser extent, *O*-linked glycans is to incubate them with a range of exoglycosidases, either sequentially or as arrays [231], monitor what is removed, usually by HPLC or MALDI, and deduce the nature of the removed fragment(s) by knowledge of the enzyme's specificity. Common enzymes are listed in several reviews [232–234]. One drawback to this approach is that it relies on the continuing supply, usually from commercial sources, of pure enzymes and that the full spectrum of activity of the enzyme is known. Several commercial enzyme preparations

have been reported to be contaminated with other enzymes. Thus, preparations of *S. pneumonia* β-*N*-acetylhexosaminidase have been found contaminated with an enzyme with endoglycosidase-H-like activity [235,236] and chicken α-fucosidase, said to be specific for fucose α1 → 2-, 4- or 6-linked to GlcNAc, has also been found to remove a considerable amount of galactose [236]. Although the method works well with conventional carbohydrates, usually from mammalian sources, some samples contain carbohydrates, or chemical modifications, that are not substrates for the common exoglycosidases. An example is the terminal mannose-phosphate group found on some hydrazine-released *N*-linked glycans from tissue plasminogen activator expressed in the yeast, *Pichia pastoris* [237]. Fortunately, however, the phosphate group, once recognised, could be hydrolysed with a phosphatase allowing the rest of the molecule to be digested with subsequent exoglycosidases.

The technique of exoglycosidase sequencing was first used with MALDI analysis of the products by Sutton et al. [235] using tryptic glycopeptides from the recombinant human tissue inhibitor of metalloproteinases (TIMP), a glycoprotein with two *N*-linked glycosylation sites. The method provided site analysis as well as glycan structure but, as with any technique involving analysis of glycopeptides, its success was dependent on an accurate knowledge of the peptide mass and of the presence of any additional post-translational modifications. In order to overcome this problem, exoglycosidase sequencing of released *N*-linked glycans was reported by our group in 1994 [238]. Biantennary *N*-linked glycans were released from human immunoglobulin (IgG) with hydrazine, incubated with exoglycosidases and the products were examined by MALDI from 2,5-DHB. Although the products of each incubation were isolated after each digestion, with consequent losses, the signal:noise ratio of the MALDI spectra rose as the result of the progressive collapse of the several peaks in the original mixture to the single peak of the final incubation product. The method was later modified so that all enzyme digests could be performed on the MALDI target [239]; the matrix was removed by drop dialysis before each incubation. In a modified procedure by Geyer et al. [240] in which 6-aza-2-thiothymine rather than the acidic DHB was used as the matrix, enzyme digests were performed in the presence of the matrix that did not affect the enzyme activities. Colangelo and Orlando [241], and Mechref and Novotny [242] have both developed similar methods using exoglycosidase arrays on different target spots in order to avoid removal of the matrix as required by the original sequential protocol. The method described by Mechref and Novotny also involved PNGase-F removal of the glycans from the glycoprotein on the MALDI target; arabinosazone was used as the matrix.

The micro-method described by Mechref and Novotny was developed in response to the need for very sensitive analytical techniques required for the minute quantities of material normally encountered in this field. Several other related methods have been described, each with its own advantages. We [100,243] have developed a method whereby glycoproteins were separated or isolated by one-dimensional SDS-PAGE gels prior to glycan release from the reduced and alkylated glycoprotein by cleavage with PNGase-F from within the gel. The glycans were extracted and subsequently desalted by passage through a mixed bed resin column of AG-3 (removal of anions), AG-50 (removal of cations), and C_{18} (removal of organic material) packed into a gel-loader pipette tip. Samples, and their products of exoglycosidase digestion, were examined directly by MALDI from DHB or by HPLC incorporating fluorescence detection of the 2-AB derivative. The AG-3 resin was found to be essential for the production of MALDI signals, but unfortunately, it removed most acidic glycans. Sensitivity was such that only 100 pmol of glycoprotein applied to the gel was sufficient for a complete glycan analysis. For retention of sialic acid-containing glycans, the methyl ester derivatization method developed by Powell and Harvey [141] was adopted and for examination of sulphated glycans, the sample was desalted with porous graphatized carbon [73].

One disadvantage of this method is that PNGase-F does not release glycans bearing a 1 → 3-linked fucose residue at the reducing-terminal GlcNAc residue.

However, these are released with PNGase-A. As this enzyme is larger than PNGase-F (75.5 as compared with 35 kDa), penetration into the gel would probably be inhibited. Also, this enzyme only releases glycans from smaller peptides. Consequently, Kolarich and Altmann [244] performed tryptic digestion of the gel-separated glycoproteins, extracted these and then incubated with PNGase-A. The products were cleaned with AG-50 and C-18 following peptide removal and examined by MALDI using a mixed matrix of DHB, HIQ and arabinosazone. Although this matrix gave results with lower laser powers than those needed for DHB, equally good spectra could often be obtained from DHB alone, although with "higher operator effort".

Charlwood et al. [245] have extended this method to the analysis of *N*-linked glycans separated by two-dimensional SDS-PAGE gels. In the first of three protocols, PNGase-F-released glycans were extracted from the gels and desalted with GlycoClean-H resin from Oxford GlycoSciences before being labelled with AA-Ac [121] and further cleaned with a Waters OASIS cartridge. In the second procedure, the glycoproteins underwent in-gel tryptic digestion and glycans were released from the resulting glycopeptides after extraction from the gel. In the third method, the gel-separated glycoproteins were blotted onto polyvinylidine difluoride (PVDF) membranes and the excised spots were subjected to PNGase-F digestion to release the glycans. In all cases, the released glycans were analysed by both MALDI and HPLC. The best results were obtained from glycoproteins containing neutral glycans when these were released from within the gel. These glycans were detected from as little as 0.5 μg of glycoprotein (ovomucoid). Glycans containing sialic acid were not recovered as effectively and better results were obtained either by release from the tryptic peptides or by desialylation prior to in-gel release. Recoveries from the PVDF membranes were poor.

In the method reported by Papac et al. [246], MALDI spectra of sialylated *N*-linked glycans were obtained from as little as 0.1 μg of recombinant tissue plasminogen activator. The method relied on the absorption of up to 50 μg of the glycoprotein directly onto PVDF (Immobilon P) membranes of a 96-well MultiScreen IP plate (pore size 0.45 μm), where the glycoprotein was reduced and alkylated before the glycans were released with PNGase-F. Tris-acetate buffer (10 mM, pH 8.5) rather than the more usual sodium phosphate was used for the incubation in order to avoid any lactone formation from sialic acid residues [63]. Samples were desalted with AG50W-X8 resin, a process that was found to cause about 3% loss of sialic acid. MALDI analysis was performed with THAP/ammonium citrate (negative ion) or with super-DHB. In an extension to this method, Callewaert et al. [247] have analysed the products as derivatives with APTS (**XXXV**), using MALDI and additionally by electrophoresis using a standard DNA analysis apparatus.

Examples of the use of MALDI for analysis of the more common *N*-linked glycans (high-mannose, di-, tri- and tetra-antennary) reported in the recent literature include those from α1-acid glycoprotein [248,249], boar seminal plasma spermadhesin [250], bovine ecto-5′-nucleotidase [251], cell adhesion molecules (CAM) [252], IgG [253], human matrix metalloproteinase [254], human α1-microglobulin [255], CD31 [256], human and bovine lung surfactant protein D [257], porcine diamine oxidase [258], siglec-5, -7 and -8 [259], human thrombopoietin [260] and goldfish neurolin [261] expressed in Chinese hamster ovary (CHO) cells and human placental arylsulphatase A [262]. A complete list from the earlier literature can be found in the review by Harvey [8].

Structurally less common *N*-linked glycans include truncated but highly branched structures from avian riboflavin-binding protein [263], phosphorylcholine-substituted glycans from filarial parasites [264], glycans, containing core-linked xylose from horseradish peroxidase [265], glycans containing antennae with *N*-acetyllactosamine extensions from human epidermal growth factor receptor [266] and human CD52 [267], glucuronic acid-containing glycans from Bowes melanoma tissue plasminogen activator (TPA) [268], sulphated GalNAc-containing glycans from bovine thyroid stimulating hormone [73], glycans with chains of at least 70 mannose residues from yeast internal

invertase [269], highly truncated glycans from human and porcine cerebroside sulphate activator protein [270] and glycans containing 6-sulphoquinovose from cytochrome $b_{558/566}$ obtained from the archaeon *Sulfolobus acidocaldarius*, an organism living at 75–80 °C at a pH of 2–2.5 [271].

7.3.4.2. O-Linked glycans. *O*-Linked glycans are typified by the mucins, on which much work has been performed in recent years. Mucins from egg jelly coats have been well studied. Thus, 19 neutral *O*-linked glycans have been identified with MALDI from the jelly-coat of the South African clawed toad (*Xenopus laevis*) [272]. Problems involving loss of fucose in this study were prevented by examination of the glycans as stable $[M + Cs]^+$ species. Tseng et al. [273] have developed a method in which groups of ions, present in fragmentation spectra, were assigned to specific structural motifs. These ion groups were then used to identify the relevant structural features in the spectra of unknown carbohydrates. Again, examples were presented from glucans of *X. laevis.* Although the method appeared to work, only four motifs were identified. The ionic patterns from other possible motifs would have to be recorded for the method to be reliable. Other recent examples of the use of MALDI to identify *O*-linked glycans include mucins from *Bufo bufo* [274], *B. arenarum* [275] and *Rana dalmatina* [276] and sulphated mucins from *R. utricularia* [275], *R. arvalis* [277], *R. temporaria* [278] and *R. ridibunda* [279].

Thirty glycans released by β-elimination from porcine stomach mucins have been identified by GC/MS by Karlsson et al. [280] but another 49 glycans, containing up to 18 residues, were detected by MALDI. Their relative composition was shown to vary with the stomach region yielding the glycans. Sixty *O*-linked glycans containing up to 15 residues were found by Thomsson et al. [281] in respiratory mucins and eight new monosulphated structures have been identified in respiratory mucus from a patient suffering from chronic bronchitis [282]. Unusually, the sulphates appeared stable under MALDI conditions from DHB.

