ANALYSIS OF CARBOHYDRATES AND GLYCOCONJUGATES BY MATRIX-ASSISTED LASER DESORPTION/IONIZATION MASS SPECTROMETRY: AN UPDATE FOR 2003–2004

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This review is the third update of the original review, published in 1999, on the application of matrix-assisted laser desorption/ionization (MALDI) mass spectrometry to the analysis of carbohydrates and glycoconjugates and brings the topic to the end of 2004. Both fundamental studies and applications are covered. The main topics include methodological developments, matrices, fragmentation of carbohydrates and applications to large polymeric carbohydrates from plants, glycans from glycoproteins and those from various glycolipids. Other topics include the use of MALDI MS to study enzymes related to carbohydrate biosynthesis and degradation, its use in industrial processes, particularly biopharmaceuticals and its use to monitor products of chemical synthesis where glycodendrimers and carbohydrate–protein complexes are highlighted. © 2008 Wiley Periodicals, Inc., Mass Spec Rev 28:273–361, 2009

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I. INTRODUCTION

This review is a continuation of the three earlier ones in this series on the application of matrix-assisted laser desorption/ionization (MALDI) mass spectrometry to the analysis of carbohydrates and glycoconjugates (Harvey, 1999, 2006, 2008) and brings the coverage of the literature to the end of 2004. The review is intended to illustrate both developments in technology and in the way in which analysis of carbohydrates contributes to scientific knowledge in general.

Matrix-assisted laser desorption/ionization (MALDI) continues to be a major technique for the analysis of carbohydrates although electrospray, particularly with quadrupole-time-of-flight (Q-TOF) instruments, is becoming increasingly popular. Advantages of the MALDI-Q-TOF combination for proteomics and glycomics have been stressed in a brief focus paper (Huang, 2003). Figure 1 shows the year-by-year increase in papers reporting use of MALDI MS for carbohydrate analysis for the period 1990–2004. As the review is designed to complement the

earlier work, structural formulae, etc. that were presented earlier are not repeated. However, a citation to the structure in the earlier works is indicated by its number with a prefix (i.e., **1/x** refers to structure **x** in the first review and **2/x** to the second). Other reviews and review-type articles directly concerned with, or including MALDI analysis of glycoconjugates that have been published during the review period include general reviews by Harvey (2003), Stuhler and Meyer (2004), and Zaia (2004), a review of capillary electrophoresis-mass spectrometry for glycoscreening (Zamfir & Peter-Katalinic, 2004) and a review on new methods for mass spectrometry of bioorganic macromolecules (Cristoni & Bernsrdi, 2003). More specific reviews include those on the glycosylation of *Caenorhabditis elegans* (Haslam & Dell, 2003), characterization of substituent distribution in starch and cellulose derivatives (Richardson & Gorton, 2003), derivatization of carbohydrates for chromatographic, electrophoretic, and mass spectral structural analysis (Lamari, Kuhn, & Karamanos, 2003), structure of bacterial lipopolysaccharides (Caroff & Karibian, 2003), analysis of post-translational modifications (Cantin & Yates, 2004; Jensen, 2004; Seo & Lee, 2004), the use of MALDI MS to detect enantioselectivity in gas-phase ion-molecule reactions with carbohydrates such as cyclodextrins (Speranza, 2004), synthesis of heparan and heparin sulfate fragments (Poletti & Lay, 2003), analysis of protein glycation products (Horvat & Jakas, 2004; Kislinger, Humeny, & Pischetsrieder, 2004), carbohydrate biosensors (Jelinek & Kolusheva, 2004), synthesis and discovery of oligosaccharides and glycoconjugates for the treatment of disease (Macmillan & Daines, 2003), dendrimers in drug research (Boas & Heegaard, 2004), combinatorial carbohydrate synthesis (Baytas & Linhardt, 2004), chemical tagging strategies for proteome analysis (Leitner & Lindner, 2004), capillary electrophoresis of biopharmaceutical products (Takechi, Kinoshita, & Nakano, 2002) and the use of mass spectrometry to study congenital disorders of glycosylation type IIx (Mills et al., 2003b).

II. INSTRUMENTATION

A. MALDI Interfaces to Trapped Ion Instruments

A comparison between spectra obtained with a conventional quadrupole ion trap (QIT) (electrospray ionization) and a MALDI-QIT-reflectron-TOF instrument has shown that the

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FIGURE 1. Yearly totals of the number of publications containing work relating to the application of MALDI mass spectrometry to carbohydrates and glycoconjugates.

latter instrument produces significant amounts of metastable fragmentation due to the longer (msec) time span of the QIT trapping process, an observation also made by Harvey et al. (2004) with released *N*-glycans. Glycopeptides were completely desialylated in the process. Fragmentation (MS^2) of glycopeptides produced fragments exclusively from the carbohydrate with the ESI-QIT instrument with peptide fragmentation occurring in MS^3 , whereas the MALDI-QIT-reflectron-TOF instrument yielded both types of fragmentation at the MS^2 stage (Demelbauer et al., 2004).

B. High-Pressure and Atmospheric Pressure MALDI (AP-MALDI)

Coupling of both vacuum and atmospheric pressure MALDI (AP-MALDI) ion sources with ion-trap instruments has been reviewed (Moyer et al., 2003) and the latter technique investigated as a method for obtaining fragmentation spectra from carbohydrates. High-pressure ion sources have been developed to cool ions in an attempt to reduce or eliminate fragmentation occurring either in-source or post-source. Such fragmentation is the primary factor limiting the use of vacuum MALDI for analysis of acidic glycans and can be considerably reduced with a high-pressure ion source but at the cost of diminished sensitivity. Arabinosazone (**1/40**) has been used as the matrix for maltohexaose (α -D-Glcp-(α -D-Glcp-)₄-(1→4)- α -D-Glcp, **1**) in positive ion mode ($[M + Na]^+$ ion) and for 6'-sialyllactose (**2**) in negative mode ($[M - H]^-$). The spectrum of the latter compound contained two abundant fragments, an O^2A_3 and a C-ion (Domon and Costello (1988) nomenclature, see first review, Scheme 3) whereas the major glycosidic fragments in the positive ion spectrum were produced by B or Y cleavages.

α -D-Glcp-(α -D-Glcp-)₄-(1→4)- α -D-Glcp

1. Maltohexaose

2. 6'-sialyllactose

Because of the near physiological conditions attainable at atmospheric pressure with glycerol as the matrix, it has been possible to observe non-covalent complexes between carbohydrates and synthetic peptides that were designed to mimic the binding sites of three members of the siglec family. 3'- (**3**) and 6'-sialyllactose (**2**), were used as the carbohydrate ligands with 3-sialyllactose maintaining its binding specificity for sialoadhesin (Von Seggern & Cotter, 2004). A new AP-MALDI technique named atmospheric pressure infrared ionization from solution (AP-IRIS) that is claimed to be 16 times as sensitive as AP UV-MALDI has been implemented on a QIT mass spectrometer (Tan et al., 2004). The method operated in the absence of an extraction

field and used a high power IR laser with aqueous glycerol as the matrix. Spectra consisting of $[M + Na]^+$ ions were obtained from several high-mannose and complex *N*-linked glycans with much better signal/noise ratios than with corresponding amounts ionized by UV-MALDI from 2,4,6-trihydroxyacetophenone (THAP, **1/44**).

C. Other Developments in Instrumentation and Techniques

Matrix-assisted laser desorption/ionization (MALDI) plates sprayed with the hydrophobic 3M product, ScotchGard™, a water repellent material for coating fabrics, has the effect of reducing the MALDI spot size and improving sensitivity (Owen et al., 2003). For cleaning, the coating could easily be removed with methyl-*t*-butyl ether and the film reapplied to the clean plate. Experiments with the carbohydrate-containing antibiotic, erythromycin A (**4**), showed increases in sensitivity of two- to threefold when ionized from the coated targets compared with signals from uncoated plates.

A microdeposition device has been constructed which mixes effluent from capillary electrochromatography or microcolumn liquid chromatography with the MALDI matrix and deposits the mixture onto the MALDI plate. Dextrin and *N*-linked glycans

from ribonuclease B were successfully analyzed (Tegeler et al., 2004). An array of 96 perforated nanovials manufactured by silicon microfabrication and filled with 40 nL of reversed-phase beads has been developed as a MALDI target for peptide analysis. It was successfully used to examine peptides and glycopeptides from prostate-specific antigen (Ekström et al., 2004).

Liquid chromatography-mass spectrometry (LC-MS) has the disadvantage that the entire sample is consumed after exiting the HPLC column. Lochnit and Geyer (2004) have used nano-LC-MALDI-TOF MS to examine tryptic peptides and glycopeptides, a technique that allows more detailed MS investigation of the effluent to be made. The technique also produced enhanced detection of glycopeptides in the presence of peptides and improved characterization of the attached glycans by optimizing the experimental conditions.

III. MATRICES

A. Theory of Matrix Action

Investigations into the mechanism of proton transfer from dihydroxybenzoic acid (DHB) isomers continues. Yassin and Marynick (2003) have calculated the gas-phase acidities of the radical cations of all six dihydroxybenzoic acid (DHB) isomers using density functional theory and found excellent agreement with experimental measurements. The 2,5-isomer (**1/26**), the most effective matrix, was the least acidic (in this review the abbreviation DHB is used for the 2,5-isomer, unless stated otherwise). The results indicate that, as proposed earlier (Gimon et al., 1992), deprotonation occurs at the phenolic rather than the acidic site as the result of resonance stabilization of the resulting ion. This conclusion is consistent with the theory proposed by Harvey (1993) that one of the reasons that the 2,5-isomer is so effective a matrix is that it is the only one that can form a *p*-benzoquinone-type (**5**) structure after photochemical decarboxylation.

Sugars, however generally ionize as $[M + Na]^+$ ions for which the factors governing formation are much less clear. A recent study by Antonopoulos et al. (2003) on the mechanism of attachment of sodium to glucose and its methylated derivatives found no simple correlation between ion yield and factors such as volatility and hydrophobicity. A study by Luxembourg et al. (2003) using the high resolution capability of matrix-assisted secondary ion mass spectrometry has demonstrated crystal inhomogeneity among DHB crystals on MALDI targets. Some crystals were free of sodium whereas others contained sodium and sample. Yet other areas of the target contained mostly sodium and little matrix. The results were thought to explain the

phenomenon of “sweet spots” frequently observed with MALDI targets.

Proton-affinity values for 15 common matrices have been measured by a kinetic method. α -Cyano-4-hydroxycinnamic acid (CHCA, **1/23**) and DHB showed the lowest affinity whereas 2-(4'-hydroxyphenyl)azobenzoic acid (HABA, **1/32**) and *nor*-harmane (**1/35**) had the highest affinity. In general, the rank order was similar to that found by other investigators. With β -cyclodextrin (**6**, cyclic-(1 $\rightarrow$ 4)- α -D-glucose)₇ as the reference compound in negative ion mode, matrices with low proton affinity, such as DHB (204.4 ± 0.17), produced no $[M-H]^-$ ions whereas those with high affinity, such as *nor*-harmane (233.0 ± 0.44), did (Mirza, Raju, & Vairamani, 2004b). Gas-phase potassium binding energies of several MALDI matrices have also been published (Zhang et al., 2003b).

Cyclic-((1 $\rightarrow$ 4)- α -D-Glc)₇

6. β -Cyclodextrin

Fournier et al. (2003) have measured the ablation volume of DHB crystals under different laser powers and found that, although the ablation volume increased only slowly with increasing laser power, the signal rose dramatically. They interpreted their results as involving a very rapid ablation to produce a plume that was further ionized by the incoming laser beam. Frankevich et al. (2003) have discussed the role of photoelectrons on MALDI sensitivity. Suppression of photoelectrons with a film of insulating material on the target; the authors used Scotch Magic Tape; was found to produce an increase of up to two orders of magnitude in sensitivity and an improvement in resolution.

B. Simple Matrices

Bashir, Mutter, and Derrick (2003b) have investigated long-chain esters of DHB as matrices and found some improvement in performance, particularly after the addition of iodine to those compounds that contained a double bond in the chain. The mechanism producing this enhancement in performance by the addition of iodine was unclear but the reason for the improved performance with some of the long-chain compounds was thought to be the formation of micelles that enhanced the matrix-analyte interactions.

5-Amino-2-mercapto-1,3,4-thiadiazole (AMT, **7**) has been described as a new matrix for carbohydrates (Mirza et al., 2004a). Its performance was similar to that of DHB although slightly higher-mass compounds could be ionized from dextran-5000. Kéki et al. (2004) have ionized simple organic molecules such as a peracetylated isoflavone glycoside (**8**) from common matrices such as DHB doped with silver trifluoroacetate to give $[M + Ag_3]^+$ species. These ions were shown to be more stable than the corresponding $[M + Ag]^+$ ions. The latter ions and their Cu^+ counterparts, fragmented in a similar manner to those from Na^+ and Li^+ (Kéki et al., 2004). Carbon nanotubes, prepared from coal by an arc discharge have been reported as a novel matrix for low molecular weight peptides and β -cyclodextrin (Xu et al., 2003). Spectra showed a very low background as the result of the absence of matrix peaks and ions produced by

fragmentation. To obtain the spectrum, a well-dispersed ethanolic solution of nanotubes was deposited onto the MALDI target and allowed to dry. The sample solution was then deposited onto the nanotubes and dried with hot air. Two papers reporting the use of fine cobalt powder for the analysis of lepidimoic acid (**9**) from okra pectic polysaccharide (Hirose et al., 2003) and of lepidimoide (**10**) from okra mucilage have appeared (Hirose, Endo, & Hasegawa, 2004).

7. 5-Amino-2-mercapto-1,3,4-thiadiazole

8. Peracetylated *iso*-flavone glycoside

9. R = H, Lepidimoic acid

10. R = Na, Lepidimoide

C. Binary Matrices

A mixture of DHB and CHCA has been shown to give enhanced performance for the ionization of glycopeptides than use of the individual matrices. The mixed matrix showed better tolerance towards the presence of salts and impurities and improved peptide sequence coverage (Laugesen & Roepstorff, 2003). A thin layer of very homogeneous crystals has been produced by mixing THAP with nitrocellulose and used to analyze pectin digests (Mohamed, Christensen, & Mikkelsen, 2003).

D. Liquid Matrices

Atmospheric pressure MALDI (AP-MALDI) has the advantage over vacuum MALDI of collisional stabilization of fragile molecules such as carbohydrates containing sialic acid residues. The technique is also compatible with the use of liquid matrices

whose volatility presents problems under vacuum conditions. Von Seggern, Moyer, and Cotter (2003a) have compared water, glycerol, and nitrobenzyl alcohol (**1/21**) for ionization of sialylated glycans with an IR laser (Er:YAG, 2,490 nm) and have found that glycerol provides the longest lasting signal with the best signal/noise ratio. Water was too volatile to survive for long under the heat produced by the laser. Intact sialylated glycans were desorbed as $[M-H]^-$ and $[M+Na]^+$ ions under negative and positive conditions respectively. In a further study (Von Seggern, Zarek, & Cotter, 2003b), the glycerol matrix was doped with alkali (Li, Na, K) alkaline earth (Ca) or transition metal cations (Ca, Co) for production of adducts containing one or two singly charged cation or one doubly charged cation. Adduction proved to be efficient with lithium or potassium being able to replace the more common sodium adduction when salts (Cl^- or I^-) containing these metals were used as the doping agents. Cobalt adduction was achieved by use of cobalt powder added to the glycerol. Fragmentation (see below) was obtained with an ion-trap instrument.

The use of glycerol as the matrix with an IR laser under atmospheric pressure conditions has also enabled non-covalent sugar–sugar complexes to be observed (Von Seggern & Cotter, 2003). The stability of the complexes varied with different sugars potentially due to the strengths of the hydrogen bonding networks. Fragmentation of the complexes was also a function of structure; some complexes, particularly those containing sialic acid, fragmented by loss of the acid before breakdown of the complex whereas complexes between neutral molecules tended preferentially to dissociate into monomer units.

E. Ionic Liquid Matrices

Ionic liquid matrices appear to show great promise for carbohydrate analysis. The matrix DHB-butylamine (DHBB) was reported to give improved shot–shot reproducibility compared with solid matrices such as DHB, and reduced the relative standard deviation by 50%. It also produced much less fragmentation from compounds such as sialylated carbohydrates (Mank, Stahl, & Boehm, 2004). Greatly improved quantitative performance has also been reported by Zabet-Moghaddam, Heinzle, and Tholey (2004).

IV. DERIVATIVES

Derivatization techniques for analysis of carbohydrates have been reviewed (Gao et al., 2003; Suzuki et al., 2003b).

A. Reducing-Terminal Derivatives

1. Reducing-Terminal Derivatives Prepared by Reductive Amination

Advantages of analytical derivatization have been emphasized by Rosenfeld (2003) with, among others, examples from the reductive amination of carbohydrates for improving the interpretation of fragmentation spectra and for modifying behavior in chromatographic systems. MALDI is frequently used to monitor reducing-terminal labeling, used to attach fluorophores or chromophores to carbohydrates for chromatographic detection

as illustrated in a recent study of glycans from the Tamm-Horsfall glycoprotein (Rohfritsch et al., 2004) and the use of 2-AA-derivatives to monitor glycosylation of therapeutic glycoproteins (Dhume et al., 2002). Benzylamine (**2/9**) derivatization was used by Morelle and Michalski (2004) mainly for ESI-MS/MS analysis of small and *N*-linked glycans. However, exoglycosidase sequencing was performed on a MALDI target and it was observed that the derivative did not inhibit the enzymic reaction. An, Franz, and Lebrilla (2003a) have derivatized several sugars, including *N*-linked glycans released from ribonuclease B, with benzylamine and quaternized the product with methyl iodide as originally performed by Broberg, Broberg, and Duus (2000) but with a procedure not involving the use of resins. The product allowed separation of isomers by capillary electrophoresis (CE) and also gave strong MALDI spectra on account of the inbuilt charge.

It is possible to remove labels attached by reductive amination; thus, 2-aminopyridine (2-AP, **1/52**)-derivatized *N*-glycans have been underivatized by a process that involved catalytic hydrogenation and hydrazinolysis using the method reported by Hase (1992) to give the reducing-terminal amine which was converted to an aldehyde using the Sommet reaction (hexamethylenetetramine and acetic acid) (Takahashi, Nakakita, & Hase, 2003a).

2. Reducing-Terminal Derivatives Prepared by Other Methods

Phenylhydrazine (**11**) has been used to form hydrazone derivatives of *N*-linked glycans for both MALDI and electrospray ionization. Because the derivatives were not reduced, as with reductive amination, no post-derivatization clean-up was necessary. Derivatives could be prepared on-target by addition of a solution (0.5 μ L), prepared by dissolving phenylhydrazine (1 μ L) in 10 μ L of water/methanol (4:1 v/v), directly to the glycan–matrix mixture on the MALDI target and allowing the mixture to dry under ambient conditions for 45 min. No osazone formation was reported and the derivatives produced $[M+Na]^+$ ions. Post-source decay (PSD) spectra were reported to be simpler than those of the underivatized glycans and to be dominated by B/Y internal fragments (Lattova & Perreault, 2003a,b).

11. Phenylhydrazine

Several hydrazones containing either a constitutive cationic charge, such as those formed from Girard's T reagent (**1/55**, quaternary ammonium) or from hydrazines carrying a guanidine group (e.g., **12**), have been shown to produce increases in sensitivity and to suppress signals from peptides, thus allowing *N*-linked glycans to be detected in the presence of tryptic peptides without extensive clean-up (Shinohara et al., 2004). Differences in sensitivity among the derivatives were observed. Among those containing a charge, and which formed M^+ ions, the pyridinium

derivative (**13**) was the most efficient, producing a detection limit of 1 fmol. The guanidine derivatives formed $[M + H]^+$ ions and some produced equal detection limits. It would appear that the most efficient derivatives possessed both good ion-forming properties and high lipophilicity. Improved detection limits have also been achieved by use of hydrazide derivatives of the cyanine dyes Cy-3 (**14**) or Cy-5 (**15**) that contain a constituent positive charge (Kameyama et al., 2004). *N*-glycans from chicken ovalbumin could be detected with near independence from the type of matrix. PSD spectra contained mainly Y-type ions consistent with localization of the positive charge on the derivative.

12. Guanidino-hydrazine reagent

13. Pyridinium-containing hydrazine reagent

14. R = C₃H₇, n = 1, Cy-3

15. R = CH₃, n = 2, Cy-5

B. Derivatives of Other Sites

Sialic acids can be stabilized for MALDI analysis by formation of methyl esters to remove the very labile acidic proton on their carboxyl groups. Migration of this hydrogen atom is largely responsible for lability of this carbohydrate. A recent example of methyl ester formation is in a study of the *N*-linked glycosylation of the glycoprotein, *N*-cadherin, from human melanoma cell lines (Ciolczyk-Wierzbicka et al., 2004). The acids were first converted into their sodium salts with a Dowex AG50 resin (Na⁺ form) and these salts were then reacted with methyl iodide. Other examples are included below.

V. CLEAN-UP OF SAMPLES PRIOR TO MALDI ANALYSIS

Removal of contaminants such as salts, buffers, etc. is essential to obtain high quality spectra. A variety of methods are used, the majority being dialysis, use of various membranes such as Nafion 117 (Börnsen, Mohr, & Widmer, 1995) or various resins. Methods for preparing samples for mass spectrometric analysis have been reviewed (Perreault, 2004). Some examples of those during the review period are listed below.

A. Membranes

Drop dialysis has been used in clean-up of lipooligosaccharide (LOS) from *Moraxella catarrhalis* by Luke et al. (2003) and a Nafion membrane for on-probe clean-up of *O*-acetylated glucomannans from birch and aspen (Teleman et al., 2003).

B. Use of Resins

Dowex AG50 resins in various forms have been used for analysis of *N*-glycans from the moss *Physcomitrella patens* (Viëtor et al., 2003) (AG 50W-X2 form), deacylated lipopolysaccharide (LPS) from *Vibrio parahaemolyticus* strains (Hashii et al., 2003a,b) (50 W × 8, H⁺ form to remove cations) and extracellular polysaccharide (EPS) from *Burkholderia cepacia* (Cescutti et al., 2003) (50 W × 8–200). AG50 combined with AG3 (anions) in a gel loader tip have been used for *N*-linked glycans (Hoja-Lukowicz et al., 2000; Ciolczyk-Wierzbicka et al., 2004). Porous graphitized carbon has been employed to desalt quaternized benzylamine derivatives (An, Franz, & Lebrilla, 2003a) and Hypercarb columns have been used by Mills et al. (2003b) for *N*-linked glycans released from human plasma glycoproteins and by Forno et al. (2004) for *N*- and *O*-linked glycans from recombinant human granulocyte-macrophage colony-stimulating factor secreted by a Chinese hamster ovary (CHO) cell line. Several examples of the use of cellulose cartridges have been published; thus, Higai et al. used them for purification of *N*-glycans from α1-acid glycoprotein (AGP) (Higai et al., 2003a) and sialyl Lewis X antigen-expressing glycoproteins secreted by human hepatoma cell line (Higai et al., 2003b) and Wada, Tajiri, and Yoshida (2004) have employed both cellulose or Sepharose for the separation of tryptic glycopeptides from tryptic peptides.

C18-solid-phase extraction followed by Carbograph column was used by Ohl et al. (2003) to remove detergents from *N*-glycans. Salts, etc. were eluted from the columns with water, neutral glycans with 25% MeCN and acidic glycans with 25% MeCN/0.05% trifluoroacetic acid (TFA). Amberlite IR-120 (H⁺) was used by Fridrich et al. (2003) to purify core oligosaccharide from *E. coli*. Release of *N*-glycans with protein-*N*-glycosidase F (PNGase F) is more efficient if the glycoprotein is pre-digested with trypsin or other protease. However, the resulting peptides can interfere with subsequent analysis and are difficult to remove with octadecyl (C₁₈) silica cartridges, cation exchange columns or graphitized carbon cartridges. To overcome this problem, Nakano, Kakehi, and Lee (2003b) have modified the amino groups of the peptides with 2,4,6-trinitrobenzene-1-sulfonate

(16) to render them more hydrophobic so that they can be more strongly retained on C₁₈ or graphitized carbon.

16. 2,4,6-trinitrobenzene-1-sulfonate

C. Bioaffinity Clean-Up

Affinity capture of glycoproteins to a MALDI target has been reported by Koopmann and Blackburn (2003). The target was coated with poly-L-lysine poly(ethylene glycol)-biotin polymer followed by tetrameric neutravidin. This surface then acted as a capturing surface for biotinylated proteins which could be adsorbed and desalted by washing. MALDI spectra were obtained following addition of CHCA to the surface. Protein–protein interactions could also be studied with this treated target and, furthermore, it could be used to isolate glycoproteins by lectin binding. Thus, biotinylated wheat germ agglutinin bound to the surface was used to further capture fetuin. The resulting MALDI spectrum contained ions from the biotinylated lectin, neutravidin and fetuin.

D. On-Target Fractionation

Glycopeptides, such as those obtained from protease digests, frequently give weak signals in the presence of hydrophobic peptides. A simple method for separating hydrophobic from hydrophilic peptides on a MALDI target has recently been devised (Kjellström & Jensen, 2003). An aqueous solution of the peptide mixture was placed on the target followed by an immiscible solvent, such as ethyl acetate with or without added matrix. The organic solvent extracted the hydrophobic peptides and was allowed to evaporate, after which, the residual aqueous phase was transferred to another part of the target and allowed to dry after addition of DHB. MALDI spectra could then be acquired from both the hydrophobic and hydrophilic fractions.

VI. QUANTIFICATION

A paper on quantitation of glucose (1/4) in a study on enzyme kinetics has highlighted problems in the use of MALDI-MS for quantitative work and how the technique can produce good quantitative results in spite of the popular belief that the technique is not quantitative. The inhomogeneity of the target and variations in the shot-to-shot laser intensity can be compensated for by averaging many shots from different target positions. It is also important to eliminate any spectrum in which the A/D converter is saturated. By use of a [U-¹³C]-labeled internal standard and measurement of the [M + Na]⁺ ions, a linear calibration curve was obtained with a correlation coefficient of $r = 0.991$. The mean standard deviation was 6% but the

authors (Bungert, Heinzle, & Tholey, 2004b) state that this could be improved if more spectra were averaged although this would have increased the analysis time.

A MALDI method for the quantification of glucose from hydrolyzed starch has been reported. Sorbitol (1/42) was used as the internal standard and DHB provided the matrix even though it produced peaks in the same region, but not at the same mass as the analytes. Calibration curves were linear over the range 0.1–10 pmol added to the target and the method compared favorably with the normal colorimetric method for measuring glucose. Even though the MALDI results showed a greater degree of variability, this was more than compensated for by the speed of analysis (Grant et al., 2003).

A quantitative MALDI-TOF MS method has also been developed for screening of ten pyranose oxidase variants. The isotopic labeled internal standard, [U-¹³C]-glucose and the ionic liquid matrix, DHB/pyridine, were used with aliquots of enzyme reaction mixtures without pre-purification steps (Bungert et al., 2004a). The ionic, liquid matrix enabled spectra to be recorded from most areas of the target (only 7 out of 200 spots failed to produce a signal). The mean standard deviation for glucosone was 11.8% and the results were in good agreement with HPLC measurements.

VII. FRAGMENTATION

Glycosidic and cross-ring cleavages produce most of the fragment ions from carbohydrates. A third type of fragmentation mechanism has now been reported involving hexacyclic hydrogen rearrangements. The products were proposed to consist of unsaturated sugar rings lacking two oxygen atoms as shown in Scheme 1 (Spina et al., 2004).

A. Post-Source Decay (PSD)

Post-source decay (PSD) fragmentation of per-acylated *iso*-flavone glycosides (e.g., 8) cationized with various metals and hydrogen have shown that the number of fragment ions decreased in the order $\text{Li}^+ = \text{Na}^+ > \text{Ag}^+ > \text{Cu}^+ > \text{H}^+ > \text{K}^+ > \text{Rb}^+ = \text{Cs}^+$, roughly in line with earlier studies of underivatized carbohydrates (Kéki et al., 2003). Fragments were largely glucosidic and losses of acetic acid and ketene but, unusually, there were a few fragments in some of the metal-cationized spectra that had lost the metal. Loss of acetic acid and ketene was highest for the lighter metals. The authors attributed this observation to elimination of metal acetates.

Neutral and some acidic carbohydrates have been shown to form stable adducts with chlorine that survive the MALDI process to give [M + Cl][−] ions from matrices such as harmine (1/36) that have gas-phase acidities lower than or close to that of HCl (Cai, Jiang, & Cole, 2003). Such adducts open the way for the production of negative ion fragmentation spectra from carbohydrates that, in many cases, produce more informative spectra than their positive ion counterparts. In the above publication, the adduct of the disaccharide D-turanose (α-D-Glcp-(1 → 3)-D-Fru, 17) was reported to fragment initially by loss of HCl to give a PSD spectrum containing both glycosidic and

SCHEME 1.

cross-ring fragments arising from what was essentially a $[M-H]^-$ ion.

α -D-Glcp-(1 $\rightarrow$ 3)-D-Fru
17. D-turanose

MS/MS analysis of *N*-acetylactosamine-6,6'-disulfate (**18**) and its 2'-epimer produce different fragmentation patterns under several mass spectrometric methods (fast-atom bombardment (FAB), ESI and MALDI-PSD). The PSD spectra were characterized by an abundant ion at m/z 431 that was produced from the 2 α -epimer by elimination of both the *N*-acetylamino-group and the 6-sulfate (Ohashi et al., 2004). Muzikar et al. (2004) have found that many carbohydrates containing an amino group at the reducing terminus show enhanced PSD fragmentation with production of more abundant cross-ring fragments. *N*-linked glycans are normally released enzymatically in this form but cross-ring fragmentation of these branched compounds was not enhanced as much as that from linear compounds and was inferior to the high-energy fragmentation obtained with a TOF/TOF mass spectrometer.