MALDI has been used to identify linear glycans with up to sixty monosaccharide residues attached to tyrosine as a novel attachment site for *O*-linked glycans in the crystalline surface layer glycoprotein from *Thermoanaerobacter thermohydrosulfuricus* LIII-69 [283] and to examine *O*-linked glycans from the hinge region of IgA_1 [284,285]. In the latter example, compounds were identified as tryptic peptides with terminal sialic acid and galactose residues removed enzymatically in order to produce fragments with masses low enough to be resolved.

7.4. Glycosaminoglycans

These highly sulphated glycans, such as heparin, consist of polymers of a uronic acid-glucosamine repeat unit in which the amino or hydroxyl groups contain various degrees of sulphation. Molecular weights can vary from 5 to over 40 kDa. These glycans are difficult to ionize by MALDI, as they tend to fragment extensively by loss of sulphate. They are normally examined as small oligomers following enzymatic digestion. Most of the successful MALDI experiments with these compounds have involved neutralization of the sulphates with natural [286] or synthetic [287,288] peptides and examination of the oligomers as the resulting ion pairs. Best results were obtained when the number of basic residues (usually arginine) equalled the number of sulphates with 3-hydroxypicolinic acid as the matrix. Even so, the larger compounds still tended to eliminate sulphate. The method was adopted by Ueoka et al. [289] to examine a condroitin-H-derived hexasaccharide from hagfish (*Eptatretus burgeri*) and by Shriver et al. [290] to study heparin decasaccharides; $(Arg\text{-}Gly)_{15}$ and $(Arg\text{-}Gly)_{19}$, respectively were used as the complexing agent in these two studies with caffeic acid (**XXXIX**) as the matrix. Only small amounts of sulphate were lost. With smaller fragments bearing few sulphate groups, more conventional methods can be used. Thus, both positive and negative ion MALDI spectra of chondroitin sulphate di- tetra- and hexa-saccharides have been obtained from DHB with only minimal loss of sulphate [291].

7.5. Sphingolipids

Sphingolipids can be examined as intact molecules from a variety of matrices (see above) and generally yield several peaks due to heterogeneity in the sphingosine or acyl chains. Such heterogeneity is often examined by techniques such as GC/MS [292,293] because fragmentation spectra tend to be dominated by ions from the sugars (Fig. 12). Nevertheless, weak lipid-derived ions are often present, particularly in the spectra of the smaller compounds. The most significant of these is that at *m*/*z* 264 derived from the sphingosine residue [294–296]. The ion is present in the fragmentation spectra of $[M + Na]^+$ ions and is interesting in that it is a protonated species derived from a sodiated precursor. CID was found by Perreault et al. [297] to give better results in this area than MALDI-PSD but a recent combination of a MALDI ion source with a Q-TOF mass spectrometer, although giving excellent CID spectra reflecting the glycan portion, produced only weak ions from the lipid [43].

MALDI is an excellent method for profiling mixtures of these compounds as well as the isolated ceramide residue. Both types of compound form $[M + Na]^+$ ions from DHB as illustrated by studies by Doering et al, [298] on glucosylceramides from mouse epidermis and by Fujiwaki et al. [299] on various sphingo- and glycosphingolipids in tissues from sphingolipidosis patients. With more complex glycolipids, containing a range of glycans or substituted glycans, this type of heterogeneity can be problematical. In order to overcome this problem for examination of only the glycan fragment, the entire ceramide can be removed enzymatically with endoglycoceramidase as illustrated by a study of large polyglycosylceramides with up to 41 sugar residues ionised from DHB by Karlsson et al. [300]. This enzyme produces glycans with an intact reducing terminus allowing them to be derivatised by reductive amination. This approach has been used to study cercarial glycolipids from *Schistosoma mansoni* [301]. The released, glycans were converted into 2-AP derivatives and profiled as positive ions from 6-aza-2-thiothymine. In a similar study, 2-AP labelled phosphorylcholine-substituted glycans from glycolipids extracted from the parasitic nematode, *Caenorhabditis elegans* were found to give strong positive ion MALDI spectra but also to exhibit considerable metastable loss of the phosphodiester groups [302]. A comparative study by electron-impact (EI) ionization, FAB and MALDI of permethylated glycosphingolipids from mouse kidney has given similar profiles although the quantitative data obtained

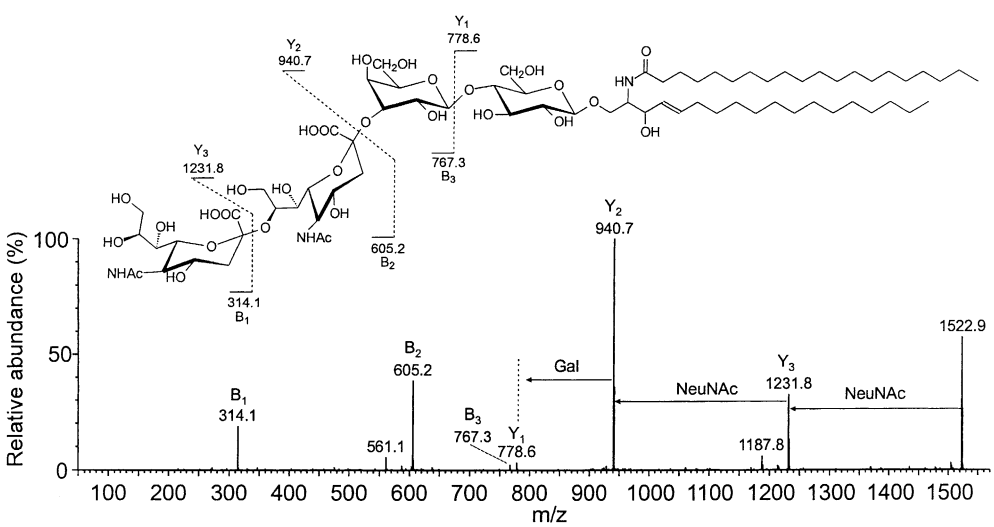


Fig. 12. Positive ion MALDI-CID spectrum of a the disialoganglioside GD3.

from FAB analysis deviated somewhat from that obtained by the other techniques [303]. The branching structure of the very large glycosphingolipids from human erythrocytes studied by Karlsson (above) have been further investigated by incubation with endo-β-galactosidase [304], an enzyme that cleaves 3Galβ1 → 4GlcNAc bond in linear but not branched poly-*N*-acetyllactosamine chains.

The presence of sialic acids in many of these compounds can cause problems because of its ready loss during MALDI ionization. However, permethylation [75] or methyl ester formation [141] stabilises the compounds. Permethylation was recently used by Nakamura et al. [305] in a study of tetra- to hexa-sialylated gangliosides from skate (*Bathyraja smirnovi*) brain; the compounds were ionized with DHB with little evidence of fragmentation.

Thin-layer chromatography (TLC) is the most common method for separating glycosphingolipids and MALDI spectra have been obtained directly from the TLC plate with DHB or a mixture of DHB and 2-amino-5-nitropyridine (XL) [306]. The method was one to two orders of magnitude more sensitive than analysis by liquid secondary ion mass spectrometry (LSIMS) but could be improved by heat-transfer of the glycolipids to a PVDF membrane attached with a thin film of adhesive to the MALDI probe.

7.6. Lipopolysaccharides (LPS) and derived glycans from bacteria

A considerable amount of work has been reported on the analysis of these compounds with MALDI playing an ever-increasing part. The field has recently been reviewed [307]. The various parts of the molecules are usually studied independently because of their size. The intact molecules are typically extracted using hot phenol and water, and purified by column chromatography or microcentrifugation. Mild acid hydrolysis is used to separate the lipid A portion, and hydrolysis with TFA can be used to isolate the carbohydrate repeat unit, when present.

These heterogeneous molecules are difficult to analyse by MALDI because of their phosphate groups and high hydrocarbon content. Nevertheless, MALDI spectra of intact core structures have been obtained from LPS from *Erwinia carotovora*, a plant pathogen that causes soft rot in vegetables, using 2,4,6-THAP as the matrix in negative ion mode [308] and from *Klebsiella pneumoniae* where L-glycero-D-mannoheptose was found [309]. A peak (negative ion) from intact LPS from *E. coli* O159 (25.0 kDa) was obtained by Linnerborg et al. from DHB [310]. LPS from *Haemophilus influenzae*, *H. ducreyi*, and *Salmonella typhimurium* have been ionized from 2,5-DHB containing 1-HIQ following removal of the *O*-linked fatty acids with hydrazine to render them water-soluble [311]. Deacylation was also used by Rund et al. [312] in a study of LPS from *Chlamydia trachomatis* with 2,4,6-THAP as the matrix for the negative ion spectrum. Both deacylation and dephosphorylation were employed by Olsthoorn et al. [313] to obtain a PSD spectrum from the LPS from a rough strain (lacking the *O*-specific chain) of *K. pneumoniae*. The spectrum of the undecasaccharide revealed the complete sequence.

Analysis of the lipid A portion of LPS is also difficult for the same reasons as those that apply to the intact molecules. Much of the earlier work [314–317] was performed with laser desorption mass spectrometry (without a matrix). Successful analyses have involved methylation of the phosphates with diazomethane [318] or deacylation of the esterified hydroxy fatty acids [319–322]. Nevertheless, spectra of the intact molecules have been reported. For example, Zhou et al. [323] obtained negative ion spectra from bis-phosphorylated species from DHB but noted some loss of aminodeoxypentose moieties that were attached via phosphate esters. Loss of phosphate groups was also noted by Aussel et al. [324] in the negative ion spectrum of lipid A from *Bordetella hinzii* ATCC 51730. One unique lipid A, from *Aquifex pyrophilus*, a bacterium that grows at 95 °C, has been found with D-galacturonic acid replacing the phosphates; the molecule gave a strong negative ion spectrum from DHB [325].