18. *N*-Acetylactosamine 6,6'-disulfate

B. Collision-Induced Decomposition (CID)

A novel method of ion isolation for CID has utilized IR-induced fragmentation and consequent removal of unwanted ions by use of a Nd:YAG laser that shone directly into the analytical cell of a modified Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer (Xie, Schuboth, & Lebrilla, 2003). The method differed from existing techniques in that it did not involve diverting the unwanted ions with magnetic or electric fields.

The paper reported the isolation of γ -cyclodextrin (**1/65**) from a mixture containing maltotetraose ((β -(1 $\rightarrow$ 4)-linked D-glucose)₄, **19**) and maltohexaose both of which also produced fragments in the analytical cell prior to irradiation with the IR laser. Infrared multiphoton dissociation (IRMPD) of alkali metal-adducted carbohydrates has also been studied in this instrument and compared with CID. Although higher energy fragmentation could be obtained with CID, IRMPD provided continuous energy transfer to the fragment ions with the result that more extensive fragmentation was achieved (Xie & Lebrilla, 2003).

Fragmentation of maltohexaose and high-mannose glycans (positive ion) with a TOF-TOF mass spectrometer with air as the collision gas has been shown to give high-energy-type fragmentation with the production of abundant cross-ring cleavage ions and, in particular, X-type cleavages not seen in the low energy spectra. Isomers of Man₇GlcNAc₂ (**20**) could be distinguished with respect to the antenna (3 or 6) to which the seventh mannose was attached by the ^{0,4}A₃ and ^{3,5}A₃ ions (Mechref, Novotny, & Krishnan, 2003). Similar results have been reported by Morelle et al. (2004) with a series of smaller sugars. A study of the effect of DHB or CHCA on fragmentation patterns of native and permethylated oligosaccharides in a TOF/TOF mass spectrometer has shown that, whereas CHCA promoted mainly glycosidic cleavages, DHB initiated glycosidic, cross-ring and internal cleavages with X-type cross-ring cleavages among the most abundant ions. Permethylated produced increased sensitivity and spectra that were easier to interpret. Studies with avidin from a DHB matrix allowed most of its *N*-glycans to be identified (Stephens et al., 2004a). A comparison of matrices used for ionization of 2-AP-labeled complex *N*-linked glycans with a TOF-TOF mass spectrometer has shown that, whereas CHCA produced only sodiated fragments in MS² spectra from [M + Na]⁺ parent ions, DHB produced both sodiated and protonated ions. It was suggested that the two matrices produced parent ions in different excited states (Kurogochi & Nishimura, 2004). However, CHCA was more effective than DHB in producing abundant PSD ions. Although providing additional information through the production of more cross-ring cleavages, acidic glycans showed extensive fragmentation before reaching the collision cell. Sialylated glycans, however, can be stabilized by permethylation or methyl ester formation, as discussed above.

20. High-mannose glycan (Man₇GlcNAc₂) with the seventh mannose residue attached to a branch of the 6-antenna.
■ = Mannose, ○ = GlcNAc

Negative ion fragmentation appears to be gaining more in popularity because of its ability to produce abundant cross-ring cleavages. The [M – H][–] ion generated by AP-MALDI from 6'-sialyl-lactose (6'-SL, **1**) using a glycerol matrix, for example,

yields the ^{0,2}A₃ ion as the most abundant fragment (Von Seggern, Moyer, & Cotter, 2003a). C-ions were prominent in the spectrum of this and larger sugars such as LSTa (Neu5Ac- α -(2 $\rightarrow$ 3)-Gal- β -(1 $\rightarrow$ 3)-GlcNAc- β -(1 $\rightarrow$ 3)-Gal- β -(1 $\rightarrow$ 4)-Glc, **21**) and LSTb (Gal- β -(1 $\rightarrow$ 3)-(Neu5Ac- α -(2 $\rightarrow$ 3))GlcNAc- β -(1 $\rightarrow$ 3)-Gal- β -(1 $\rightarrow$ 4)-Glc, **22**) and the spectra easily allowed the location of sialic acids to be determined. Cross-ring and C-type fragments were also prominent in the spectra of lithiated adducts and, in particular, the spectra of lithium salts of sialic acids (Von Seggern, Zarek, & Cotter, 2003b). Fragmentation of the [M + Li]⁺ and [M + Co]⁺ ions showed no significant difference between 3'- (**3**) and 6'-SL (**2**) but the spectra of the [M – H + 2Li]⁺ ions were different. In general, replacing the acidic proton of the sialic acid group by salt formation prevented ready loss of sialic acid under positive ion conditions and accentuated differences between the spectra of isomeric sugars containing sialic acid at different positions.

1. Multiple Successive Fragmentation (MSⁿ)

Studies with a MALDI-ion-trap-TOF instrument have shown that many of the fragments in the positive ion spectra of *N*-glycans originate from multiple sites. In the spectra of high-mannose glycans an ion formed by loss of the chitobiose core and 3-antenna is diagnostic for the composition of the 6-antenna. However, in the spectra of biantennary glycans (**23**), the abundant ion at the equivalent mass (*m/z* 712, Hex₃-HexNAc) was shown to be formed predominantly from the B₅ ion (loss of the reducing-terminal GlcNAc) and Gal-GlcNAc from each antenna (Harvey et al., 2004).

23. Biantennary *N*-linked glycan
Symbols as for **20** plus % = galactose

C. Computer Analysis of Fragmentation Spectra

The programming language Java has been used to write a program for identifying the total monosaccharides present in a tryptic glycopeptide containing up to six *O*-linked glycans from the hinge region of human serum immunoglobulin 1 (IgG1). The program also calculated the amount of each glycopeptide as a percentage of total peak area (Pouria et al., 2004).

A modification and extension to the StrOligo algorithm (Ethier et al., 2002) for assigning structures of *N*-linked glycans from their CID spectra has been published (Ethier et al., 2003). Spectra were recorded with a MALDI-Q-TOF instrument, isotope-stripped and examined for peaks corresponding to monosaccharide losses. The program then built a relationship tree that was analyzed with respect to fragment ion types and adduct. $[M + Na]^+$, the ions usually encountered in MALDI spectra of carbohydrates are now catered for as well as the $[M + H]^+$ ions featured in the original version of the program. Negative ion fragmentation was also accommodated. Greater attention to known biochemistry was incorporated in the new algorithm and the program was able to annotate spectra with the most probable structure.

A web-based tool entitled GLYCO-FRAGMENT, to be found at <http://www.dkfz.de/spec/projekte/fragments/> accepts carbohydrate structures in the extended IUPAC nomenclature (<http://www.chem.qmw.ac.uk/lupac/2carb/38.html>), as developed for the carbohydrate database, CarbBank, and calculates the masses of all possible fragments. The presence of reducing-terminal derivatives, various adducts such as sodium or lithium, persubstituted (e.g., permethyl) derivatives and substituents such as sulfate, acetate and phosphate are also accepted. B- and Y-type fragments are listed by default and masses of C-, Z- and cross-ring fragments can be obtained on demand. Internal fragments are not catered for. Output is in the form of a list or, in "view as structure" mode, ions associated with cleavage of each bond are shown when the user moves a cursor over a specific glycosidic linkage (Lohmann & von der Lieth, 2003). GlycoSearchMS (<http://www.dkfz.de/spec/glycosciences.de/sweetdb/ms/>) compares each peak in a mass spectrum with calculated fragments from all structures in the SweetDB database and the best matches are displayed (Lohmann & von der Lieth, 2004). Constituent monosaccharides can be obtained from the program GlycoMod (<http://www.expasy.ch>) as demonstrated by Ma et al. (2003a) to assign compositions to *N*-glycans released from the glycoprotein rat selenoprotein P. Reviews of available databases relating to glycomics have been published (von der Lieth, 2004a,b).

VIII. STUDIES ON SPECIFIC CARBOHYDRATE TYPES

A. Polysaccharides

Work in this area is summarized in Table 1. A comparison of the behavior of dextrans in positive and negative ion MALDI has shown the expected formation of $[M + Na]^+$ ions in positive mode whereas the negative ion spectra contained ions at $[M - 120]^-$ as the result of fragmentation. Under ESI conditions on a Shimadzu-Kratos SEQ instrument, positive ionization again produced $[M + Na]^+$ ions but with a bias towards the lower masses. The negative ion spectra were dominated by extensive A-type fragment ions. MALDI was reported to be more sensitive than ESI but ESI was more useful for structural studies (Cmelík, Stikarovská, & Chmelík, 2004). Comparisons of fragmentation modes for the $[M + Na]^+$ ions from these compounds have shown that, whereas PSD, ISD, and

CID all produced glycosidic cleavages, only ISD and CID produced significant cross-ring cleavage. Localization of the charge to the reducing terminus by formation of a 1-phenyl-3-methyl-5-pyrazolone derivative (see **1/61**) restricted cleavage to the non-reducing terminus (Bashir et al., 2004).

1. Polysaccharides from Plants

The β -D-fructan (inulin; for fructose, see **1/16**) from dahlia tubers and glucose syrup from potatoes, together with similar polysaccharides from red onion and Jerusalem artichokes, were used as reference compounds by Stikarovská and Chmelík (2004) to evaluate the best matrices for these compounds. Linear-MALDI-TOF analysis gave stronger signals than reflectron-TOF; DHB was found to be the best matrix for starch and THAP for inulin. Less effective matrices were CHCA, HABA, 3-aminoquinoline (3-AQ, **1/24**) and sinapinic acid (**1/48**). Depolymerization of most of these very large molecules is necessary before MALDI analysis. Enzymatic treatment is usually used (details in Table 1) but heat treatment is also common. Acetylation of glucuronoxylans and glucomannans has received considerable attention (Table 1) with some evidence of acetyl group migration during depolymerization (Kabel et al., 2003). Acetylated glucuronoxylans and glucomannans have been recovered from flax shive following hydrothermal treatment but in this case, acetyl migration was not reported (Jacobs et al., 2003). Acetylation has also been found on the mannose residues of glucomannans from birch and aspen wood and appear to be randomly distributed (Teleman et al., 2003). MALDI-TOF spectra from these two papers consisted of an extensive series of peaks with a mass separation of 42 units (masses up to 5,500) but considerable simplification was found after deacetylation.

Fernández et al. (2003) have examined arabinoxylans from wheat by MALDI and ESI mass spectrometry and detected structures with up to 22 monosaccharide residues. As arabinose (**1/2**) and xylose (**1/3**) are isobaric, it was not possible to study the distribution of arabinose residues along the xylose backbone. However, this problem was overcome by permethylation; arabinose residues attracted three methyl groups, unsubstituted internal xylose residues, two and substituted residues one group.

2. Synthetic (Modified) Polysaccharides

Matrix-assisted laser desorption/ionization (MALDI) conditions for determination of the substitution patterns in methyl- (Momcilovic et al., 2003b) and carboxymethyl-cellulose (Momcilovic et al., 2003a) have been evaluated. Depolymerization was achieved both by use of enzymes or acid and the products fractionated by size-exclusion chromatography (SEC). DHB, CHCA, HABA and indoleacrylic acid (IAA, **3/5**) were tested as matrices for methylcellulose but little difference was found between them. The choice of solvent, however, did have a significant effect, probably due to differential solubility of the variously methylated compounds. Measurements on two acid-depolymerized samples of methylcellulose with different degrees of substitution gave values that agreed well with the

TABLE 1. Use of MALDI MS for examination of carbohydrate polymers from plants, fungi, and lichens

Species	Carbohydrate	Depolymerization	Techniques ¹	Glycans	Notes	Reference
<i>Agave tequilana</i> Weber var. <i>azul</i>	Fructans	-	TOF (+ve) (DHB) NMR, GC/MS	β -D-Fruf	Structural characterization. Not inulin type as originally thought. Fructans with DP from 3 to 30	(Lopez, Mancilla- Margalli & Mendoza- Diaz, 2003)
<i>Agropyron</i> <i>cristatum</i> (Crested wheatgrass)	Fructans	-	TOF (DHB)	Glc, Fru	Structural determination from <i>Agropyron cristatum</i> L. $\times$ 4. <i>desertorum</i> (Fisch.) Schult. Hybrid variety CD-II.	(Chatterton & Harrison, 2003)
<i>Arabidopsis</i> <i>thaliana</i>	Xyloglucans	<i>Endo</i> - β -(1 $\rightarrow$ 4)- glucanase	R-TOF (DHB)	Glc, Xyl	β -Xylosidase gene <i>AtBXL1</i> involved in 2 nd cell wall metab.	(Goujon, <i>et al.</i> , 2003)
<i>Argania spinosa</i> (Argan tree)	Hemicellulose	<i>Endo</i> - β -D-(1 $\rightarrow$ 4)- xylanase	R-TOF (DHB), CID, PSD, NMR, GLC	Xyl, 4-OMe-GlcA, Ara, Rha, Fuc, Glc GalA, Man, Gal	Identification of novel XUFG motif	(Ray, <i>et al.</i> , 2004)
<i>Aspergillus</i> <i>wentii</i>	Galactomannans	TFA, Acetolysis	R-TOF (DHB), NMR	β -D-Gal, Man	Structural characterization. Same as <i>Chaetosartorya chrysella</i>	(Gómez-Miranda, <i>et</i> <i>al.</i> , 2004)
<i>Atractylodes</i> <i>lancea</i> DC	Arabinogalactan	<i>Endo</i> - β -D-(1 $\rightarrow$ 6)- galactanase	TOF (DHB), (2- AB, ABOE)	Araf, Gal	Structure of oligosaccharides that modulate intestinal immune system	(Taguchi, <i>et al.</i> , 2004)
<i>Azorhizobium</i> <i>caulinodans</i>	Cyclic β -glucan	-	TOF (DHB) NMR, GC/MS	β -D-Glc	Consist of 10 to 13 β -(1 $\rightarrow$ 3)- and β -(1 $\rightarrow$ 6)-linked glucose residues	(Komaniecka & Choma, 2003)
Aspen wood	Glucomannan	-	TOF (DHB) SEC, HPAEC, NMR	Glc, Man	Random acetylation on Man residues of glucomannans.	(Teleman, <i>et al.</i> , 2003)
Barley	Starch	Enzyme	L-TOF (+ve) (DHB)	Glc	Quantification of glucose from starch. Sorbitol as internal stand.	(Grant, <i>et al.</i> , 2003)
Barley (hull-less)	Starch	Heat	MALDI	Glc	Study of structural changes caused by heating	(Li, <i>et al.</i> , 2004c)
Birch wood	Glucomannan	-	TOF (DHB) SEC, HPAEC, NMR	Glc, Man	Random acetylation on Man residues of glucomannans.	(Teleman, <i>et al.</i> , 2003)
<i>Brassica</i> <i>campestris</i> L. (Field mustard)	Hemicellulose	<i>Endo</i> -(1 $\rightarrow$ 4)- β - xylanase	TOF(DHB) GLC, GC/MS, SEC	Rha, Fuc, Ara, Xyl, Man, Gal, Glc	Structural characterization of polysaccharides from seed cake	(Ghosh, <i>et al.</i> , 2004)
<i>Chaetosartorya</i> <i>chrysella</i>	Galactomannans	Acetolysis	R-TOF (DHB), NMR	β -D-Gal, Man	Structural characterization. Same as <i>Aspergillus wentii</i>	(Gómez-Miranda, <i>et</i> <i>al.</i> , 2004)
<i>Chlorella</i> (green alga)	GlcA-containing	vAL-1 (novel polysaccharide lyase)	R-TOF (DHB), PSD	GlcA	vAL-1, encoded by chlorovirus CVK2 cleaves GlcA-chains	(Sugimoto, <i>et al.</i> , 2004)
Citrus pectin	Pectin	Galacturonase from <i>Aspergillus niger</i>	TOF, ESI, NMR	GalA, Me-GalA	New alcoholic pectins prepared from natural pectin. Low activity.	(Rosenbohm, <i>et al.</i> , 2003)
<i>Coffea arabica</i> (green coffee)	Galactomannans	Endomannase	TOF (DHB/HIQ)	Man, Gal, Acetyl groups	Structural characterization	(Oosterveld, <i>et al.</i> , 2004)
Commercial sample	Hyaluronic acid	Hyaluronic acid lyase from <i>Streptococcus</i> <i>agalactiae</i>	R-TOF, L-TOF (s-DHB)	GlcA, GlcNAc	Structural determination in pharmaceutical formulations	(Kühn, <i>et al.</i> , 2003)

(Continued)

TABLE 1. (Continued)

Species	Carbohydrate	Depolymerization	Techniques ¹	Glycans	Notes	Reference
Commercial sample	Tannin	-	TOF (DHB)	Gallic acid (26), quinic acid (27)Glc	Structural characterization. Inhibition of salivary α -amylase	(Kandra, <i>et al.</i> , 2004)
Corn	Starch	Enzyme	L-TOF (+ve) (DHB)	Glc	Quantification of glucose from starch. Sorbitol as internal standard.	(Grant, <i>et al.</i> , 2003)
<i>Craterostigma wilmsii</i>	Xyloglucans	<i>Endo</i> -glucanase	R-TOF (DHB)	Ara, Rha, Xyl, Man, Gal, Glc, GlcA, GalA	Study of cell-wall composition of dehydrated resurrection plant. More Gal than hydrated leaves.	(Vicré, <i>et al.</i> , 2004)
<i>Eleusine coracana</i> (Finger millet)	Arabinoxylans	<i>Endo</i> -xylanase from <i>Trichoderma viridae</i>	TOF, GC/MS, IR, OR	Ara, Xyl	Structural characterization	(Subba Rao & Muralikrishna, 2004)
<i>Eucalyptus</i> wood	Xylooligosaccharides	Hydrothermal	TOF (DHB) SEC, HPLC, NMR	Xyl, Ac-Xyl	<i>O</i> -Acetyl groups migrate during hydrothermal depolymerization	(Kabel, <i>et al.</i> , 2003)
Flax shive	Acetyl-glucomannan	Hydrothermal	L-TOF (+ve, -ve) (DHB) SEC	Glc, Man	No acetyl migration after hydrothermal treatment	(Jacobs, <i>et al.</i> , 2003)
Flax shive	Xylooligosaccharides	Hydrothermal	L-TOF (+ve, -ve) (DHB) SEC	Xyl, Me-GlcA	Isolation and characterization of water-soluble hemicelluloses	(Jacobs, <i>et al.</i> , 2003)
<i>Gelidium amansii</i>	Agar	Agarase	TOF (DHB)	D-Gal, sulfate	To study antioxidative properties	(Wang, <i>et al.</i> , 2004a)
<i>Gramineae</i> and <i>Solanaceae</i>	Xyloglucans	Three xyloglucan-specific enzymes	TOF	Glc, Xyl	Structure and comparison with other dicotyledonous plants	(Kato, Ito & Mitsuishi, 2004)
Grape	Xyloglucans	Xyloglucan-specific endoglucanase	TOF (DHB/HIQ) GC/MS	Xyl, Glc, Gal, Fuc (Man. Ara, Rha)	XXXG, XXFG and XLFG major in mesocarp and exocarp cell wall	(Doco, <i>et al.</i> , 2003)
<i>Lasallia pustulata</i> (Lichen)	Pustulan Galactomannan	Acetolysis	R-TOF (DHB) NMR, IR, GLC	β -glucan-chitin Gal, α -Man	Main constituents are pustulan, a β -glucan-chitin complex and a β -(1 $\rightarrow$ 3)-glucan	(Pereyra, <i>et al.</i> , 2003)
<i>Lycopersicon esculentum</i> (Tomato)	Xyloglucans	Xyloglucan-specific endoglucanase	TOF (DHB/HIQ) NMR	α -Ara, β -Ara, Xyl, Glc, Gal	Structure of xyloglucan produced by suspension-cultured tomato cells.	(Jia, <i>et al.</i> , 2003)
<i>Manihot esculenta</i> Crantz, (Cassava)	Starch	Iso-amylase	TOF ("standard matrix")	Glc	Study of different strains. One has more water-soluble glycans and unusual starch with low branching	(Carvalho, <i>et al.</i> , 2004)
<i>Mesorhizobium haukuui</i>	Cyclic- β -glucan	-	TOF (DHB) NMR, GLC	β -D-Glc	Consist of 17 - 28 β -(1 $\rightarrow$ 2)-linked glucose residues	(Choma & Komaniecka, 2003)
<i>Mycobacterium bovis</i>	Anti-neoplastic glucans	Pullulanase	R-TOF (DHB), NMR, GC/MS	β -D-Glc	Polymers of β -(1 $\rightarrow$ 4)-linked Glc with branches at 6-position	(Dinadayala, <i>et al.</i> , 2004)
<i>Mycobacterium kansasii</i>	Lipomannan Lipoarabinomannan	Mild acid hydrolysis	TOF (DHB) ESI, GC/MS	α -Man, Araf, 5-Me-thiopentose	Identification of novel features in a clinical isolate	(Guérardel, <i>et al.</i> , 2003)
<i>Nothogenia erinacea</i>	Xylan	Xylanase	R-TOF (DHB) ESI, NMR	Xyl	Comparison of xylanases from different families	(Nerinckx, <i>et al.</i> , 2004)
<i>Olea europaea</i> (Olive)	Pectic polysaccharides	Polygalacturonase, arabinofuranosidase	MALDI, HPLC	Rha, Gal, Fuc, Xyl, Ara, Man, Glc	Structural determination related to oil extraction	(Vierhuis, <i>et al.</i> , 2003)

Species	Carbohydrate	Depolymerization	Techniques ¹	Glycans	Notes	Reference
Olive pulp	Xylooligosaccharides	Mild acid hydrolysis	L-TOF (DHB/CMBT) layer, SEC	Xyl, GlcA, Me- GlcA	GlcA and Me-GlcA residues irregularly distributed. Pulp has mainly Me-GlcA, seed hull, GlcA	(Reis, <i>et al.</i> , 2003)
<i>Picea abies</i> (Spruce wood)	Galactoglucomannan	Hydrothermal	L-TOF (+ve, -ve) (DHB) SEC	Man, Glc, Gal, Xyl, Ara	Effect of temperature and pH for extn. of hemicelluloses. 2% NaOH increased high mass (14 kDa) extn. but removed OAc groups	(Lundqvist, <i>et al.</i> , 2003)
<i>Picea abies</i> (Spruce wood)	Hemicellulosic oligomers	Steam	SEC-MALDI	Mannan and xylan	Investigations of compounds that can replace fossil-based products	(Palm & Zacchi, 2004)
Potato, Sweet potato	Acylation starch	α -, β -amylase, amylglucosidase	TOF	Glc	Differently sized granules differ in acyl substitution pattern	(Chen, Schols & Voragen, 2004)
Red seaweed from China sea	κ -Carrageenans	Carrageenase from marine <i>Cytophaga</i> MCA2	TOF (DHB)	Sulfated D- galactose	Structural determination	(Mou, <i>et al.</i> , 2004)
Red wine	Rhamnogalacturonan II	Endogalacturonase Acid hydrolysis	TOF (+ve) (DHB) NMR	β -L-Araf, Me- α -L- Fucp, α -L-Arap, α - L-Rhap, β -L-Rhap β -D-Galp, α -L- AcetfA, β -D-Apif	Structural determination of heptasaccharide from red wine rhamnogalacturonan	(Glushka, <i>et al.</i> , 2003)
<i>Scheelea phalerata</i> (Palm)	Heteroxylan	Partial acid hydrolysis	TOF (DHB), NMR, GC/MS	GlcApA, α -GalpA, β -Xylp, α -GlcApA, Araf	Structural characterization	(Simas, <i>et al.</i> , 2004)
<i>Schizosacharo- myces pombe</i>	Glucans	NaOH/Zymolyase	TOF (CHCA), NMR	Glc, Man	Structural characterization	(Sugawara, <i>et al.</i> , 2004)
<i>Solanum tuberosum</i> (potato)	Phosphorylated starch	Isoamylase	TOF (DHB)	Glc-phosphate	Phosphorylation of starch is required for degradation	(Ritte, <i>et al.</i> , 2004)
Sugar beet pulp	Acetylated homogalacturonan	Endogalacturonase from <i>Fusarium moniliforme</i>	TOF (THAP), HPAEC	GalA, Me-GalA	Products of enzymolysis show decreasing hydrolysis for more highly acetylated compounds	(Bonnin, <i>et al.</i> , 2003)
Sweet potato	Starch	Water	TOF (DHB)	Glc	Investigation of hydrothermal conditions for glucose production	(Nagamori & Funazukuri, 2004)
Tamarind	Xyloglucan	<i>Pyrococcus furiosus</i> β 1 $\rightarrow$ 4-endoglucanase	TOF (DHB) GLC	Xyl, Glc	Structural determination. Fragments with 2-9 residues.	(Marry, <i>et al.</i> , 2003)
Wheat	Arabinoxylans	Endoxylanase A	R-TOF (DHB), ESI, MS/MS	Ara, Xyl	Structures with up to 22 monosaccharide residues detected.	(Fernández, <i>et al.</i> , 2003)
Wood, various species	Hemicelluloses	-	MALDI, SEC	Xyl, HexA	Xylan more abundant in surface layers of pulp fibres	(Dahlman, Jacobs & Sjöberg, 2003)

(1) Instrument (matrix) other technique, sample (derivative).

data supplied by the manufacturers. However, data from the carboxymethyl-celluloses was less satisfactory even though samples with different degrees of substitution could easily be distinguished from each other.

A method has been published for detecting hydroxyethyl starch in urine. The compound is used as a plasma volume expander by athletes and has been banned by the International Olympic Committee. Partial acid hydrolysis of urine was carried out with TFA and the product was examined directly by MALDI-TOF using super-DHB (s-DHB). The method was claimed to provide unambiguous identification in only 90 min (Gallego & Segura, 2004).

3. Cyclodextrins and Related Compounds

Of the matrices DHB, *nor*-harmane (**1/35**), CHCA, HABA, IAA, and sinapinic acid, DHB and *nor*-harmane were found by Xiong et al. (2003b) to give the strongest and best resolved spectra from macrocyclic polysaccharides containing from 19 to 25 sugar residues. Sinapinic acid gave a very weak signal and was only recorded in linear mode. The liquid matrix, CHCA/3-AQ/glycerol also gave a strong and long-lasting signal but with inferior resolution. Addition of alkali metal salts produced the expected adducts.

A glass slide containing a UV absorbing TiO₂ sol–gel film from which imprinted α -cyclodextrin (CD (cyclic-(1 $\rightarrow$ 4)- α -D-glucose)₆, **24**) molecules had been removed has been used to selectively adsorb α -CD from a mixture of α -, β -, and γ -CD. The α -CD was detected directly by MALDI-MS without the addition of extra matrix (Chen & Chen, 2004).

Cyclic-(1 $\rightarrow$ 4)- α -D-Glc)₆

24. α -Cyclodextrin

Experiments with α -, β -, and γ -cyclodextrins have shown that the larger molecules have greater affinity for larger alkali metals suggesting that the products are inclusion complexes that can be ionized intact. Sinapinic acid was used as the matrix and under these conditions, dextran, a linear polymer, produced only [M + H]⁺ ions (Bashir et al., 2003a). Inclusion complexes with cyclodextrins have recently been used to investigate the composition of humic acids from Antarctica. The structure of humic acids is still largely unknown even though they are widely distributed in nature. Gajdošová et al. (2003) recorded the MALDI spectra of humic acids with and without γ -cyclodextrin and found that several constituents appeared to form inclusion complexes. Thus, for example, a shift in the cyclodextrin peak by 66.0 mass units after addition of humic acid from a standard soil sample was interpreted as consistent with inclusion of a cyclopentadiene radical (C₅H₆⁺). Results from the soils from Antarctica were similar. MALDI spectra were recorded without a matrix to simplify the spectra. An investigation of the mechanism of the well-known migration of alkyl-silyl groups from the two- to the three position in cyclodextrins has shown that the receiving oxygen is that from the same ring as the migrating silyl group and not to the adjacent ring (Teranishi & Ueno, 2003).

4. Other Polysaccharides

Matrix-assisted laser desorption/ionization (MALDI) sample preparation protocols for examination of Curdlan ((($\rightarrow$ 3)- β -D-Glc-(1 $\rightarrow$))_n, **25**), a polyglucose obtained from the bacterium *Alcaligenes faecalis* var. *myxogenes* 10C3, have been reported. The crude sample was separated into a low molecular weight, water-soluble portion and a high-molecular weight, water-insoluble portion. The low molecular weight portion was examined from DHB containing ammonium fluoride and gave two low-mass (<4 kDa) polysaccharide distributions differing by 16 Da. The high molecular weight, water-insoluble portion was found to produce good signals from a mixture of DHB and 3-AQ in dimethylsulfoxide (DMSO) that was dried on a hot-plate at 70°C. Ions with masses of up to 15,000 were observed (Chan & Tang, 2003). Spectra of extracellular polysaccharides from the colony-forming prymnesiophyte algae *Phaeocystis globosa* and *P. antarctica* showed peaks of less than 1,000 Da, some of which had a repeat pattern of 192 Da but the monosaccharide units were not identified (Solomon et al., 2003).

(($\rightarrow$ 3)- β -D-Glc-(1 $\rightarrow$))_n

25. Curdlan

Supplementation of a pre-term formula with a mixture of galacto- and fructo-oligosaccharides with a molecular weight range similar to that of carbohydrates found in human milk as determined by MALDI-TOF analysis has been found to have a stimulating effect on the growth of bifidobacteria in the intestine and results in more frequent produced and softer stools. Thus, prebiotic mixtures such as the studied oligosaccharide mixture might help in improving intestinal tolerance to enteral feeding in pre-term infants (Boehm et al., 2003).