Analysis of the *O*-specific chains, on the other hand, does not present such serious problems. For example,

MALDI analysis from 2,5-DHB of the chain from several strains of *Hafnia alvei* [326–331] and *Plesiomonas shigelloides* [332] has revealed series of ions up to a mass of 16 kDa with repeat unit mass of around 1 kDa.

7.7. Other glycolipids

The first successful study of a lipoteichoic acid was recently reported by Roethlisberger et al. [333]. The molecule, from *Streptococcus*, contained a diacylglycerol-glucofuranose core attached to a string of from 7 to 17 glycerophosphates moieties. The MALDI spectrum was recorded from sinapinic acid.

Among other glycolipids to have been studied by MALDI are phenolic glycolipids [334] and lipoarabinomannan [335–337], from mycobacteria. The lipoarabinomannans are large molecules, but have given broad peaks with masses of up to 50 kDa from super-DHB [336]. Other molecules include glucose polymers esterified with a long-chain fatty acid from Alzheimer paired helical filaments [338], extracellular polysaccharides (EPS) from *E. chrysanthemi* [339], phosphoinositolmannosides containing long 1 → 2-linked chains [340], lipophosphoglycans from Leishmania [341] and 6′-*O*-(3″-phosphocholine-2″-amino-1″-phospho-1″,3″-propanediol)-α-D-glucopyranosyl-(1″ → 3)-1,2-giacylglycerol, found as a major polar lipid in the cell wall of *Mycoplasma fermentans* [342].

7.8. Glycated proteins

Protein glycation refers to the non-enzymatic reaction between sugars and proteins, and is of particular importance in the pathology of diabetes, where proteins can be exposed to high levels of circulating reducing sugars. The main reaction is between hexoses and the ϵ-amino group of lysine to form an Amadori complex. Various oxidations and dehydrations follow [343], leading eventually to protein cross-linking and tissue damage, which produce the well-characterized complications of the disease. MALDI has characterised glycation with glucose on bovine serum albumin (66.4 kDa, with 51 residues) [344,345], bovine pancreatic ribonuclease (four residues) [346], lysozyme [347], IgG [348], globins [349], haemoglobin [350], major intrinsic protein from calf lens [351] and advances glycation end-products derived from serum albumin [352]. Sinapinic acid was the best matrix for the majority of these assays although 4-HCCA was used for studies of the α- and β-chains of haemoglobin [353] and β-2-microglobulin [354], and to characterise products responsible for oxidative stress [355].

7.9. Other carbohydrate–protein complexes

The ability of MALDI to measure increases in molecular weight of proteins, when they are conjugated to other molecules, has been utilised by a number of investigators for studies of drug binding and to protein–protein interactions. For example, up to 24 molecules of lactose [356] and 15 molecules of steroidal alkaloid glycosides [357] have been found to bind to human serum albumin and loading values for conjugates to monoclonal antibodies measured by MALDI have been shown to parallel those values found by other techniques [358]. Up to 29 antigenic monosaccharides were found to be covalently attached to chicken serum albumin in an experiment aimed at the synthesis of new potential vaccines [359].

8. Applications of MALDI mass spectrometry to monitoring the products of chemical synthesis

The speed and convenience of MALDI has been used to advantage by several investigators to monitor products from synthetic reactions involving carbohydrates. Most reactions involve the use of glycosyl transferases. Recent examples include fucosylated biantennary glycans [360], large octa-antennary glycans [361], lacto-globo-type pentasaccharides [362], lacto-*N*-neotetraose [363] sialyl-Lewis a and x glycoconjugates [364,365], Lewis b hexasaccharide [366], carbamate-containing cyclodextrin analogues [367] and seven-membered cyclodextran rings with

two thioalkylglucopyranase groups attached to each glucose residue [368] and *Neisseria meningitides* pentasaccharide [369].

9. Conclusions

MALDI mass spectrometry has proved itself to be one of the most valuable techniques for the analysis of carbohydrates and their conjugates with proteins and lipids. Unlike other forms of mass spectrometry, it is capable of producing ions from underivatised molecules. The resulting spectra accurately reflect the composition of the sample and the technique can conveniently be combined with other methods such as HPLC, GC/MS or exoglycosidases digestion to provide more detailed analysis of carbohydrate structure. With instrumentation introduced in the last few years, high quality fragmentation spectra can now be obtained, offering the possibility of more complete analysis by mass spectrometry alone. Over the next few years, MALDI is expected to make an even greater contribution, not only to the analysis of basic carbohydrates, but also to ever larger and more complicated molecules incorporating these complex and structurally diverse compounds.

Acknowledgements

I thank Professor Raymond Dwek, Director of the Oxford Glycobiology Institute for his help and encouragement.

References

[1] R.A. Laine, Glycobiology 4 (1994) 759.
[2] M. Barber, R.S. Bordoli, R.D. Sedgwick, A.N. Tyler, J. Chem. Soc., Chem. Commun. (1981) 325.
[3] M. Karas, D. Bachmann, U. Bahr, F. Hillenkamp, Int. J. Mass Spectrom. Ion Process. 78 (1987) 53.
[4] M. Karas, F. Hillenkamp, Anal. Chem. 60 (1988) 2299.
[5] B. Stahl, M. Steup, M. Karas, F. Hillenkamp, Anal. Chem. 63 (1991) 1463.
[6] U. Bahr, M. Karas, F. Hillenkamp, Fresenius J. Anal. Chem. 348 (1994) 783.
[7] D.J. Harvey, J. Chromatogr. A 720 (1996) 429.
[8] D.J. Harvey, Mass Spectrom. Rev. 18 (1999) 349.
[9] D.J. Harvey, B. Küster, T.J.P. Naven, Glycoconjugate J. 15 (1998) 333.
[10] D. Garozzo, NATO ASI Ser. C 504 (1997) 477.
[11] K.K. Mock, M. Davy, J.S. Cottrell, Biochem. Biophys. Res. Commun. 177 (1991) 644.
[12] M.C. Huberty, J.E. Vath, W. Yu, S.A. Martin, Anal. Chem. 65 (1993) 2791.
[13] D. Garozzo, E. Spina, L. Sturiale, G. Montaudo, R. Rizzo, Rapid Commun. Mass Spectrom. 8 (1994) 358.
[14] X. Shen, H. Perreault, J. Chromatogr. A 811 (1998) 47.
[15] A. Tsarbopoulos, M. Karas, K. Strupat, B.N. Pramanik, T.L. Nagabhushan, F. Hillenkamp, Anal. Chem. 66 (1994) 2062.
[16] J.S. Cottrell, Org. Mass Spectrom. 27 (1992) 343.
[17] R.S. Bordoli, K. Howes, R.G. Vickers, R.H. Bateman, D.J. Harvey, Rapid Commun. Mass Spectrom. 8 (1994) 585.
[18] R.S. Bordoli, K. Howes, R.G. Vickers, R.H. Bateman, D.J. Harvey, NATO ASI Ser. C 475 (1996) 51.
[19] V.S.K. Kolli, R. Orlando, Rapid Commun. Mass Spectrom. 10 (1996) 923.
[20] V.S.K. Kolli, R. Orlando, Anal. Chem. 69 (1997) 327.
[21] D.J. Harvey, A.P. Hunter, Rapid Commun. Mass Spectrom. 12 (1998) 1721.
[22] D.J. Harvey, A.P. Hunter, Adv. Mass Spectrom. 14 (1998) BO6 TUP0083.
[23] J.A. Carroll, S.G. Penn, S.T. Fannin, J. Wu, M.T. Cancilla, M.K. Green, C.B. Lebrilla, Anal. Chem. 68 (1996) 1798.
[24] M.L. Vestal, P. Juhasz, S.A. Martin, Rapid Commun. Mass Spectrom. 9 (1995) 1044.
[25] V.M. Doroshenko, T.J. Cornish, R.J. Cotter, Rapid Commun. Mass Spectrom. 6 (1992) 753.
[26] K.A. Cox, J.D. Williams, R.G. Cooks, R.E. Kaiser, Biol. Mass Spectrom. 21 (1992) 226.
[27] D.M. Chambers, D.E. Goeringer, S.A. McLuckey, G.A. Glish, Anal. Chem. 65 (1993) 14.
[28] V.M. Doroshenko, R.J. Cotter, Rapid Commun. Mass Spectrom. 7 (1993) 822.
[29] K. Jonscher, G. Currie, A.L. McCormack, J.R. Yates III, Rapid Commun. Mass Spectrom. 7 (1993) 20.
[30] J.C. Schwartz, M.E. Bier, Rapid Commun. Mass Spectrom. 7 (1993) 27.
[31] V.M. Doroshenko, R.J. Cotter, Anal. Chem. 67 (1995) 2180.
[32] V.M. Doroshenko, R.J. Cotter, Rapid Commun. Mass Spectrom. 10 (1996) 65.
[33] V.M. Doroshenko, R.J. Cotter, Anal. Chem. 68 (1996) 463.
[34] V.M. Doroshenko, R.J. Cotter, J. Mass Spectrom. 32 (1997) 602.
[35] J. Qin, B.T. Chait, Anal. Chem. 69 (1997) 4002.
[36] J. Qin, R.J.J.M. Steenvoorden, B.T. Chait, Anal. Chem. 68 (1996) 1784.
[37] A.N. Krutchinsky, A.V. Loboda, V.L. Spicer, R. Dworschak, W. Ens, K.G. Standing, Rapid Commun. Mass Spectrom. 12 (1998) 508.
[38] A. Shevchenko, A. Loboda, A. Shevchenko, W. Ens, K.G. Standing, Anal. Chem. 72 (2000) 2132.