B. Glycoproteins

1. Intact Glycoproteins

It is unusual for all but the simplest, low mass glycoproteins to produce MALDI-TOF spectra with resolved glycoforms but broad peaks and significant mass differences between observed and calculated protein masses for larger glycoproteins suggest glycosylation. Thus, epoetins (erythropoietins), with several glycosylation sites occupied by sialylated complex glycans, produced only broad peaks with masses in the range of 30–35 kDa and half-height peak widths of up to 5 kDa when examined by MALDI-TOF (Stanley & Poljak, 2003). Deglycosylation, however, produced sharp peaks from the protein with only minimal tailing. Human follistatin expressed in CHO cells has two *N*-linked glycosylation sites at Asn-95 and 259. Its MALDI spectrum contained three peaks; the first at *m/z* 31,525 corresponded to the unglycosylated protein and the other two at *m/z* 33,804 and 35,600 were produced by unresolved glycopeptides. LC/MS analysis identified nine complex glycans (Hyuga et al., 2004). Several partially resolved peaks from 52 to 55.5 kDa suggest glycosylation of human β -secretase catalytic domain, confirmed as *N*-linked by digestion with PNGase F (Wang et al., 2004b). Reduction in mass of 1.4 kDa of a humanized anti-HBs Fab fragment by treatment with Endo-H gave a mass that was still

3.7 kDa above that of the protein. The authors speculated that the extra mass might indicate *O*-linked glycosylation but no proof was provided (Ning et al., 2003a). Similarly, a reduction in the mass of approximately 4.5 kDa of a lysosomal serine carboxypeptidase from *Trypanosoma cruzi* following Endo-H treatment suggested the presence of two to three *N*-linked glycans (Parussini et al., 2003). Other carbohydrates detected by mass-difference measurements of proteins before and after deglycosylation include GlcNAc in Cu/Zn-superoxide dismutase from fungus *Humicola lutea* 103 (Dolashka-Angelova et al., 2004b) and the high-mannose glycan Man₁₀GlcNAc₂ in ovotransferrin expressed in *Pichia pastoris* (Mizutani et al., 2004).

Matrix-assisted laser desorption/ionization (MALDI) analysis is also useful for detecting the absence (Perteguer et al., 2004) or modification of glycosylation. Thus, the mass of human transferrin produced in insect (*Drosophila melanogaster* S2) cells is consistent with lack of sialic acids on its *N*-glycans (Lim et al., 2004).

2. *N*-Linked Glycans

a. Analysis of derived glycopeptides and site occupancy. Although *N*-linked glycans have long been known to attach to a consensus sequence consisting of an Asn-Xxx-Ser(Thr) motif, where Xxx can be any amino acid except proline, evidence is now emerging that Asn-Xxx-Cys can also act as a motif. Satomi, Shimonishi, and Takao (2004) have identified a third site based on this motif, occupied by approximately 2% of the glycosylation, in human transferrin.

Proteolysis, usually with trypsin, to localize each consensus sequence site to an individual glycopeptide is the classical method for determination of the glycosylation at each site. Detection of glycopeptides by HPLC following proteolysis is often difficult because of effects such as signal suppression. Monitoring of oxonium ions (163 for hexose, 204 for GlcNAc, etc.) is a standard method for tracking the glycopeptides and has been extended to monitoring with an ion trap mass spectrometer by generating them in the ion-source region (Sullivan, Addona, & Carr, 2004). Peptide masses were confirmed by MALDI-TOF MS after desalting with C18 ZipTips. A method for predicting peptide retention times in reversed-phase HPLC has been published (Krokhin et al., 2004b) following examination of 346 tryptic peptides and deglycosylated glycopeptides identified by MALDI-TOF MS from 17 proteins. Glycopeptides eluted slightly earlier than their deglycosylated versions. Liu, Feasley, and Regnier (2004) have evaluated the technique of diagonal chromatography which involves comparisons of HPLC chromatograms run under identical conditions before and after enzymatic removal of the glycans and have concluded that, although the technique worked well for detection of protein phosphorylation, retention times of the glycopeptides and derived peptides, identified by MALDI-TOF MS, were too similar for diagonal chromatography to be a reliable technique for detecting glycopeptides. MALDI-TOF MS has the advantage over ESI-MS for the detection of glycopeptides in that the spectra usually only contain singly charged ions unlike ESI with its tendency to produce multiply charged ions. Glycopeptides are often revealed in MALDI profiles by their relatively high mass

and by peak spacing corresponding to monosaccharide residues (e.g., 162 for hexose) (Krokhin et al., 2004a).

Rather than attempt detection of glycopeptides in the presence of peptides, some investigators employ fractionation techniques to isolate the glycopeptides before mass spectrometric analysis. For example, Wada et al. (2004) have developed a method whereby glycoproteins are reduced and alkylated, cleaved with trypsin and lysylendopeptidase and partitioned with cellulose or Sepharose to bind the glycopeptides. The isolated glycopeptides could then be examined by LC or linear MALDI-TOF MS/MS in an ion-trap-TOF instrument provided peptide fragments for protein identification and carbohydrate ladders that gave information on the glycan composition. The method was applied to transferrin, IgG and β_2 -glycoprotein 1. A method has been reported for identification and quantification of *N*-linked glycoproteins following their specific immobilization on a solid support (Zhang et al., 2003a). *Cis*-diol groups in the carbohydrate were oxidized with periodate to aldehydes allowing the carbohydrate to be captured by hydrazide chemistry. The protein was then cleaved with trypsin or another suitable enzyme and the resulting peptides were released with PNGase F for analysis by MALDI or ESI mass spectrometry. Deuterium labeling of the peptide with succinic anhydride allowed quantitative studies to be made.

The frequent absence of glycopeptides in LC/MS analyses has been used to advantage in indicating which peptide might be glycosylated. Thus, peptides containing the consensus *N*-glycosylation sites at Asn-142, 173, and 178 were not observed by Hambrock et al. (2004) from the tryptic digest of the recombinant protein TSC-36/Flik expressed in human cells suggesting that these sites were glycosylated.

An et al. (2003b) have used pronase to digest two well-characterized glycoproteins and found that steric factors prevented complete digestion in the region of the glycosylation site leaving the carbohydrate attached to a short peptide. *N*-glycan masses were obtained by MALDI-FT MS from a separate experiment involving glycan removal by PNGase F and subtracted from those of the glycopeptides to obtain the peptide mass and, hence, its structure. The method was claimed to be more rapid than the traditional method of proteolysis and the results easier to interpret because no large peptides appeared in the final sample. The method was applied to the study of site heterogeneity of *Xenopus laevis* egg cortical granule lectin.

Asn to Asp conversion after PNGase digestion effectively labels the percentage of a given site that is occupied and is detected by a mass difference of one unit. The technique has been used, for example, to show glycosylation in 10 of the 11 consensus sequence sites of the SU component of avian sarcoma/leukosis virus envelope glycoprotein (Kvaratskhelia et al., 2004) and to identify occupied glycosylation sites in membrane-bound glycoproteins. Normally this latter task is difficult because 2D sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gels are ineffective in separating these compounds. To overcome the problem, the proteins were solubilized in guanidine-HCl, digested with trypsin and the glycopeptides were recovered by lectin binding. Following removal of the *N*-glycans with PNGase F, the peptides with the Asn to Asp conversion were detected by MALDI-Q-TOF MS (Fan et al., 2004). Investigations of the spike glycoprotein from the SARS

virus have revealed four occupied and one unoccupied *N*-linked sites by molecular weight measurements made before and after glycan removal by PNGase F and by observing the Asn to Asp conversion (Ying et al., 2004). In a similar experiment, digestion of the glycoprotein N-CAM with AspN and trypsin resulted in the identification of glycopeptides, each containing a single glycosylation site. They were separated by HPLC and analyzed by MALDI-TOF MS. Five of the six sites were identified but glycopeptides from site Asn-4 were missing. This site was eventually characterized by a direct MALDI-FTICR analysis of the glycopeptides released in-gel by trypsin (Albach et al., 2004). Incorporation of ^{18}O into the aspartic acid from H_2^{18}O can be used to label the newly produced aspartic acid and was used by Barinka et al. (2004) to identify and confirm glycosylation on each of the 10 consensus sequence sites of glutamate carboxypeptidase II.

b. N-Linked glycan composition from glycopeptide analysis. Measurement of glycan masses directly or by subtraction of the mass of the peptide from those of glycopeptides leads directly to the constituent isobaric monosaccharide composition as illustrated by a recent study of *N*-glycan masses from the androgenic hormone of *Porcellio scaber* (Crustacea) (Grève et al., 2004). Sometimes, with large glycopeptides, peaks are poorly resolved but can often be improved by chemical treatments. Thus, for example, the core fragment of human luteinizing hormone gave a broad, poorly resolved peak (m/z 8,700–10,700) when examined from sinapinic acid with a linear TOF instrument (Jacoby, Kicman, & Iles, 2003). The compound consisted of two peptide chains, only one of which was glycosylated, linked with four disulfide bonds. These bonds were reduced on the MALDI target with dithiothreitol (DTT) to give a more fully resolved envelope of peaks from which the *N*-linked glycan composition was deduced by subtraction of the peptide masses.

c. Glycan release. Most structural determinations of these compounds are performed on released glycans. Experimental details for chemical and enzymatic release of *N*-glycans have been described (Merry & Astrautsova, 2003). Release with PNGase F (or PNGase A if the glycans contain fucose in α -(1 $\rightarrow$ 3)-linkage to the core GlcNAc) or hydrazine are the preferred enzymatic and chemical release methods respectively. No new chemical release techniques or modifications were found during the review period reflecting the increasing popularity of enzymatic release, particularly with PNGase F. Additionally, PNGase F release is not accompanied with the potential hazards of hydrazine. However, there is some controversy as to whether this enzyme releases all glycans, particularly from larger glycoproteins. Thus, it has been shown not to release $\text{Glc}_1\text{Man}_9\text{GlcNAc}_2$ (**28**) from arylophorin under non-denaturing conditions (Kim et al., 2003) and, in addition, Reinhold et al. (Zhu et al., 2004a) assert that PNGase F fails to release a subset of glycans from *C. elegans* and thus use hydrazine. Artifacts detected by MALDI-TOF analysis after in-gel release with PNGase F have included glycans with urea attached to the reducing terminus (Omtvedt et al., 2004) as the result of its inclusion in an earlier procedure for glycoprotein purification. Deglycosylation with trifluoromethanesulfonic acid, a method

that leaves the protein intact for further study has been reviewed (Edge, 2003).

26. Gallic acid

27. Quinic acid

28. High-mannose glycan $\text{Glc}_1\text{Man}_9\text{GlcNAc}_2$
Symbols as for structure **20** plus $\square$ = glucose

d. Applications of MALDI MS to the detailed structural determination of N-linked glycans. The ability to carry out the classical method of exoglycosidase sequencing by performing the digestions on the MALDI target, with DHB as the matrix, has been demonstrated by Morelle and Michalski (2004) using the well-studied glycoproteins α 1-antitrypsin and ribonuclease B. Gutternigg et al. (2004), however, preferred ATT as the matrix because of its neutrality.

A large number of studies have been conducted on the structural determination of *N*-glycans from isolated glycoproteins and from intact organisms or tissues; these are summarized in Tables 2 and 3 respectively. Among the more unusual glycosylations to have been reported are the presence of $\text{Glc}_1\text{Man}_9\text{GlcNAc}_2$ (**28**) in the insect *Antheraea pernyi* (Chinese oak silkworm) (Kim et al., 2003), the presence of 3-*O*-Methylgalactose and 3-*O*-Me-GlcNAc (bisecting GlcNAc) in biantennary glycans from the marine snail *Rapana venosa* (Dolashka-Angelova et al., 2004a) and 3-*O*-Me-galactose and -mannose from high-mannose glycans of the slug *Arion lusitanicus* (Gutternigg et al., 2004). 3- and 6-difucosylation have been found on the core GlcNAc residue of $\text{Man}_3\text{GlcNAc}_2\text{Fuc}_2$ (**29**) from *Todarodes pacificus* (Japanese flying squid) together with attachment of a β 4-linked galactose to the 6-linked fucose

(text continued on page 297)

29. Fucosylated *N*-glycan from *Todarodes pacificus*
Symbols as for structure **20** plus $\diamond$ = fucose

TABLE 2. Use of MALDI MS for examination of *N*-glycans from specific glycoproteins (see also Table 10, biopharmaceuticals)

Glycoprotein	Type	Release, Peptide cleavage	Instrument (Matrix) Other technique Sample (derivative)	Glycans	Notes	Reference
Acetylcholine esterase	Rhesus macaques	?	TOF (DHB), Glycans (2-AB, Me)	Complex, di-, tri-, tetra-antennary	Peptides, not glycosylation control residence time	(Cohen, <i>et al.</i> , 2004)
β -(1→6)- <i>N</i> -Ac-glucosaminyl-transferase V	Human recombinant in CHO cells	PNGase F Trypsin	R-TOF (DHB), ESI, MS/MS, LC/MS, Glycans (2-AP, Per-Me)	Complex, Asn-334 biantennary, 433, 447 (mixed)	Site-specific glycan characterization.	(Kamar, <i>et al.</i> , 2004)
α 1-Acid glycoprotein	Human	PNGase F	R-TOF (DHB), Glycans and Me esters	Complex	Altered glycosylation in patients with inflammation and diabetes.	(Higai, <i>et al.</i> , 2003a)
α 1-Acid glycoprotein	Human, ox, sheep, rat	PNGase F Trypsin, chymotrypsin	L-TOF (DHB), Glycans (2-AA)	Complex	Comparison of glycosylation in four species. Different glycosylation in each.	(Nakano, <i>et al.</i> , 2004)
α 1-Acid glycoprotein	Human	PNGase F Trypsin	L- + R-TOF (DHB), Glycans (various hydrazones)	High-mannose hybrid, complex	Use of cationic or hydrophobic derivs. to enhance signal	(Shinohara, <i>et al.</i> , 2004)
α 1-Acid glycoprotein	Human	PNGase F	TOF, Glycans (2-AA)	Complex, fucosylated di- and tri- antennary	New separation method. Fuc-glycans up in cancer	(Szöllosi, <i>et al.</i> , 2004)
α 1-Acid glycoprotein	Chicken	Trypsin	R-TOF, L-TOF (CHCA), Glycopeptides	-	Det ⁿ . of glycosylation sites (Asn-16, 70, 77, 87, 62 (partially))	(Matsunaga, Sadakane & Haginaka, 2004)
α 1-Acid glycoprotein	Human	PNGase F	R-TOF (DHB), Glycans (2-AA)	Complex	Cancer patients showed raised AGP with abnormal glycans	(Kremmer, <i>et al.</i> , 2004)
Aminopeptidase N	<i>Manduca sexta</i>	PNGase A PNGase F Trypsin	TOF/TOF (CHCA, DHB), Glycans (Per-Me)	Paucimannosidic, Asn-295, 609, 623, 752	Structural identification with TOF/TOF. Location of fucose residues	(Stephens, <i>et al.</i> , 2004b)
α 1-Antitrypsin	Human plasma	Hydrazine	R-TOF (DHB), MALDI-Q-TOF, LC/MS, Glycans (2-AB)	High-mannose, hybrid, complex	Identification of congenital disorders of glycosylation	(Butler, <i>et al.</i> , 2003)
α 1-Antitrypsin	Human hepatoma cells	PNGase F	R-TOF (DHB), Glycans	Complex	Increase in 1→3-fucosylation in hepatoma cell line	(Higai, <i>et al.</i> , 2003b)
α 1-Antitrypsin	-	PNGase F Trypsin	TOF (DHB), Glycans (benzylamine)	Complex (di-, tri-, tetra-antennary)	As reference compound to demonstrate on-target digestion	(Morelle & Michalski, 2004)
Amyloid fibril	Human	Trypsin	TOF, Glycopeptide	Complex	Detection of one occupied glycosylation site	(Karimi, Sletten & Westermarck, 2003)
Androgenic hormone	<i>Porcellio scaber</i> (Crustacea)	-	L-TOF (CHCA), Glycopeptide (by difference)	Complex, Asn-111	Structural identification	(Grève, <i>et al.</i> , 2004)
α 1-Antitrypsin	Human plasma	PNGase (In gel), Trypsin	L-TOF (-ve) (THAP/NH ₄ -citrate), Glycans	Complex, Asn-46, 83, 247	Under-glycosylation of plasma α 1-antitrypsin not random	(Mills, <i>et al.</i> , 2003a)