[39] A.V. Loboda, A.N. Krutchinsky, M. Bromirski, W. Ens, K.G. Standing, Rapid Commun. Mass Spectrom. 14 (2000) 1047.
[40] P. Verhaert, S. Uttenweiler-Joseph, M. de Vries, A. Loboda, W. Ens, K.G. Standing, Proteomics 1 (2001) 118.
[41] T.J. Griffin, S.P. Gygi, B. Rist, R. Aebersold, A. Loboda, A. Jilkine, W. Ens, K.G. Standing, Anal. Chem. 73 (2001) 978.
[42] D.J. Harvey, R.H. Bateman, R.S. Bordoli, R. Tyldesley, Rapid Commun. Mass Spectrom. 14 (2000) 2135.
[43] V. Hunnam, D.J. Harvey, D.A. Priestman, R.H. Bateman, R.S. Bordoli, R. Tyldesley, J. Am. Soc. Mass Spectrom. 12 (2001) 1220.
[44] V.V. Laiko, M.A. Baldwin, A.L. Burlingame, Anal. Chem. 72 (2000) 652.
[45] C.S. Creaser, J.C. Reynolds, D.J. Harvey, Rapid Commun. Mass Spectrom. 16 (2002) 176.
[46] M. Kussmann, E. Nordhoff, H. Rehbek-Nielsen, S. Haebel, M. Rossel-Larsen, L. Jakobsen, J. Gobom, E. Mirgorodskaya, A. Kroll-Kristensen, L. Palm, P. Roepstorff, J. Mass Spectrom. 32 (1997) 593.
[47] R.C. Beavis, B.T. Chait, Rapid Commun. Mass Spectrom. 3 (1989) 432.
[48] P. Juhasz, C.E. Costello, K. Biemann, J. Am. Soc. Mass Spectrom. 4 (1993) 399.
[49] J.J. Pitt, J.J. Gormon, Rapid Commun. Mass Spectrom. 10 (1996) 1786.
[50] C. Sottani, M. Fiorentino, C. Minoia, Rapid Commun. Mass Spectrom. 11 (1997) 907.
[51] K. Strupat, M. Karas, F. Hillenkamp, Int. J. Mass Spectrom. Ion Process. 111 (1991) 89.
[52] A. Mele, L. Malpezzi, J. Am. Soc. Mass Spectrom. 11 (1999) 228.
[53] M. Karas, H. Ehring, E. Nordhoff, B. Stahl, K. Strupat, F. Hillenkamp, M. Grehl, B. Krebs, Org. Mass Spectrom. 28 (1993) 1476.
[54] M.D. Mohr, K.O. Börnsen, H.M. Widmer, Rapid Commun. Mass Spectrom. 9 (1995) 809.
[55] Y. Mechref, M.V. Novotny, J. Am. Soc. Mass Spectrom. 9 (1998) 1292.
[56] A.I. Gusev, W.R. Wilkinson, A. Proctor, D.M. Hercules, Anal. Chem. 67 (1995) 1034.
[57] C. Köster, J.A. Castoro, C.L. Wilkins, J. Am. Chem. Soc. 114 (1992) 7572.
[58] D.J. Harvey, Rapid Commun. Mass Spectrom. 7 (1993) 614.
[59] J. Krause, M. Stoeckli, U.P. Schlunegger, Rapid Commun. Mass Spectrom. 10 (1996) 1927.
[60] V. Horneffer, K. Dreiswerd, H.-C. Lüdemann, F. Hillenkamp, M. Läge, K. Strupat, Int. J. Mass Spectrom. 185/186/187 (1999) 859.
[61] J.O. Metzger, R. Woisch, W. Tuszynski, R. Angermann, Fresenius J. Anal. Chem. 349 (1994) 473.
[62] B. Stahl, S. Thurl, J. Zeng, M. Karas, F. Hillenkamp, M. Steup, G. Sawatzki, Anal. Biochem. 223 (1994) 218.
[63] D.I. Papac, A. Wong, A.J.S. Jones, Anal. Chem. 68 (1996) 3215.
[64] B. Stahl, A. Linos, M. Karas, F. Hillenkamp, M. Steup, Anal. Biochem. 246 (1997) 195.
[65] P. Zöllner, E.R. Schmid, G. Allmaier, Rapid Commun. Mass Spectrom. 10 (1996) 1278.
[66] P. Zöllner, G. Stübiger, E. Schmid, E. Pittenauer, G. Allmaier, Int. J. Mass Spectrom. Ion Process. 169/170 (1997) 99.
[67] T.L. Williams, C. Fenselau, Eur. Mass Spectrom. 4 (1998) 379.
[68] Y.F. Zhu, N.I. Taranenko, S.L. Allman, S.A. Martin, L. Haff, C.H. Chen, Rapid Commun. Mass Spectrom. 10 (1996) 1591.
[69] H. Nonami, K. Tanaka, Y. Fukuyama, R. Erra-Balsells, Rapid Commun. Mass Spectrom. 12 (1998) 285.
[70] C. Hao, X. Ma, S. Fang, Z. Liu, S. Liu, F. Song, J. Liu, Rapid Commun. Mass Spectrom. 12 (1998) 345.
[71] P. Chen, A.G. Baker, M.V. Novotny, Anal. Biochem. 244 (1997) 144.
[72] S.F. Wheeler, D.J. Harvey, Anal. Chem. 72 (2000) 5027.
[73] S.F. Wheeler, D.J. Harvey, Anal. Biochem. 296 (2001) 92.
[74] N. Xu, Z.-H. Huang, J.T. Watson, D.A. Gage, J. Am. Soc. Mass Spectrom. 8 (1997) 116.
[75] P. Juhasz, C.E. Costello, J. Am. Soc. Mass Spectrom. 3 (1992) 785.
[76] Y. Dai, R.M. Whittal, C.A. Bridges, Y. Isogai, O. Hindsgaul, L. Li, Carbohydr. Res. 304 (1997) 1.
[77] K.O. Börnsen, M.D. Mohr, H.M. Widmer, Rapid Commun. Mass Spectrom. 9 (1995) 1031.
[78] M.T. Cancilla, S.G. Penn, J.A. Carroll, C.B. Lebrilla, J. Am. Chem. Soc. 118 (1996) 6736.
[79] C.K.L. Wong, T.-W.D. Chan, Rapid Commun. Mass Spectrom. 11 (1997) 513.
[80] A.W. Wong, M.T. Cancilla, L.R. Voss, C.B. Lebrilla, Anal. Chem. 71 (1999) 205.
[81] D.J. Harvey, J. Mass Spectrom. 30 (1995) 1311.
[82] E. Sugiyama, A. Hara, K.-I. Uemura, T. Taketomi, Glycobiology 7 (1997) 719.
[83] S.G. Penn, M.T. Cancilla, M.K. Green, C.B. Lebrilla, Eur. Mass Spectrom. 3 (1997) 67.
[84] J.J. Pitt, J.J. Gormon, Anal. Biochem. 248 (1997) 63.
[85] H. Bartsch, W.A. König, M. Strassner, U. Hintze, Carbohydr. Res. 286 (1996) 41.
[86] D. Garozzo, G. Impallomeni, E. Spina, L. Sturiale, F. Zanetti, Rapid Commun. Mass Spectrom. 9 (1995) 937.
[87] P.M. Rudd, I.G. Scragg, E. Coghill, R.A. Dwek, Glycoconjugate J. 9 (1992) 86.
[88] T.J.P. Naven, D.J. Harvey, Rapid Commun. Mass Spectrom. 10 (1996) 1361.
[89] T. Kazmaier, S. Roth, J. Zapp, M. Harding, R. Kuhn, Fresenius J. Anal. Chem. 361 (1998) 473.
[90] P.K. Chan, T.-W.D. Chan, Rapid Commun. Mass Spectrom. 14 (2000) 1841.
[91] M. Field, D. Papac, A. Jones, Anal. Biochem. 239 (1996) 92.
[92] F. Dubois, R. Knochenmuss, R.J.J.M. Steenvoorden, K. Breuker, R. Zenobi, Eur. Mass Spectrom. 2 (1996) 167.
[93] R.C. Beavis, B.T. Chait, Anal. Chem. 62 (1990) 1836.
[94] K.K. Mock, C.W. Sutton, J.S. Cottrell, Rapid Commun. Mass Spectrom. 6 (1992) 233.

[95] J.-M. Strub, Y. Goumon, K. Lugardon, C. Capon, M. Lopez, M. Moniatte, A. Van Dorsselaer, D. Aunis, M.-H. Metz-Boutigue, J. Biol. Chem. 271 (1996) 28533.
[96] R.M. Whittal, M.M. Palcic, O. Hindsgaul, L. Li, Anal. Chem. 67 (1995) 3509.
[97] R. Marusyk, A. Sergeant, Anal. Biochem. 105 (1980) 403.
[98] H. Görisch, Anal. Biochem. 173 (1988) 393.
[99] D.J. Harvey, B. Küster, S.F. Wheeler, A.P. Hunter, R.H. Bateman, R.A. Dwek, in: A.L. Burlingame, S.A. Carr, M.A. Baldwin (Eds.), Mass Spectrometry in Biology and Medicine, Humana Press, Totowa, 2000, p. 403.
[100] B. Küster, S.F. Wheeler, A.P. Hunter, R.A. Dwek, D.J. Harvey, Anal. Biochem. 250 (1997) 82.
[101] J. Charlwood, H. Birrell, P. Camilleri, J. Chromatogr. B 734 (1999) 169.
[102] N.H. Packer, M.A. Lawson, D.R. Jardine, J.W. Redmond, Glycoconjugate J. 15 (1998) 737.
[103] J.C. Rouse, J.E. Vath, Anal. Biochem. 238 (1996) 82.
[104] Y. Huang, Y. Mechref, J. Tian, H. Gong, W.J. Lennarz, M.V. Novotny, Rapid Commun. Mass Spectrom. 14 (2000) 1233.
[105] H. Wang, K. Tseng, C.B. Lebrilla, Anal. Chem. 71 (1999) 2014.
[106] J. Bundy, C. Fenselau, Anal. Chem. 71 (1999) 1460.
[107] T.A. Worrall, R.J. Cotter, A.S. Woods, J. Mass Spectrom. 35 (2000) 647.
[108] A. Jacobs, O. Dahlman, Anal. Chem. 73 (2001) 405.
[109] S. Hakomori, J. Biochem. (Tokyo) 55 (1964) 205.
[110] I. Ciucanu, F. Kerek, Carbohydrate Res. 131 (1984) 209.
[111] H. Perreault, C.E. Costello, Org. Mass Spectrom. 29 (1994) 720.
[112] H. Perreault, C.E. Costello, J. Mass Spectrom. 34 (1999) 184.
[113] P. Chen, U. Werner-Zwanziger, D. Wiesler, M. Pagel, M.V. Novotny, Anal. Chem. 71 (1999) 4969.
[114] S. Hase, J. Chromatogr. A 720 (1996) 173.
[115] T. Takao, Y. Tambara, A. Nakamura, K.-I. Yoshino, H. Fukuda, M. Fukuda, Y. Shimonishi, Rapid Commun. Mass Spectrom. 10 (1996) 637.
[116] M. Okamoto, K. Takahashi, T. Doi, Y. Takimoto, Anal. Chem. 69 (1997) 2919.
[117] G. Okafo, L. Burrow, S.A. Carr, G.D. Roberts, W. Johnson, P. Camilleri, Anal. Chem. 68 (1996) 2230.
[118] G. Okafo, J. Langridge, S. North, A. Organ, A. West, M. Morris, P. Camilleri, Anal. Chem. 69 (1997) 4985.
[119] S. North, G. Okafo, H. Birrell, N. Haskins, P. Camilleri, Rapid Commun. Mass Spectrom. 11 (1997) 1635.
[120] S. North, H. Birrell, P. Camilleri, Rapid Commun. Mass Spectrom. 12 (1998) 349.
[121] J. Charlwood, H. Birrell, A. Gribble, V. Burdes, D. Tolson, P. Camilleri, Anal. Chem. 72 (2000) 1453.
[122] J.C. Bigge, T.P. Patel, J.A. Bruce, P.N. Goulding, S.M. Charles, R.B. Parekh, Anal. Biochem. 230 (1995) 229.
[123] T.J.P. Naven, D.J. Harvey, Rapid Commun. Mass Spectrom. 10 (1996) 829.
[124] S. Broberg, A. Broberg, J.Ø. Duus, Rapid Commun. Mass Spectrom. 14 (2000) 1801.
[125] K.R. Anumula, S.T. Dhume, Glycobiology 8 (1998) 685.
[126] J. Lemoine, M. Cabanes-Macheteau, M. Bardor, J.-C. Michalski, L. Faye, P. Lerouge, Rapid Commun. Mass Spectrom. 14 (2000) 100.
[127] H. Suzuki, O. Müller, A. Guttman, B.L. Karger, Anal. Chem. 69 (1997) 4554.
[128] Y. Zhao, S.B.H. Kent, B.T. Chait, Proc. Natl. Acad. Sci. U.S.A. 94 (1997) 1629.
[129] T. Tamura, M.S. Wadhwa, K.G. Rice, Anal. Biochem. 216 (1994) 335.
[130] Y.H. Ahn, J.S. Yoo, Rapid Commun. Mass Spectrom. 13 (1999) 1985.
[131] V.N. Reinhold, B.B. Reinhold, C.E. Costello, Anal. Chem. 67 (1995) 1772.
[132] M.T. Cancilla, A.W. Wong, L.R. Voss, C.B. Lebrilla, Anal. Chem. 71 (1999) 3206.
[133] B. Domon, C.E. Costello, Glycoconjugate J. 5 (1988) 397.
[134] R. Orlando, C.A. Bush, C. Fenselau, Biomed. Environ. Mass Spectrom. 19 (1990) 747.
[135] L.C. Ngoka, J.-F. Gal, C.B. Lebrilla, Anal. Chem. 66 (1994) 692.
[136] T.J.P. Naven, D.J. Harvey, J. Brown, G. Critchley, Rapid Commun. Mass Spectrom. 11 (1997) 1681.
[137] D.J. Harvey, T.J.P. Naven, B. Küster, R.H. Bateman, M.R. Green, G. Critchley, Rapid Commun. Mass Spectrom. 9 (1995) 1556.
[138] E. Mortz, T. Sareneva, I. Julkunen, P. Roepstorff, J. Mass Spectrom. 31 (1996) 1109.
[139] G. Talbo, M. Mann, Rapid Commun. Mass Spectrom. 10 (1996) 100.
[140] Z. Szilágyi, J.E. Varney, P.J. Derrick, K. Vékey, Rapid Commun. Mass Spectrom. 12 (1998) 489.
[141] A.K. Powell, D.J. Harvey, Rapid Commun. Mass Spectrom. 10 (1996) 1027.
[142] P.B. O'Connor, C.E. Costello, Rapid Commun. Mass Spectrom. 15 (2001) 1862.
[143] P.B. O'Connor, E. Mirgorodskaya, C.E. Costello, J. Am. Soc. Mass Spectrom. 13 (2002) 402.
[144] A. Tsarbopoulos, U. Bahr, B.N. Pramanik, M. Karas, Int. J. Mass Spectrom. Ion Process. 169/170 (1997) 251.
[145] W.J. Mo, T. Takao, H. Sakamoto, Y. Shimonishi, Anal. Chem. 70 (1998) 4520.
[146] D.J. Harvey, A.P. Hunter, R.H. Bateman, J. Brown, G. Critchley, Int. J. Mass Spectrom. Ion Process. 188 (1999) 131.
[147] T. Yamagaki, H. Nakanishi, Rapid Commun. Mass Spectrom. 13 (1999) 2199.
[148] R. Kaufmann, P. Chaurand, D. Kirsch, B. Spengler, Rapid Commun. Mass Spectrom. 10 (1996) 1199.
[149] T.J. Cornish, R.J. Cotter, Rapid Commun. Mass Spectrom. 7 (1993) 1037.
[150] T.J. Cornish, R.J. Cotter, Rapid Commun. Mass Spectrom. 8 (1994) 781.
[151] T. Yamagaki, H. Nakanishi, J. Mass Spectrom. 35 (2000) 1300.
[152] N. Xu, Z.-H. Huang, B.L.M. de Jonge, D.A. Gage, Anal. Biochem. 248 (1997) 7.

[153] S. Lopaticki, C.J. Morrow, J.J. Gorman, J. Mass Spectrom. 33 (1998) 950.
[154] J.C. Rouse, A.-M. Strang, W. Yu, J.E. Vath, Anal. Biochem. 256 (1998) 33.
[155] F. Zal, B. Küster, B.N. Green, D.J. Harvey, F.H. Lallier, Glycobiology 8 (1998) 663.
[156] T. Pfeifer, M. Drewello, A. Schierhorn, J. Mass Spectrom. 34 (1999) 644.
[157] R.D. Burton, C.H. Watson, J.R. Eyler, G.L. Lang, D.H. Powell, M.Y. Avery, Rapid Commun. Mass Spectrom. 11 (1997) 443.
[158] T.J.D. Jorgensen, G. Bojesen, H. Rahbek-Nielsen, Eur. Mass Spectrom. 4 (1998) 39.
[159] S.-L. Wu, Anal. Biochem. 253 (1997) 85.
[160] A. Pfenninger, M. Karas, B. Finke, B. Stahl, G. Sawatzki, J. Mass Spectrom. 34 (1999) 98.
[161] W. Mo, H. Sakamoto, A. Nishikawa, N. Kagi, J.I. Langridge, Y. Shimonishi, T. Takao, Anal. Chem. 71 (1999) 4100.
[162] B. Spengler, D. Kirsch, R. Kaufmann, J. Lemoine, J. Mass Spectrom. 30 (1995) 782.
[163] Y. Mechref, A.G. Baker, M.V. Novotny, Carbohydr. Res. 313 (1998) 145.
[164] E. Spina, R. Cozzolino, E. Ryan, D. Garozzo, J. Mass Spectrom. 35 (2000) 1042.
[165] J. Lemoine, F. Chirat, B. Domon, J. Mass Spectrom. 31 (1996) 908.
[166] G.E. Hofmeister, Z. Zhou, J.A. Leary, J. Am. Chem. Soc. 113 (1991) 5964.
[167] N. Viseux, C.E. Costello, B. Domon, J. Mass Spectrom. 34 (1999) 364.
[168] H. Egge, J. Peter-Katalinic, Mass Spectrom. Rev. 6 (1987) 331.
[169] A. Dell, J.E. Thomas-Oates, in: C.J. Biermann, G.D. McGinnis (Eds.), Analysis of Carbohydrates by GLC and MS, CRC Press, Boca Raton, 1989, p. 217.
[170] B. Domon, D.R. Müller, W.J. Richter, Biomed. Environ. Mass Spectrom. 19 (1990) 390.
[171] R.A. Laine, E. Yoon, T.J. Mahier, S. Abbas, B. de Lappe, R. Jain, K. Matta, Biol. Mass Spectrom. 20 (1991) 505.
[172] D. Garozzo, G. Impallomeni, G. Montaudo, E. Spina, Rapid Commun. Mass Spectrom. 6 (1992) 550.
[173] D.J. Harvey, R.H. Bateman, M.R. Green, J. Mass Spectrom. 32 (1997) 167.
[174] D.J. Harvey, D.R. Wing, B. Küster, I.M. Wilson, J. Am. Soc. Mass Spectrom. 11 (2000) 572.
[175] Y. Sato, M. Suzuki, T. Nirasawa, A. Suzuki, T. Endo, Anal. Chem. 72 (2000) 1207.
[176] Y. Mizuno, T. Sasagawa, N. Dohmae, K. Takio, Anal. Chem. 71 (1999) 4764.
[177] D.J. Harvey, J. Mass Spectrom. 35 (2000) 1178.
[178] D.J. Harvey, Analyst 125 (2000) 609.
[179] D.J. Harvey, J. Am. Soc. Mass Spectrom. 11 (2000) 900.
[180] E. Clayton, R.H. Bateman, Rapid Commun. Mass Spectrom. 6 (1992) 719.
[181] B. Küster, T.J.P. Naven, D.J. Harvey, Rapid Commun. Mass Spectrom. 10 (1996) 1645.
[182] V. Kovácik, J. Hirsch, P. Kovác, W. Heerma, J. Thomas-Oates, J. Haverkamp, J. Mass Spectrom. 30 (1995) 949.
[183] L.P. Brüll, W. Heerma, J. Thomas-Oates, J. Haverkamp, V. Kovácik, P. Kovác, J. Am. Soc. Mass Spectrom. 8 (1997) 43.
[184] B.M. Warrack, M.E. Hail, A. Triolo, F. Animati, R. Seraglia, P. Traldi, J. Am. Soc. Mass Spectrom. 9 (1998) 710.
[185] Y.-L. Ma, I. Vedernikova, H. Van den Heuvel, M. Claeys, J. Am. Soc. Mass Spectrom. 11 (2000) 136.
[186] L.P. Brüll, V. Kovácik, J.E. Thomas-Oates, W. Heerma, J. Haverkamp, Rapid Commun. Mass Spectrom. 12 (1998) 1520.
[187] T.S. Mattu, L. Royle, J. Langridge, M.R. Wormald, P.E. Van den Steen, J. Van Damme, G. Opdenakker, D.J. Harvey, R.A. Dwek, P.M. Rudd, Biochemistry 39 (2000) 15695.
[188] T. Yamagaki, M. Maeda, K. Kanazawa, Y. Ishizuka, H. Nakanishi, Biosci. Biotechnol. Biochem. 60 (1996) 1222.
[189] W.S. York, V.S.K. Kolli, R. Orlando, P. Albersheim, A.G. Darvill, Carbohydr. Res. 285 (1996) 99.
[190] A. Rydlund, O. Dahlman, Carbohydr. Res. 300 (1997) 95.
[191] W.S. York, G. Impallomeni, M. Hisamatsu, P. Albersheim, A.G. Darvill, Carbohydr. Res. 267 (1995) 79.
[192] P.J.H. Daas, P.W. Arisz, H.A. Schols, G.A. De Ruiter, A.G.J. Voragen, Anal. Biochem. 257 (1998) 195.
[193] R. Körner, G. Limberg, J.D. Mikkelsen, P. Roepstorff, J. Mass Spectrom. 33 (1998) 836.
[194] G.-J.W.M. van Alebeek, O. Zabotina, G. Beldman, H.A. Schols, A.G.J. Voragen, J. Mass Spectrom. 35 (2000) 831.
[195] E. Vinogradov, K. Bock, Carbohydr. Res. 309 (1998) 57.
[196] B. Finke, B. Stahl, A. Pfenninger, M. Karas, H. Daniel, G. Sawatzki, Anal. Chem. 71 (1999) 3755.
[197] B. Finke, M. Mank, H. Daniel, B. Stahl, Anal. Biochem. 284 (2000) 256.
[198] A. Klein, A. Lebreton, J. Lemoine, J.M. Perini, P. Roussel, J.C. Michalski, Clin. Chem. 44 (1998) 2422.
[199] B. Chankvetadze, G. Endresz, G. Blaschke, M. Juza, H. Jakubetz, V. Schurig, Carbohydr. Res. 287 (1996) 139.
[200] K. Linnemayr, A. Rizzi, G. Allmaier, J. Chromatogr. A 791 (1997) 299.
[201] T. Yamagaki, Y. Ishizuka, S.-I. Kawabata, H. Nakanishi, Rapid Commun. Mass Spectrom. 10 (1996) 1887.
[202] J.P. Mercier, J.L. Debrun, M. Dreux, C. Elfakir, B. Hakim, Rapid Commun. Mass Spectrom. 14 (2000) 68.
[203] P.J.H. Daas, H.A. Schols, H.H.J. de Jongh, Carbohydr. Res. 329 (2000) 609.
[204] A. Dell, H.R. Morris, Science 291 (2001) 2351.
[205] Y. Wada, J. Gu, N. Okamoto, K. Inui, Biol. Mass Spectrom. 23 (1994) 108.
[206] K. Yamashita, T. Ohkura, H. Ideo, K. Ohno, M. Kanai, J. Biochem. (Tokyo) 114 (1993) 766.
[207] Y. Yang, R. Orlando, Rapid Commun. Mass Spectrom. 10 (1996) 932.
[208] J. Zaia, R.E. Boynton, D.R. Marshak, H. Olsson, D. Heinegard, F.P. Barry, J. Biol. Chem. 272 (1997) 14120.
[209] P. Juhasz, S.A. Martin, Int. J. Mass Spectrom. Ion Process. 169/170 (1997) 217.