(Continued)

TABLE 2. (Continued)

Glycoprotein	Type	Release, Peptide cleavage	Instrument (Matrix) Other technique Sample (derivative)	Glycans	Notes	Reference
Acetylcholine esterase	Human in HEK293 cells	-	TOF, Glycans (2-AB, Me ester)	Hybrid, complex (di-, tri-, tetra-antennary)	Effect of post-translational modifications on circulatory life	(Shafferman, <i>et al.</i> , 2004)
Arylphorin	<i>Antherara pernyi</i> (Chinese oak silkworm)	PNGase F, (In-gel)	Sector (DHB), Glycans (2-AB)	High-mannose	Identification of Glc ₁ Man ₉ GlcNAc ₂	(Kim, <i>et al.</i> , 2003)
Asialofetuin	Fetal calf serum	PNGase F Trypsin	L- + R-TOF (DHB), Glycans (hydrazones)	Complex	Use of cationic or hydrophobic derivatives, to enhance signal	(Shinohara, <i>et al.</i> , 2004)
Avidin	Chicken egg white	PNGase F	TOF/TOF (DHB) Glycans (free and per-Me)	High-mannose, complex	Study of the effects of DHB and CHCA on fragmentation	(Stephens, <i>et al.</i> , 2004a)
Bile salt-stimulated lipase	Human recombinant in <i>Pichia pastoris</i>	Endo H	R-TOF (DHB), Glycans	High-mannose, Asn-187	Structural determination. Polymannose containing glycans	(Trimble, <i>et al.</i> , 2004)
BM-40 (SPARC, osteonectin)	Human bone, platelets, osteosarcoma cells	PNGase F	R-TOF (Sinapinic acid), Glycans	High-mannose, hybrid, complex	Glycosylation tissue-dependent suggesting different functional roles.	(Kaufmann, <i>et al.</i> , 2004)
CA125	Ovarian tumour	PNGase F Trypsin	R-TOF (DHB), GC/MS, FAB, CID, Glycans (Per-Me)	Complex	Glycan identification in human ovarian tumour marker	(Wong, <i>et al.</i> , 2003)
N-Cadherin	Human melanoma cells	PNGase-F, (On blot)	TOF (DHB), Glycans	Complex	Difference in glycan structures between primary and metastatic sites in melanoma cell lines	(Ciolek-Wierzbicka, <i>et al.</i> , 2004)
CD8	Mouse	Hydrazine	R-TOF (DHB), ESI, Glycans	High-mannose, complex	Structural characterization	(Mery, <i>et al.</i> , 2003)
CD24 (Heat stable antigen)	Human Mouse	PNGase F	R-TOF (DHB), Glycans	Complex	Structural determination from different cell lines	(Ohl, <i>et al.</i> , 2003)
Chorionic gonadotropin	Horse	PNGase F	R-TOF (DHB), ESI, MS/MS Glycans	High-Man, hybrid, complex (SO ₃), N-56	Structural determination. Effect on receptor-binding	(Bousfield, <i>et al.</i> , 2004)
Chorionic gonadotropin	Human in choriocarcinoma cell line JEG-3	Hydrazine	L-, R-TOF (DHB), Glycans (2-AB)	Complex	Abnormal (branched on 3-arm) biantennary glycans in choriocarcinoma JEG-3 cells	(Takamatsu, <i>et al.</i> , 2004)
Cortical granule lectin	<i>Xenopus laevis</i>	PNGase F Pronase, Trypsin	FT-ICR (DHB), Glycopeptides	High-mannose, complex Asn-154, 217	N-Glycan ident. method based on pronase digestion to determine site occupancy	(An, <i>et al.</i> , 2003b)
Cytotoxin 3	<i>Naja kaouthia</i> (Cobra) venom	PNGase F Trypsin	L-, R-TOF (DHB, Sinapinic acid) Glycopep.	Complex, Asn-29	First ident. of glycosylation in three-fingered toxin	(Osipov, <i>et al.</i> , 2004)
Decay-accelerating factor (CD55)	Recombinant in <i>E. coli</i>	PNGase F (In gel)	R-TOF (DHB), Glycans (2-AB)	Complex	Structural identification	(Lukacik, <i>et al.</i> , 2004)
Ecto-5'-nucleotidase	Bull seminal plasma	CNBr, trypsin	L-, R-TOF (CHCA), Glycopeptides	Oligo-Man, hybrid, complex, Asn-403	Structural identification	(Finii, <i>et al.</i> , 2003)

Glycoprotein	Type	Release, Peptide cleavage	Instrument (Matrix) Other technique Sample (derivative)	Glycans	Notes	Reference
Fetuin	Bovine	PNGase F Trypsin, chymotrypsin	L-, R-TOF (DHB), Glycans	Complex	Contaminating peptides removed with C ₁₈ after hydrophobic derivatization Used as model for a study of N-glycans from α 1-acid glycoprotein	(Nakano, <i>et al.</i> , 2003b)
Fetuin	Bovine	PNGase F Trypsin, chymotrypsin	L-, R-TOF (DHB), Glycans (2-AA)	Complex		(Nakano, <i>et al.</i> , 2004)
Fetuin	Bovine	β -Elimination	TOF (DHB, 3-AQ), ESI, MS/MS, Glycans (ANTS, AMAC)	Complex	Method for glycan release by β -elimination (no reduction) and AMAC labelling	(Robbe, <i>et al.</i> , 2003a)
Fillitropin	Horse	PNGase F	R-TOF (DHB), ESI, MS/MS, Glycans	High-mannose, hybrid, complex (SO ₃) Asn-56	Structural identification. effect on receptor-binding	(Bousfield, <i>et al.</i> , 2004)
Follistatin	Human in CHO cells	PNGase F	TOF (Sinapinic acid), Reduced glycans	Complex (bi-, tri-antennary (Asn-95))	Site-specific structure determination.	(Hyuga, <i>et al.</i> , 2004)
α -Galactosidase	Recombinant human	PNGase F	R-TOF (s-DHB), Glycans	Complex (bi-, tri-antennary)	For study of quantification by HPAEC-PAD	(Ganesa, Granda & Mattaliano, 2003)
GCDFP-15/PIP	Human breast cyst	PNGase F Trypsin	SELDI, L-TOF (CHCA), CID, Glycans (Per-Me)	Complex	Different N-linked glycan profile in pathological state	(Caputo, <i>et al.</i> , 2003)
Glutamate carboxypeptidase II	Recombinant S2 cells	Trypsin, Asp-A	TOF (CHCA, ferulic acid, DHB), Glycopeptides	All 10 sites occupied but nature of glycans not stated	Identification of glycosylation sites by ¹⁸ O-incorporation	(Barinka, <i>et al.</i> , 2004)
β ₂ -Glycoprotein I	Human	Trypsin, Lysylendo-peptidase	TOF (DHB), MS ⁿ	Complex	As test compound for method based on hydrophilic affinity isolation	(Wada, <i>et al.</i> , 2004)
Gp17/SABP	Human seminal fluid	PNGase F Trypsin	SELDI, L-TOF (CHCA) CID Glycans (Per-Me)	Complex	Different N-linked glycan profile in pathological state	(Caputo, <i>et al.</i> , 2003)
GP120	HIV-1 _{sf2}	Trypsin	L-TOF (CHCA, +ve, DHB, -ve), LC/MS (ESI), Glycopeptides	Complex, high-Man, Asn-99, 110, 154, 160, 170, 203, 214, 235, 249, 274, 304, 328, 334, 358, 370, 378, 415, 428, 431	Structure identification. Nano-capillary LC/MS to characterise sialylated sites.	(Cutalo, Deterding & Tomer, 2004)
Granulocyte-macrophage colony-stimulating factor	Human recombinant in CHO cells	PNGase F Trypsin	TOF/TOF (CHCA, Sinapinic acid), ESI MS/MS, Glycopeptides, Glycans	Complex, Asn-37	Structural identification of zwitterionic carbohydrates	(Forno, <i>et al.</i> , 2004)
Hemocyanin	<i>Rapana venosa</i> (marine snail)	PNGase F Trypsin	TOF (CHCA) ESI Glycans, Glycopeptides	Complex, Asn-262, 401	Structural identification	(Dolashka-Angelova, <i>et al.</i> , 2004a)

(Continued)

TABLE 2. (Continued)

Glycoprotein	Type	Release, Peptide cleavage	Instrument (Matrix) Other technique Sample (derivative)	Glycans	Notes	Reference
Hemocyanin	<i>Megathura crenulata</i> (Keyhole limpet)	PNGase F	R-TOF, TOF/TOF (ATT), ESI, MS/MS, GC/MS, Glycans (2-AB)	High-mannose complex (Gal-Fuc)	Structural identification. Novel galactose-substitution on core fucose	(Wuhrer, <i>et al.</i> , 2004c)
Hemocyanin	<i>Megathura crenulata</i> (Keyhole limpet)	Hydrazine	MALDI, Glycans (2-AP)	High-mannose, hybrid	First Gal- β -(1 $\rightarrow$ 6)-Man and Fuc- α -(1 $\rightarrow$ 3)-GalNAc epitopes	(Geyer, <i>et al.</i> , 2004)
Hemocyanin	<i>Rapana venosa</i> (marine snail)	Trypsin	TOF (CHCA), glycopeptides	Complex (biantennary)	Structural identification of <i>N</i> -glycans from functional unit RvH1-a	(Dolashka-Angelova, <i>et al.</i> , 2003)
Hemocyanin	<i>Rapana thomasi</i>	PNGase F Trypsin	TOF (CHCA), Glycopeptides (by diff.)	High-mannose, Asn-394, 405	Structural identification and comp. with other hemocyanins	(Idakieva, <i>et al.</i> , 2004)
Horseradish peroxidase	Horseradish	Trypsin	TOF/TOF (DHB), Glycopeptides	Paucimannosidic truncated, Asn-285, 298, 316	Structural identification. Novel Fuc-GlcNAc disaccharide	(Wuhrer, Hokke & Deelder, 2004b)
IgA (secretory)	Human colostrum	PNGase F	R-TOF (DHB), LC/MS, ESI, Glycans (2-AB)	High-mannose, complex	Structural identification mainly by HPLC.	(Royle, <i>et al.</i> , 2003)
IgD/IgE	Human	PNGase F, In-gel	R-TOF (DHB), HPLC (2-AB)	High-mannose, complex	Identification of glycans. Interaction with lectins.	(Arnold, <i>et al.</i> , 2004)
IgG	Human (Sjögren's disease)	Hydrazine	TOF, Glycans, (ABEE)	Complex	Structural ident. Lower Gal on glycans from patient	(Kuroda, <i>et al.</i> , 2002)
IgG	Human	Hydrazine	R-TOF (DHB), MALDI-Q-TOF, LC/MS (2-AB)	Hybrid, complex	Identification of congenital disorders of glycosylation	(Butler, <i>et al.</i> , 2003)
IgG	Chicken	PNGase F Papain, Trypsin	L-TOF (Sinapinic acid, CHCA, DHB, THAP/NH ₄ -Citrate), Glycopep Glycans (2-AP)	High-mannose, complex, Fc, Fab	Site-specific structural identification. Pattern similar to mammalian IgE	(Suzuki & Lee, 2004)
IgG	Pigeon	Trypsin, chymotrypsin, PNGase F	R-TOF (DHB), Glycans (2-AP, Per-Me), Q-TOF (CHCA), MS/MS	High-Man, complex (bi- and triantennary with α -Gal-caps)	Structural identification	(Suzuki, <i>et al.</i> , 2003a)
IgG	Human	Trypsin, Lysylendopeptidase	TOF (DHB), MS ⁿ	Complex	As reference compound for method based on hydrophilic affinity isolation	(Wada, <i>et al.</i> , 2004)
IgM	Mouse	Glu-C	TOF (DHB, CHCA), LC/MS, MS/MS, Glycopeptides	High-Man, hybrid, complex Asn-166, 331, 363, 378, 401	Determination of site-specific glycosylation	(Wang, <i>et al.</i> , 2003b)
IL-22	Wild and from <i>D. melanogaster</i>	Endo-D, PNGase F	TOF (sinapinic acid)	Complex	Fuc-GlcNAc on Asn-54 needed for max. IL-10R2 binding	(Logsdon, <i>et al.</i> , 2004)
Integrin $\alpha_3\beta_1$	Human melanoma cells	PNGase F	TOF, (+ve) (DHB), Glycans	High-mannose, complex	Glycans from melanoma integrins have β 1 $\rightarrow$ 6-branches	(Pochee, <i>et al.</i> , 2003)