[210] S.A. Carr, J.R. Barr, G.D. Roberts, K.R. Anumula, P.B. Taylor, Methods Enzymol. 193 (1990) 501.
[211] M.J. Huddleston, M.F. Bean, S.A. Carr, Anal. Chem. 65 (1993) 877.
[212] S.A. Carr, M.J. Huddleston, M.F. Bean, Protein Sci. 2 (1993) 183.
[213] A.P. Hunter, D.E. Games, Rapid Commun. Mass Spectrom. 9 (1995) 42.
[214] J.P. Liu, K.J. Volk, E.H. Kerns, S.E. Klohr, M.S. Lee, I.E. Rosenberg, J. Chromatogr. 632 (1993) 45.
[215] P. Ferranti, P. Pucci, G. Marino, I. Fiume, B. Terrana, C. Ceccarini, A. Malorni, J. Mass Spectrom. 30 (1995) 632.
[216] J. Gonzalez, T. Takao, H. Hori, V. Besada, R. Rodriguez, G. Padron, Y. Shimonishi, Anal. Biochem. 205 (1992) 151.
[217] B. Küster, M. Mann, Anal. Chem. 71 (1999) 1431.
[218] K.D. Greis, B.K. Hayes, F.I. Comer, M. Kirk, S. Barnes, T.L. Lowary, G.W. Hart, Anal. Biochem. 234 (1996) 38.
[219] G.J. Rademaker, J. Haverkamp, J. Thomas-Oates, Org. Mass Spectrom. 28 (1993) 1536.
[220] S. Müller, S. Goletz, N. Packer, A. Gooley, A.M. Lawson, F.-G. Hanisch, J. Biol. Chem. 272 (1997) 24780.
[221] K. Alving, R. Körner, H. Paulsen, J. Peter-Katalinic, J. Mass Spectrom. 33 (1998) 1124.
[222] F.G. Hanisch, M. Jovanovic, J. Peter-Katalinic, Anal. Biochem. 290 (2001) 47.
[223] S. Takasaki, T. Misuochi, A. Kobata, Methods Enzymol. 83 (1982) 263.
[224] T. Patel, J. Bruce, A. Merry, C. Bigge, M. Wormald, A. Jaques, R. Parekh, Biochemistry 32 (1993) 679.
[225] B. Bendiac, D.A. Cumming, Carbohydr. Res. 144 (1985) 1.
[226] J. Costa, D.A. Ashford, M. Nimtz, I. Bento, C. Frazao, C.L. Esteves, C.J. Faro, J. Kervinen, E. Pires, P. Verissimo, A. Wlodawer, M.A. Carrondo, Eur. J. Biochem. 243 (1997) 695.
[227] A.L. Tarentino, C.M. Gómez, T.H. Plummer Jr., Biochemistry 24 (1985) 4665.
[228] B. Küster, D.J. Harvey, Glycobiology 7 (1997) vii.
[229] V. Tretter, F. Altmann, L. März, Eur. J. Biochem. 199 (1991) 647.
[230] I.B.H. Wilson, R. Zeleny, D. Kolarich, E. Staudacher, C.J.M. Stroop, J.P. Kamerling, F. Altmann, Glycobiology 11 (2001) 261.
[231] C.J. Edge, T.W. Rademacher, M.R. Wormald, R.B. Parekh, T.D. Butters, D.R. Wing, R.A. Dwek, Proc. Natl. Acad. Sci. U.S.A. 89 (1992) 6338.
[232] J. Montreuil, S. Bouquelet, H. Debray, B. Fournet, G. Spik, G. Strecker, in: M.F. Chaplin, J.F. Kennedy (Eds.), Carbohydrate Analysis: A Practical Approach, IRL Press: Oxford, 1986, p. 143.
[233] R.A. Dwek, C.J. Edge, D.J. Harvey, M.R. Wormald, R.B. Parekh, Annu. Rev. Biochem. 62 (1993) 65.
[234] B. Küster, T.N. Krogh, E. Mortz, D.J. Harvey, Proteomics 1 (2001) 350.
[235] C.W. Sutton, J.A. O'Neill, J.S. Cottrell, Anal. Biochem. 218 (1994) 34.
[236] G.D. Roberts, W.P. Johnson, S. Burman, K.R. Anumula, S.A. Carr, Anal. Chem. 67 (1995) 3613.
[237] R.G. Miele, F.J. Castellino, R.K. Bretthauer, Biotechnol. Appl. Biochem. 26 (1997) 79.
[238] D.J. Harvey, P.M. Rudd, R.H. Bateman, R.S. Bordoli, K. Howes, J.B. Hoyes, R.G. Vickers, Org. Mass Spectrom. 29 (1994) 753.
[239] B. Küster, T.J.P. Naven, D.J. Harvey, J. Mass Spectrom. 31 (1996) 1131.
[240] H. Geyer, S. Schmitt, M. Wuhrer, R. Geyer, Anal. Chem. 71 (1999) 476.
[241] J. Colangelo, R. Orlando, Anal. Chem. 71 (1999) 1479.
[242] Y. Mechref, M.V. Novotny, Anal. Chem. 70 (1998) 455.
[243] B. Küster, A.P. Hunter, S.F. Wheeler, R.A. Dwek, D.J. Harvey, Electrophoresis 19 (1998) 1950.
[244] D. Kolarich, F. Altmann, Anal. Biochem. 285 (2000) 64.
[245] J. Charlwood, J.M. Skehel, P. Camilleri, Anal. Biochem. 284 (2000) 49.
[246] D.I. Papac, J.B. Briggs, E.T. Chin, A.J.S. Jones, Glycobiology 8 (1998) 445.
[247] N. Callewaert, S. Geysens, F. Molemans, R. Contreras, Glycobiology 11 (2001) 275.
[248] K. Kakehi, M. Kinoshita, D. Kawakami, J. Tanaka, K. Sei, K. Endo, Y. Oda, M. Iwaki, T. Masuko, Anal. Chem. 73 (2001) 2640.
[249] H.L. Byers, E. Tarelli, K.A. Homer, D. Beighton, Glycobiology 9 (1999) 469.
[250] M. Nimtz, E. Grabenhorst, H.S. Conradt, L. Sanz, J.J. Calvete, Eur. J. Biochem. 265 (1999) 703.
[251] C. Fini, A. Amoresano, A. Andolfo, S. D'Auria, A. Floridi, S. Paolini, P. Pucci, Eur. J. Biochem. 267 (2000) 4978.
[252] C. Kannicht, L. Lucks, R. Nuck, W. Reutter, M. Gohlke, Glycobiology 9 (1999) 897.
[253] T.S. Raju, J.B. Briggs, S. Borge, A.J.S. Jones, Glycobiology 10 (2000) 477.
[254] J. Saarinen, H.G. Welgus, C.A. Flizar, N. Kalkkinen, J. Helin, Eur. J. Biochem. 259 (1999) 829.
[255] A. Amoresano, L. Minchiotti, M.E. Cosulich, M. Campagnoli, P. Pucci, A. Andolfo, E. Gianazza, M. Galliano, Eur. J. Biochem. 267 (2000) 2105.
[256] J.P. Newton, A.P. Hunter, D.L. Simmons, C.D. Buckley, D.J. Harvey, Biochem. Biophys. Res. Commun. 261 (1999) 283.
[257] R. Leth-Larsen, U. Holmskov, P. Højrup, Biochem. J. 343 (1999) 645.
[258] Y. Huang, Y. Mechref, M.V. Novotny, Carbohydr. Res. 323 (2000) 111.
[259] S. Freeman, H.C. Birrell, K. D'Alessio, C. Erickson-Miller, K. Kikly, P. Camilleri, Eur. J. Biochem. 268 (2001) 1228.
[260] N. Inoue, T. Watanabe, T. Kutsukake, H. Saitoh, H. Tsumura, H. Arai, M. Takeuchi, Glycoconjugate J. 16 (1999) 707.
[261] T. Denzinger, H. Diekmann, K. Bruns, U. Laessing, C.A. Stuermer, M. Przybylski, J. Mass Spectrom. 34 (1999) 435.
[262] D. Hoja-Lukowicz, D. Ciolczyk, J. Bergquist, A. Litynska, P. Laidler, Glycobiology 10 (2000) 551.
[263] A. Amoresano, A. Brancaccio, A. Andolfo, M. Perduca, H.L. Monaco, G. Marino, Eur. J. Biochem. 263 (1999) 849.
[264] S.M. Haslam, K.M. Houston, W. Harnett, A.J. Reason, H.R. Morris, A. Dell, J. Biol. Chem. 274 (1999) 20953.