Glycoprotein	Type	Release, Peptide cleavage	Instrument (Matrix) Other technique Sample (derivative)	Glycans	Notes	Reference
Interferon- β	Mouse in <i>Bombyx mori</i>	Hydrazine	TOF, Glycans (2-AP)	High-mannose	Structural characterization	(Misaki, <i>et al.</i> , 2003)
Lipase	Dog, gastric in tobacco	PNGase A Trypsin	L-, R-TOF, Glycans (ANTS)	High-mannose, paucimannosidic, Asn-15, 80, 252, 308	Structural characterization. MALDI-TOF effective for determination of glycoprotein	(Mokrzycki-Issartel, <i>et al.</i> , 2003)
Luteinizing hormone	Human	DTT	L-TOF (Sinapinic acid), Glycoprotein	Complex, truncated, Asn-30	Ident. of glycans resulting from LH β polymorphisms	(Jacoby, <i>et al.</i> , 2003)
Luteinizing hormone	Horse	PNGase F	R-TOF (DHB), ESI, MS/MS	High-Man, hybrid, complex SO ₃ , Asn-56	Structural identification. Effect on receptor-binding	(Bousfield, <i>et al.</i> , 2004)
Lyc e 2	<i>Lycopersicon esculentum</i> (tomato)	PNGase A Trypsin	TOF, Glycans	Paucimannosidic	Structural identification and allergenic activity	(Westphal, <i>et al.</i> , 2003)
Lysozyme mutant (G49N)	Yeast	PNGase F (In gel)	TOF, Glycans	High-mannose plus GlcNAc	Yeast shown to be capable of adding GlcNAc to glycans	(Yoko-o, <i>et al.</i> , 2003)
Matrix metallo-proteinase-9	Human	PNGase F, trypsin	TOF, Glycans	Complex (biantennary)	Structural determination. Asn-28, 120	(Kotra, <i>et al.</i> , 2002)
Neoculin	<i>Curculigo latifolia</i> fruit	-	R-TOF, Glycopeptides	Paucimannosidic-deduced from mass	Structural characterization of taste-modifying protein	(Shirasuka, <i>et al.</i> , 2004)
Neural cell adhesion molecule	Calves	Endo N PNGase F Trypsin	TOF (ATT), ESI, Ion trap, Glycans (2-AP)	Complex HNK-1 Epitope - sites 2,4,5,6	Structural identification. Polysialic acid removed by Endo N treatment	(Wuhrer, <i>et al.</i> , 2003a)
Ovalbumin	Chicken	PNGase F	L- + R-TOF (DHB), ESI, Glycans, (Phenylhydrazones)	Truncated-complex, high-mannose	Structural characterization. Model for the use of phenylhydrazone label	(Lattova & Perreault, 2003a, Lattova & Perreault, 2003b)
Ovalbumin	Chicken	PNGase F Pronase, Trypsin	FTICR (DHB), Glycopeptides	High-mannose, hybrid, complex Asn-292	Identification of N-glycosylation sites and glycan heterogeneity	(An, <i>et al.</i> , 2003b)
Ovalbumin	Chicken	PNGase F Trypsin	L- + R-TOF (DHB), Glycans (hydrazones)	High-mannose, hybrid, complex	Use of cationic or hydrophobic derivs. to enhance signal	(Shinohara, <i>et al.</i> , 2004)
Peripheral myelin glycoprotein P0	Bovine	PNGase A	TOF (DHB)	Complex	Ident. of novel trisulfated hybrid glycan with GlcA	(Yan, <i>et al.</i> , 2003)
Peroxidase	Soybean seed-coat	Trypsin	TOF, Glycopeptides	Paucimannosidic	Structural identification and site analysis	(Welinder & Larsen, 2004)
Phaiodactylipin (phospholipase A ₂)	<i>Anuroctonus phaiodactylus</i> (scorpion) venom	Trypsin, Lys-C, V8, Arg-C	L-, R-TOF (Sinapinic acid, DHB), ESI, Ion trap, MS/MS, Glycopeptide	Truncated	Structural identification	(Valdez-Cruz, <i>et al.</i> , 2004)
Phl p 1 and 13	Timothy grass pollen	PNGase A, Trypsin	R-TOF (sinapinic acid), FTMS	Paucimannosidic	Phl p 1 has one glycan and Phl p13 has four. Can cross-link IgE receptors.	(Wicklein, <i>et al.</i> , 2004)

(Continued)

TABLE 2. (Continued)

Glycoprotein	Type	Release, Peptide cleavage	Instrument (Matrix) Other technique Sample (derivative)	Glycans	Notes	Reference
Plasminogen	Human	PNGase F	TOF	High-mannose	Reporter protein for yeast with deleted mannosidase genes	(Davidson, <i>et al.</i> , 2004)
Procyclin	<i>Trypanosoma brucei</i>	HF, PNGase F	TOF (CHCA), Glycopeptides, Glycans	High-mannose	Identification of glycosylation mutants resistant to Con A	(Leal, <i>et al.</i> , 2004)
Prostate-specific antigen (PSA)	Human	PNGase-F (In gel)	R-TOF (DHB), LC/MS, Glycans	Complex	Identification of glycans. Fuc- α -(1 $\rightarrow$ 2)-Gal in PSA	(Peracaula, <i>et al.</i> , 2003b)
Prostate-specific antigen (PSA)	Human	PNGase F	L-, R-TOF (DHB), Glycans (2-AP)	Hybrid, complex	Structure of glycans in normal and prostate cancer	(Ohyama, <i>et al.</i> , 2004)
Receptor for AGEs	Mouse	PNGase F Trypsin,	Q-TOF	Complex (biant.) high-mannose	Characterization of receptor glycoprotein	(Hanford, <i>et al.</i> , 2004)
Ricin	<i>Ricinus communis</i>	-	L-TOF (Sinapinic acid)	Paucimannosidic	Molecular weight measurement consistent with carbohydrate	(Na, <i>et al.</i> , 2004)
Rhodopsin	<i>Todarodes pacificus</i> (Japanese flying squid)	PNGase A Trypsin, Chymotrypsin	Q-TOF (CHCA) Nanoflow ESI, GC/MS, Glycans (Per-Me, 2-AP)	Fucosylated core	Structural identification. Novel α (1 $\rightarrow$ 3)- and α (1 $\rightarrow$ 6)-linked fucose on core GlcNAc	(Takahashi, <i>et al.</i> , 2003b)
Ribonuclease	Human pancreatic	PNGase F (in gel)	R-TOF (DHB), HPLC, LC/MS, Glycans	Complex, high-mannose	Modified glycosylation, e.g. increased sialic acid in cancer	(Peracaula, <i>et al.</i> , 2003a)
Ribonuclease B	-	PNGase F Pronase Trypsin	FT-ICR (DHB), Glycopeptides	High-mannose, Asn-60	N-Glycan ident. method based on pronase digestion to determine site occupancy	(An, <i>et al.</i> , 2003b)
Ribonuclease B	-	PNGase F	FT-ICR (DHB), Glycans (Quaternized Bz)	High-mannose	CE Separation of derivatized glycans with MALDI ident.	(An, <i>et al.</i> , 2003a)
Ribonuclease B	-	PNGase F	TOF, Glycans	High-mannose	Yeast shown to be capable of adding GlcNAc to glycans	(Yoko-o, <i>et al.</i> , 2003)
Ribonuclease B	-	Endo-H	TOF, Glycans and glycoprotein	High-mannose, Asn-34	Dimer formed by N-termini favoured by glycosylation	(Gotte, <i>et al.</i> , 2003)
Ribonuclease B	Bovine pancreas	PNGase F Trypsin	L- + R-TOF (DHB), Glycans (hydrazones)	High-mannose	Use of cationic or hydrophobic derivs. to enhance signal	(Shimohara, <i>et al.</i> , 2004)
Ribonuclease B	Bovine pancreas	Trypsin	TOF, Glycopeptides	High-mannose	Confirmation of glycans for study of Smith degradation	(Pitchayawasin & Isobe, 2004)
Ribonuclease B	-	PNGase F Trypsin,	TOF (DHB), Glycans (benzylamine)	High-mannose	As reference compound to demonstrate on-target digestion	(Morelle & Michalski, 2004)
Rhodopsin	<i>Todarodes pacificus</i> (squid)	PNGase A	Q-TOF (CHCA), Glycans (Per-Me)	Unusual fucosylated Man ₃ GlcNAc ₂	Structural identification	(Takahashi, <i>et al.</i> , 2003b)
Selenoprotein P	Rat	PNGase F Glu-C Trypsin	L-TOF (CHCA), Glycopeptides	Complex, Asn-64, 155, 169 (351, 356 not occupied)	Structural identification	(Ma, <i>et al.</i> , 2003a)
Spike protein	SARS virus	PNGase F Trypsin	Q-TOF, MS/MS	High-mannose, complex	Structural identification	(Krokin, <i>et al.</i> , 2003)

Glycoprotein	Type	Release, Peptide cleavage	Instrument (Matrix) Other technique Sample (derivative)	Glycans	Notes	Reference
Thyroid stimulating hormone	Human, recombinant	PNGase F	L-TOF (CHCA), Glycans (Per-Me)	Complex	To investigate the effect of charged (sialylated) glycans on glycoprotein migration in isoelectric focusing gels	(Zhou, <i>et al.</i> , 2004)
Thy-1	Avian in Lec1 or Tn5 cells	Asp-N, Glu-C	TOF (CHCA), glycopeptides	Paucimannosidic	Structural identification	(Mehndiratta, <i>et al.</i> , 2004)
Transferrin	Human	PNGase F	R-TOF (DHB, 3-AQ), Glycans (Me ester, ANTS)	Complex	Multiple changes in glycosylation explains poor sensitivity as EtOH-use marker	(Flahaut, <i>et al.</i> , 2003)
Transferrin	Human serum	Hydrazine	R-TOF (DHB), MALDI-Q-TOF, LC/MS, Glycans (2-AB)	High-mannose, hybrid, complex	Identification of congenital disorders of glycosylation	(Butler, <i>et al.</i> , 2003)
Transferrin	Human plasma	PNGase-F	L + R-TOF, (+ve, -ve) (THAP, NH ₄ citrate, DHB), Glycans	Complex	Use of MALDI to monitor congenital disorders of glycosylation	(Mills, <i>et al.</i> , 2003b)
Transferrin	Human	PNGase F Lysylendo-peptidase	R-TOF (CHCA), LC/MS/MS, Glycopeptides	Complex, Asn-432, 630, 491	Structural identification. Glycosylation at Asn-491 in Asn-Xxx-Cys motif.	(Satomi, <i>et al.</i> , 2004)
Transferrin	Human	Trypsin, Lysylendo-peptidase	TOF (DHB), MS ⁿ	Complex	As reference compound for method based on hydrophilic affinity isolation	(Wada, <i>et al.</i> , 2004)
Transferrin	Human hepatoma cells	PNGase F	R-TOF (DHB), Glycans	Complex	Increase in (1→3)-fucosylation in hepatoma cell line	(Higai, <i>et al.</i> , 2003b)
Vicilin Cor a 11	<i>Corylus avellana</i> (Hazelnut)	Trypsin	TOF, Glycopeptides	Paucimannosidic	To study allergenic behaviour.	(Lauer, <i>et al.</i> , 2004)
Vitelline	<i>Xenopus laevis</i> envelope glycoprotein	PNGase F	FTMS (DHB)	High-mannose, complex	Study of the effect of glycan structure on sperm-binding	(Vo, <i>et al.</i> , 2003)
Vitellogenin	<i>Cherax quadricarinatus</i> (Crayfish)	PNGase F (In gel)	R-TOF (DHB) ESI, MS/MS, LC/MS, Glycans (2-AB)	High-mannose	Structural identification	(Khalaila, <i>et al.</i> , 2004)
β-Xylosidase	<i>Solanum tuberosum</i> (Potato)	PNGase A	TOF (DHB), Glycans (2-AP)	Paucimannosidic	Purification and characterization of the enzyme.	(Peyer, Bonay & Staudacher, 2004)

TABLE 3. Use of MALDI MS for examination of *N*-glycans from intact organisms, tissues or protein mixtures

Organism	Type	Release, Peptide cleavage	Instrument (Matrix) Other technique Sample (derivative)	Glycans	Notes	Reference
<i>Arion lusitanicus</i> (Slug)	Skin, viscera, eggs	PNGase A	L-TOF (DHB, ATT), Glycans (2-AP)	High-Man (Me-Man) paucimannosidic	Structural identification	(Guttermigg, <i>et al.</i> , 2004)
<i>Caenorhabditis elegans</i>	Total proteins	Hydrazine	TOF, Glycans (Per-Me and reduced)	High-mannose (with Fuc), hybrid, complex	Glycan synthesis in a GnT I mutant	(Zhu, <i>et al.</i> , 2004a)
<i>Caenorhabditis elegans</i>	Total proteins	PNGase A	TOF (ATT), Glycans	Complex, High-mannose	Anti-horseradish peroxidase staining needs $\alpha(1\rightarrow3)$ -Fuc	(Paschinger, <i>et al.</i> , 2004)
<i>Caenorhabditis elegans</i>	Total proteins	PNGase F PNGase A	TOF, Q-TOF (DHB)	High-mannose, hybrid, complex	Srf-3 Mutant, resistant to bacterial infection is deficient in glycoconj	(Cipollo, <i>et al.</i> , 2004b)
<i>Caenorhabditis elegans</i>	Membrane glycoproteins	Hydrazine	R-TOF (DHB), Glycans (2-AP)	High-mannose, truncated complex	Structural identification	(Natsuka, <i>et al.</i> , 2002)
<i>Caenorhabditis elegans</i>	Soluble glycoproteins	Hydrazine	TOF (DHB), Glycans (2-AP)	High-mannose, truncated complex	Structural identification and genetics	(Hirabayashi, <i>et al.</i> , 2002)
Chinese hamster	Ovary cells with GlcNAcT-III	PNGase F	L TOF (s- DHB), Glycans	Complex (with bisect)	Structural identification. GlcNAc-TIII produces bisects	(Lee, Park & Stanley, 2003b)
Chinese hamster, (wild and Lec19	Ovary cells	PNGase F	TOF (+ve, -ve) (s- DHB, THAP), Glycans	High-mannose, complex	Lec19 CHO cells have reduced ability to add galactose	(Lee, <i>et al.</i> , 2003c)
Chinese hamster, (Lec23)	Ovary cells	PNGase F	TOF (arabinoxazone), Glycans (Me ester)	High-mannose, complex	For testing mutations in α -glucosidase 1 (CDG IIb)	(Hong, <i>et al.</i> , 2004)
Grass	Pollen	PNGase A	L-TOF (CHCA), Glycoproteins	Paucimannosidic	Use of 2D electrophoresis replica blotting to study allergens	(Petersen, 2003)
Hepatitis B virus	Surface glycoproteins	Trypsin PNGase F	TOF, Glycans	Complex, biantennary	Structural identification	(Schmitt, <i>et al.</i> , 2004a)
Human	Serum	Hydrazine	R-TOF (DHB), Q-TOF, LC/MS, Glycans (2-AB)	High-mannose, hybrid, complex	Identification of congenital disorders of glycosylation	(Butler, <i>et al.</i> , 2003)
Maize	Glycoproteins	PNGase A	R-TOF (DHB), Glycans (Reduced, Per-Me)	Paucimannosidic	For detection of immunogenic core (1 $\rightarrow$ 3)-Fuc and core Xyl	(Bardor, <i>et al.</i> , 2003a)
Mice	Erythrocytes	Hydrazine	TOF (DHB)	Complex	β -Gal-transferase-1 deficient mice produced more <i>N</i> -Ac-lactosamine	(Kotani, <i>et al.</i> , 2004)
<i>Nicotiana benthamiana</i>	Leaves	PNGase A	TOF (DHB), Glycans	Paucimannosidic	<i>N</i> -glycan profile unaltered in plants with reduced GlcNAc-T I	(Strasser, <i>et al.</i> , 2004a)
<i>Pisum sativum</i>	Pea Glycoproteins	PNGase A	R-TOF (DHB), Glycans (Reduced, Per-Me)	Paucimannosidic	For detection of immunogenic core (1 $\rightarrow$ 3)-Fuc and core Xyl	(Bardor, <i>et al.</i> , 2003a)
<i>Physcomitrella patens</i> (Moss)	Glycoproteins	PNGase A (Pepsin)	R-TOF (DHB) PSD Glycans (Reduced, Per-Me)	Paucimannosidic, high-mannose, complex	Structural identification. Glycans similar to those of higher plants	(Viřtor, <i>et al.</i> , 2003)
<i>Oryza sativa</i> (Rice)	Glycoproteins	PNGase A	R-TOF (DHB), Glycans (Reduced, Per-Me)	Paucimannosidic	For detection of immunogenic core (1 $\rightarrow$ 3)-Fuc and core Xyl	(Bardor, <i>et al.</i> , 2003a)
Rice, maize, wheat, date	Leaves and grain	PNGase A	L-TOF, R-TOF (DHB/HIQ), GC/MS, Glycans	Paucimannosidic	<i>N</i> -Glycan structure similar between mono- and di-cotyledons	(Lřonard, <i>et al.</i> , 2004)