[265] J.S.S. Gray, B.Y. Yang, R. Montgomery, Carbohydr. Res. 311 (1998) 61.
[266] C.J.M. Stroop, W. Weber, G.J. Gerwig, M. Nimtz, J.P. Kamerling, J.F.G. Vliegenthart, Glycobiology 10 (2000) 901.
[267] S. Schröter, P. Derr, H.S. Conradt, M. Nimtz, G. Hale, C. Kirchhoff, J. Biol. Chem. 274 (1999) 29862.
[268] S. Zamze, D.R. Wing, M.R. Wormald, A.P. Hunter, R.A. Dwek, D.J. Harvey, Eur. J. Biochem. 268 (2001) 4063.
[269] C.H. Zeng, K. Biemann, J. Mass Spectrom. 34 (1999) 311.
[270] K.F. Faull, J. Johnson, M.J. Kim, T. To, J.P. Whitelegge, R.L. Stevens, C.B. Fluharty, A.L. Fluharty, J. Mass Spectrom. 35 (2000) 1416.
[271] U. Zähringer, H. Moll, T. Hettmann, Y.A. Knirel, G. Schäfer, Eur. J. Biochem. 267 (2000) 4144.
[272] K. Tseng, L.L. Lindsay, S. Penn, J.L. Hendrick, C.B. Lebrilla, Anal. Biochem. 250 (1997) 18.
[273] K. Tseng, J.L. Hedrick, C.B. Lebrilla, Anal. Chem. 71 (1999) 3747.
[274] W. Morelle, G. Strecker, Glycobiology 7 (1997) 1129.
[275] W. Morelle, G. Strecker, Biochem. J. 330 (1998) 469.
[276] W. Morelle, M.O. Cabada, G. Strecker, Eur. J. Biochem. 252 (1998) 253.
[277] A. Coppin, E. Maes, C. Flahaut, B. Coddeville, G. Strecker, Eur. J. Biochem. 266 (1999) 370.
[278] E. Maes, D. Florea, F. Delplace, J. Lemoine, Y. Plancke, G. Strecker, Glycoconjugate J. 14 (1997) 127.
[279] R. Mourad, W. Morelle, A. Neveu, G. Strecker, Eur. J. Biochem. 268 (2001) 1990.
[280] N.G. Karlsson, H. Nordman, H. Karlsson, I. Carlsted, G.C. Hansson, Biochem. J. 326 (1997) 911.
[281] K.A. Thomsson, I. Carlstedt, N.G. Karlsson, H. Karlsson, G.C. Hansson, Glycoconjugate J. 15 (1998) 823.
[282] J.-M. Lo-Guidice, H. Herz, G. Lamblin, Y. Plancke, P. Roussel, M. Lhermitte, Glycoconjugate J. 14 (1997) 113.
[283] K. Bock, J. Schuster-Kolbe, E. Altman, G. Allmaier, B. Stahl, R. Christian, U.B. Sleytr, P. Messner, J. Biol. Chem. 269 (1994) 7137.
[284] H. Iwase, A. Tanaka, Y. Hiki, T. Kokubo, I. Ishii-Karakasa, Y. Kobayashi, K. Hotta, J. Biochem. (Tokyo) 120 (1996) 393.
[285] H. Iwase, A. Tanaka, Y. Hiki, T. Kokubo, I. Ishii-Karakasa, Y. Kobayashi, K. Hotta, J. Biochem. (Tokyo) 120 (1996) 92.
[286] P. Juhasz, K. Biemann, Proc. Natl. Acad. Sci. U.S.A. 91 (1994) 4333.
[287] P. Juhasz, K. Biemann, Carbohydr. Res. 270 (1995) 131.
[288] A.J. Rhomberg, S. Ernst, R. Sasisekharan, K. Biemann, Proc. Natl. Acad. Sci. U.S.A. 95 (1998) 4176.
[289] C. Ueoka, S. Nadanaka, N. Seno, K.-H. Khoo, K. Sugahara, Glycoconjugate J. 16 (1999) 291.
[290] Z. Shriver, R. Raman, G. Venkataraman, K. Drummond, J. Turnbull, T. Toida, R. Linhardt, K. Biemann, R. Sasisekharan, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 10359.
[291] J. Schiller, J. Arnhold, S. Benard, S. Reichl, K. Arnold, Carbohydr. Res. 318 (1999) 116.
[292] S. Gerdt, G. Lochnit, R.D. Dennis, R. Geyer, Glycobiology 7 (1997) 265.
[293] G. Lochnit, S. Nispel, R.D. Dennis, R. Geyer, Glycobiology 8 (1998) 891.
[294] A. Olling, M.E. Breimer, E. Peltomaa, B.E. Samuelsson, S. Ghardashkhani, Rapid Commun. Mass Spectrom. 12 (1998) 637.
[295] A.A. Karlsson, P. Michelsen, G. Odham, J. Mass Spectrom. 33 (1998) 1192.
[296] S.B. Levery, M.S. Toledo, R.L. Doong, A.H. Straus, H.K. Takahashi, Rapid Commun. Mass Spectrom. 14 (2000) 551.
[297] H. Perreault, X.L. Hronowski, O. Koul, J. Street, R.H. McCluer, C.E. Costello, Int. J. Mass Spectrom. Ion Process. 169/170 (1997) 351.
[298] T. Doering, W.M. Holleran, A. Potrarz, G. Vielhaber, P.M. Elias, K. Suzuki, K. Dandhoff, J. Biol. Chem. 274 (1999) 11038.
[299] T. Fujiwaki, S. Yamaguchi, K. Sukegawa, T. Taketomi, J. Chromatogr. B 731 (1999) 45.
[300] H. Karlsson, L. Johansson, H. Miller-Podraza, K.A. Karlsson, Glycobiology 9 (1999) 765.
[301] M. Wuhrer, R.D. Dennis, M.J. Doenhoff, G. Lochnit, R. Geyer, Glycobiology 10 (2000) 89.
[302] S. Gerdt, R.D. Dennis, G. Borgonie, R. Schnabel, R. Geyer, Eur. J. Biochem. 266 (1999) 952.
[303] B.M. Olsson, H. Karlsson, T. Larsson, B. Lanne, J. Mass Spectrom. 34 (1999) 942.
[304] H. Karlsson, T. Larsson, K.-A. Karlsson, H. Miller-Podraza, Glycobiology 10 (2000) 1291.
[305] K. Nakamura, H. Kojima, M. Suzuki, A. Suzuki, Y. Tamai, Eur. J. Biochem. 267 (2000) 5198.
[306] J. Guittard, X. Hronowski, C.E. Costello, Rapid Commun. Mass Spectrom. 13 (1999) 1839.
[307] B.L.M. van Baar, FEMS Microbiol. Rev. 24 (2000) 193.
[308] S. Fukuoka, Y.A. Knirel, B. Lindner, H. Moll, U. Seydel, U. Zähringer, Eur. J. Biochem. 250 (1997) 55.
[309] M. Susskind, B. Lindner, T. Weimar, H. Brade, O. Holst, Carbohydr. Res. 312 (1998) 91.
[310] M. Linnerborg, A. Weintraub, G. Widmalm, Eur. J. Biochem. 266 (1999) 460.
[311] B.W. Gibson, J.J. Engstrom, C.M. John, W. Hines, A.M. Falick, J. Am. Soc. Mass Spectrom. 8 (1997) 645.
[312] S. Rund, B. Lindner, H. Brade, O. Holst, J. Biol. Chem. 274 (1999) 16819.
[313] M.M.A. Olsthoorn, J. Haverkamp, J.E. ThomasOates, J. Mass Spectrom. 34 (1999) 622.
[314] U. Seydel, B. Lindner, H.-W. Wollenweber, E.T. Rietschel, Eur. J. Biochem. 145 (1984) 505.
[315] G. Allmaier, E.R. Schmid, K. Hagspiel, C.P. Kubicek, M. Karas, F. Hillenkamp, Anal. Chim. Acta 241 (1990) 321.
[316] V.A. Kulshin, U. Zähringer, B. Lindner, C.E. Frasch, C.-M. Tsai, B.A. Dmitriev, E.T. Rietschel, J. Bacteriol. 174 (1992) 1793.
[317] A.P. Moran, B. Lindner, E.J. Walsh, J. Bacteriol. 179 (1997) 6453.
[318] I.A. Kaltashov, V. Doroshenko, R.J. Cotter, K. Takayama, N. Qureshi, Anal. Chem. 69 (1997) 2317.
[319] K.A. White, I.A. Kaltashov, R.J. Cotter, C.R.H. Raetz, J. Biol. Chem. 272 (1997) 16555.

[320] S. Rund, B. Lindner, H. Brade, O. Holst, Eur. J. Biochem. 267 (2000) 5717.
[321] W. Brabetz, B. Lindner, H. Brade, Eur. J. Biochem. 267 (2000) 5458.
[322] C. Oertelt, B. Lindner, M. Skurnik, O. Holst, Eur. J. Biochem. 268 (2001) 554.
[323] Z. Zhou, S. Lin, R.J. Cotter, C.R.H. Raetz, J. Biol. Chem. 274 (1999) 18503.
[324] L. Aussel, J.-R. Brisson, M.B. Perry, M. Caroff, Rapid Commun. Mass Spectrom. 14 (2000) 595.
[325] B.M. Plötz, B. Lindner, K.O. Stetter, O. Holst, J. Biol. Chem. 275 (2000) 11222.
[326] W. Jachymek, C. Petersson, A. Helander, L. Kenne, T. Niedziela, C. Lugowski, Carbohydr. Res. 292 (1996) 117.
[327] W. Jachymek, C. Petersson, A. Helander, L. Kenne, C. Lugowski, T. Niedziela, Carbohydr. Res. 269 (1995) 125.
[328] W. Jachymek, J. Czaja, T. Niedziela, C. Lugowski, L. Kenne, Eur. J. Biochem. 266 (1999) 53.
[329] C. Petersson, T. Niedziela, W. Jachymek, L. Kenne, P. Zarzecki, C. Lugowski, Eur. J. Biochem. 244 (1997) 580.
[330] C. Petersson, W. Jachymek, A. Klonowska, C. Lugowski, T. Niedziela, L. Kenne, Eur. J. Biochem. 245 (1997) 668.
[331] T. Niedziela, C. Petersson, A. Helander, W. Jachymek, L. Kenne, C. Lugowski, Eur. J. Biochem. 237 (1996) 635.
[332] J. Czaja, W. Jachymek, T. Niedziela, C. Lugowski, E. Aldova, L. Kenne, Eur. J. Biochem. 267 (2000) 1672.
[333] P. Roethlisberger, N. Iida-Tanaka, K. Hollemeyer, E. Heinzle, I. Ishizuka, W. Fischer, Eur. J. Biochem. 267 (2000) 5520.
[334] S. Hartmann, D.E. Minnikin, A.I. Mallet, M. Ridell, L. Rigouts, F. Portaels, Biol. Mass Spectrom. 23 (1994) 362.
[335] A. Venisse, J.-M. Berjeaud, P. Chaurand, M. Gilleron, G. Puzo, J. Biol. Chem. 268 (1993) 12401.
[336] J. Nigou, M. Gilleron, T. Brando, A. Vercellone, G. Puzo, Glycoconjugate J. 16 (1999) 257.
[337] P. Ludwiczak, T. Brando, B. Monsarrat, G. Puzo, Anal. Chem. 73 (2001) 2323.
[338] W.J. Goux, S. Rodriguez, D.R. Sparkman, J. Neurochem. 67 (1996) 723.
[339] B.Y. Yang, J.S.S. Gray, R. Montgomery, Carbohydr. Res. 316 (1999) 138.
[340] P.-A. Trinel, Y. Plancke, P. Gerold, T. Jouault, F. Delplace, R.T. Schwarz, G. Strecker, D. Poulain, J. Biol. Chem. 274 (1999) 30520.
[341] A.B. Mahoney, D.L. Sacks, E. Saraiva, G. Modi, S.J. Turco, Biochemistry 38 (1999) 9813.
[342] U. Zähringer, F. Wagner, E.T. Rietschel, G. Ben-Menachem, J. Deutsch, S. Rottem, J. Biol. Chem. 272 (1997) 26262.
[343] L.C. Maillard, C. R. Acad. Sci. 154 (1912) 66.
[344] A. Lapolla, C. Gerhardinger, L. Baldo, D. Fedele, A. Keane, R. Seraglia, S. Catinella, P. Traldi, Biochim. Biophys. Acta 1225 (1993) 33.
[345] A. Lapolla, D. Fedele, R. Seraglia, S. Catinella, L. Baldo, R. Aronica, P. Traldi, Diabetologia 38 (1995) 1076.
[346] A. Lapolla, L. Baldo, R. Aronica, C. Gerhardinger, D. Fedele, G. Elli, R. Seraglia, S. Catinella, P. Traldi, Biol. Mass Spectrom. 23 (1994) 241.
[347] A. Lapolla, D. Fedele, R. Aronica, L. Baldo, M. D'Alpaos, R. Seraglia, P. Traldi, Rapid Commun. Mass Spectrom. 10 (1996) 1512.
[348] A. Lapolla, D. Fedele, R. Aronica, M. Garbeglio, M. D'Alpaos, R. Seraglia, P. Traldi, Rapid Commun. Mass Spectrom. 11 (1997) 1342.
[349] A. Lapolla, D. Fedele, M. Plebani, R. Aronica, M. Garbeglio, R. Seraglia, M. D'Alpaos, P. Traldi, Clin. Chem. 45 (1999) 288.
[350] A. Lapolla, D. Fedele, R. Aronica, M. Garbeglio, M. D'Alpaos, R. Seraglia, P. Traldi, Rapid Commun. Mass Spectrom. 10 (1996) 1133.
[351] S. Swamy-Mruthinti, K.L. Schey, Curr. Eye Res. 16 (1997) 936.
[352] A. Lapolla, D. Fedele, L. Martano, N.C. Arico', M. Garbeglio, P. Traldi, R. Seraglia, D. Favretto, J. Mass Spectrom. 36 (2001) 370.
[353] A. Lapolla, D. Fedele, R. Aronica, M. Garbeglio, M. D'Alpaos, M. Plebani, R. Seraglia, P. Traldi, Rapid Commun. Mass Spectrom. 11 (1997) 613.
[354] X. Tang, M. Sadeghi, Z. Olumee, A. Vertes, J.A. Braatz, L.K. McIlwain, P.A. Dreifuss, Anal. Chem. 68 (1996) 3740.
[355] A. Lapolla, D. Fedele, M. Plebani, M. Garbeglio, R. Seraglia, M. D'Alpaos, C.N. Aricò, P. Traldi, Rapid Commun. Mass Spectrom. 13 (1999) 8.
[356] M.M. Siegel, H.-R. Tsou, B. Lin, I.J. Hollander, A. Wissner, M. Karas, A. Ingendoh, F. Hillenkamp, Biol. Mass Spectrom. 22 (1993) 369.
[357] H. Tanaka, W. Putalun, C. Tsuzaki, Y. Shoyama, FEBS Lett. 404 (1997) 279.
[358] M.M. Siegel, I.J. Hollander, P.R. Hamann, J.P. James, L. Hinman, B.J. Smith, A.P.H. Farnsworth, A. Phipps, D.J. King, M. Karas, A. Ingendoh, F. Hillenkamp, Anal. Chem. 63 (1991) 2470.
[359] J. Zhang, Yergey, J. Kowalak, P. Kovác, Carbohydr. Res. 313 (1998) 15.
[360] R. Niemelä, J. Räbinä, A. Lappänen, H. Maaheimo, C.E. Costello, O. Renkonen, Carbohydr. Res. 279 (1995) 331.
[361] J. Helin, H. Maaheimo, A. Seppo, A. Keane, O. Renkonen, Carbohydr. Res. 266 (1995) 191.
[362] J. Natunen, A. Seppo, J. Helin, B.B. Reinhold, J. Räbinä, C.E. Costello, O. Renkonen, Glycobiology 7 (1997) 711.
[363] M.R.E. Aly, E.-S.I. Ibrahim, E.-S.H. El Ashry, R.R. Schmidt, Carbohydr. Res. 316 (1999) 121.
[364] M. Bårström, M. Bengtsson, O. Blixt, T. Norberg, Carbohydr. Res. 328 (2000) 525.
[365] S. Zeng, R.G. Gallego, A. Dinter, M. Malissard, J.P. Kamerling, J.F.G. Vliegenthart, E.G. Berger, Glycoconjugate J. 16 (1999) 487.
[366] A. Chernyak, S. Oscarson, D. Turek, Carbohydr. Res. 329 (2000) 309.
[367] P.Y. Chong, P.A. Petillo, Org. Lett. 2 (2000) 1093.
[368] D.A. Fulton, J.F. Stoddart, Org. Lett. 2 (2000) 1113.
[369] F. Yan, W.W. Wakarchuk, M. Gilbert, J.C. Richards, D.M. Whitfield, Carbohydr. Res. 328 (2000) 3.
[370] G.R. Guile, D.J. Harvey, N. O'Donnell, A.K. Powell, A.P. Hunter, S. Zamze, D.L. Fernandes, R.A. Dwek, D.R. Wing, Eur. J. Biochem. 258 (1998) 623.