(30) (Takahashi et al., 2003b). Paucimannosidic glycans with similar Gal- β -(1 $\rightarrow$ 4)-Fuc and Gal- β -(1 $\rightarrow$ 4)-Gal- β -(1 $\rightarrow$ 4)-Fuc groups at the reducing-terminal 6-position have been found in keyhole limpet (*Megathura crenulata*) hemocyanin (Wuhrer et al., 2004c). An unusual difucosylated core structure Man₃-GlcNAc₂dHex₂ in which one of the fucose residues is attached to a mannose residue has been reported as a constituent of phaiodactylipin (phospholipase A₂) from scorpion *Anuroctonus phaiodactylus* venom (Valdez-Cruz, Batista, & Possani, 2004). High-mannose glycans including the first report of a glycan with a Gal- β -(1 $\rightarrow$ 6)-Man moiety have been reported from keyhole limpet hemocyanin together with glycans with a Fuc- α -(1 $\rightarrow$ 3)-GalNAc epitope which causes cross-reactivity with *Schistosoma mansoni* glycans which share same epitope (Geyer et al., 2004). Some *N*-glycans from *C. elegans* and related organisms are substituted with phosphorylcholine (PC, 3/13) which is thought to modulate the host immune response. Studies on their biosynthesis have shown that *C. elegans* microsomes transfer PC from L- α -dipalmitoylphosphatidylcholine (31) to both hybrid and complex *N*-glycans containing GlcNAc (Cipollo et al., 2004a). HL60 cells treated with the glucosidase inhibitor *n*-butyl-deoxynojirimycin (NB-DNJ, 32) produced a series of compounds with the structure of high-mannose glycans but with only one GlcNAc residue as the result of cytosolic cleavage from misfolded glycoproteins that had been exported from the endoplasmic reticulum (Mellor et al., 2004b). A novel trisulfated hybrid glycan containing a GlcA residue capping the Gal-GlcNAc antenna has been identified from bovine myelin glycoprotein P0 (Yan, Kitamura, & Nomura, 2003).

30. Galactosylated, fucosylated *N*-glycan from *Todarodes pacificus*
Symbols as for structures 23 and 29.

31. L- α -dipalmitoyl-phosphatidylcholine

32. *n*-butyl-deoxynojirimycin (NB-DNJ)

e. Applications of MALDI MS to the study of N-glycan function. The presence of *N*-glycans on ribonuclease has been shown to reduce oligomerization (Gotte, Libonati, & Laurents, 2003). Engineered follitropins containing variable numbers of *N*- and *O*-linked glycosylation sites, expressed in CHO cells, have been used to show that increasing numbers of attached carbohydrates lengthened the elimination half-life by up to twofold. Numbers of attached carbohydrates were estimated by MALDI-TOF measurements on the intact glycoproteins (Weenen et al., 2004). Gårdsvoll et al. (2004) have used site-directed mutagenesis to remove the five *N*-linked sites of soluble human urokinase receptor expressed in *Drosophila* S2 cells either individually or in various combinations and have shown that only when all glycans are removed was there an impaired level of secretion. Monitoring of the levels of glycosylation of the 35 kDa protein was made with MALDI-TOF MS. Although there was incomplete resolution of the glycoforms, site occupation was clear by the appearance of peaks or shoulders coinciding with the calculated mass, assuming that the average mass of a glycan was 1,039 Da.

3. *O*-linked Glycans

O-Linked glycans continue to receive less attention than their *N*-linked counterparts, one factor being their tendency to occur in groups that make site analysis difficult. As with *N*-linked glycans, much structural analysis is conducted on released glycans. A method for the identification of sites modified by *O*-GlcNAc that relies on mild β -elimination followed by Michael addition with dithiothreitol and known as BEMAD has been described (Wells et al., 2002). The method was validated by mapping three previously identified *O*-GlcNAc sites, as well as three novel sites, on Synapsin I purified from rat brain and on the Lamin B receptor and the nucleoporin Nup155.

a. Studies on intact glycoproteins and glycopeptides. Collision-induced decomposition (CID) spectra of glycopeptides obtained by ESI from doubly charged ions frequently display prominent loss of the sugar moieties as the result of the labile protons. Czeszak et al. (2004) have used MALDI-PSD on glycopeptides derivatized with a phosphonium group at the amino-terminus and have shown that the resulting singly charged phosphonium ions and a-type fragments retain the sugar. In contrast, when doubly charged ions were generated by proton addition, loss of the glycan was again seen. The method was claimed to be an effective alternative to electron-capture dissociation (ECD) and was used to locate up to three GalNAc residues on the full tandem repeat peptide derived from the MUC5AC mucin. Kuroguchi, Matsushita, and Nishimura (2004) have reported that LIFT-TOF with DHB as the matrix is an ideal system for fragmenting glycopeptides (b and y series ions) and have used it to determine the *O*-linked glycosylation sites in mucin-type glycopeptides.

Up to six *O*-linked glycosylation sites have been found in the hinge region of IgA1 from human serum (Tarelli et al., 2004). Tryptic digestion yielded a 33-mer glycopeptide that was examined by MALDI-TOF MS from THAP/ammonium citrate. This matrix gave more highly resolved spectra than s-DHB or

CHCA; the CHCA spectrum, in particular, was very poorly resolved (Pouria et al., 2004). Structural analysis was by exoglycosidase digestion (sialidase and galactosidase) and site analysis was performed on the degraded glycans by further cleavage with the endoproteases Glu-C from *Staphylococcus aureus* and proteinase K from *Tritirachium album* (Tarelli et al., 2004). MALDI-TOF analysis of the stalk-region glycopeptide (APTPVPPPTGTPTPL) from murine CD8 has shown that all three threonine residues are glycosylated with HexNAc. Four peaks were obtained and attributed to molecules containing 0–3 additional hexose residues (Merry et al., 2003).

The 47 kDa portion of the 45/47 kDa glycoprotein from *Mycobacterium tuberculosis* expressed in *Streptomyces lividans* has been found to contain glycans on both the *N*- and *C*-terminal tryptic peptide; MALDI-TOF analysis showed from 0 to 9 hexose residues at the *N*-terminus but the glycosylation site was not determined (Lara et al., 2004). The 13-amino acid-containing peptide isolated from *Conus textile* contains a Gal-GalNAc residue attached to threonine but with an unknown linkage between the sugar rings. MALDI-TOF analysis of the products of a β -galactosidase digest on a synthetic analogue containing the common β -D-Gal-(1 $\rightarrow$ 3)- α -D-GalNAc-carbohydrate showed that the enzyme was unable to remove the galactose residue suggesting the presence of α 1 $\rightarrow$ 3-linked galactose and this was confirmed by subsequent NMR analysis (Kang et al., 2004). Tryptic glycopeptides from surface glycoproteins of the hepatitis B virus were found by MALDI-TOF MS and on-target exoglycosidase digestion to be *O*-glycosylated with Gal-GalNAc or Neu5Ac-Gal-GalNAc. The *N*-glycans were mainly biantennary complex (Schmitt et al., 2004a).

b. Applications of MALDI to the structural determination of O-linked glycans. Studies on the structural determination of *O*-glycans from isolated glycoproteins and from intact organisms or tissues are summarized in Tables 4 and 5 respectively. Studies on some specific structural types are summarized below.

i. Mucins. The ability of MALDI MS to resolve complex mixtures of glycans has been utilized in studies of mucins from the jelly coats of amphibian eggs. Thus, three layers of the jelly coat from *X. laevis* have been shown to contain different but overlapping glycan structures; 40 neutral and 30 sulfated compounds were identified (Zhang et al., 2004b) by HPLC (PGC column), MALDI-FT-ICR, CID and the “catalog-library” approach of CID peak matching reported earlier (Tseng, Hedrick, & Lebrilla, 1999). Thirty-five neutral, sulfated and sialylated glycans have been identified from *X. tropicalis* by similar techniques combined with exoglycosidase digestion (Zhang et al., 2004a). Sialylated glycans were stabilized by formation of methyl esters. Sulfated core-2 and core-4 glycans have been identified by MALDI-TOF MS and NMR from respiratory mucins from a patient suffering from chronic bronchitis but, unlike the structures of glycans identified earlier from a cystic fibrosis patient (Lo-Guidice et al., 1994), these glycans contained no sialic acid (Degroote et al., 2003). MALDI-TOF data were recorded in negative ion mode and no loss of sulfate was reported. Human intestinal mucins show an acidic gradient along the intestinal tract which may explain the region-specific colonization of the gut by various bacteria (Robbe et al., 2003b). *O*-Glycans of stomach mucosa have an antibacterial action

against *Helicobacter pylori* by inhibiting biosynthesis of cholesteryl- α -D-glucoside (identified by MALDI-TOF MS), a major cell wall component (Kawakubo et al., 2004).

ii. Glycosaminoglycans (GAGs). Methods for the analysis of hyaluronan and its fragments have been reviewed (Capila & Sasisekharan, 2004).

α . Unsulfated GAGs. Hyaluronan oligomers ((($\rightarrow$ 3)- β -D-GlcNAc-(1 $\rightarrow$ 4)- β -D-GlcAp-(1 $\rightarrow$))_n, **33**), with masses up to 8 kDa, obtained from hyaluronic acid by the action of bovine testicular hyaluronidase have been examined by electrospray and MALDI-TOF MS. Electrospray showed the presence of oligomers with both odd and even numbers of sugar units whereas MALDI and high-performance anion exchange chromatography (HPAEC) showed only even numbered oligomers. The discrepancy was traced to the electrospray ion source which was producing cone-voltage fragmentation. It was recommended that for ESI studies of compounds of this type, the cone voltage be kept low and precisely controlled (Prebyl et al., 2003). An LC/MS method for quantification of hyaluronic acid fragments in pharmaceutical preparations produced by the action of hyaluronate lyase from *Streptococcus agalactiae* has been reported, with negative ion MALDI-TOF from DHB being used to record the glycan profiles of three fractions. The largest fraction showed peaks to 15 kDa (Kühn et al., 2003). A model disaccharide, Δ UA-GlcNAc (10 mmol), derived from heparan sulfate by heparitinase enzyme digestion and bearing an unsaturated 4,5-uronic acid (Δ UA) at the non-reducing end has been adducted to mercuric acetate and the product characterized by MALDI-TOF MS, confirming the formation of a mercury adduct (*m/z* calc. 639.9, obs. 640 with the expected spread of 6 mass units for Hg₁₉₈ to Hg₂₀₄, consistent with the production of a cyclic 4,5-mercurinium intermediate (Skidmore et al., 2004).

β . Sulfated GAGs. A common method for analysis of these glycans is the use of basic peptides for ion pairing as outlined in the earlier reviews in this series. Thus, for example, cleavage of heparin by controlled γ -irradiation has produced fragments enriched in highly sulfated sequences which were examined by MALDI-TOF MS using ion-pairing with (Arg-Gly)₁₉-Arg to stabilize the sulfates (Bisio et al., 2004). Studies by MALDI-TOF MS and HPLC of the serine protease inhibitor and its chondroitinase and hyaluronidase digestion products have shown that, in acute inflammation, its chondroitin-4 sulfate chain is both longer and undersulfated compared with control glycoproteins (Capon et al., 2003). Combined MALDI-TOF and enzyme digestion have also been used by Kett and Coombe (2004) for GAG analysis.

4. Studies Involving Both *N*- and *O*-Linked Glycans

A method for simultaneous analysis of both *N*- and *O*-linked glycans has been published by Robbe et al. (2003a). Glycans were released by non-reductive β -elimination using the ammonia-based method described earlier by Huang, Mechref, and Novotny (2001) and labeled by reductive amination with either

TABLE 4. Use of MALDI MS for examination of O-glycans from specific glycopeptides

Glycoprotein	Type	Cleavage/ release	Instrument (Matrix) Other technique Sample (derivative)	Glycans	Notes	Reference
N-Acetyl-hexosaminidase	<i>Aspergillus oryzae</i>	—	MALDI, Glycopeptide	Mannose	O- but not N-glycosylation required for activity	(Plihal, <i>et al.</i> , 2004)
Bile salt-stimulated lipase	Human recombinant in <i>Pichia pastoris</i>	β-Elimination	R-TOF, PSD, Glycans	(Man) ₁₋₆ (Man) ₆ -PO ₃ H ₂	Structural identification. First report of β-Man and PO ₄ in <i>P. pastoris</i>	(Trimble, <i>et al.</i> , 2004)
CA125	Human ovarian tumour	Trypsin, β-Elimination	R-TOF (DHB), FAB, GC/MS, ESI/CID, Glycans, (Per-Me)	Core-1 core-2	Structural identification. Branched core I in cancer	(Wong, <i>et al.</i> , 2003)
C1 Inhibitor	Human in rabbit mammary gland	β-Elimination	R-TOF (DHB (+ve) THAP/NH ₄ citrate), Glycans	Core-1	Structural ident. of glycans expressed in milk	(Koles, <i>et al.</i> , 2004b)
Carbohydrate-binding module mutant	<i>Cellulomonas fimi</i> xylanase in <i>Pichia pastoris</i>	Trypsin, chymotrypsin	TOF (Cinnamic acid), SELDI, Glycopeptides	Mannose	Structural identification. 3 Regions of glycans with maximum of four mannose	(Boraston, <i>et al.</i> , 2003)
κ-Casein	Ovine	Trypsin	L-TOF (-/+ve) (3,4-DHB, CHCA), ESI, MS/MS, Glycopeptide	Neu5Gc-Gal-GalNAc	Structural identification	(Mamone, <i>et al.</i> , 2003)
κ-Casein	Bovine	Trypsin, GluC	R-TOF (CHCA), Glycopeptides	Neu5Gc-Gal-GalNAc	Structural identification	(Holland, Deeth & Alewood, 2004)
CD8	Mouse	Trypsin	R-TOF (DHB), ESI, Glycopeptides	Core-1	Structural identification	(Merry, <i>et al.</i> , 2003)
Chorionic gonadotrophin	Human in CHO cells	Hydrazine	R-TOF (DHB CMBT), SSI, Ion trap, Glycans (2-AB)	2 to 6 residues, Sialylated	Structural identification	(Gervais, <i>et al.</i> , 2003)
Coagulation factor V	Human	Trypsin	R-TOF, Glycopeptides	GalNAc or GlcNAc	Attached to Thr-402	(Gould, Silveira & Tracy, 2004)
Cyclic AMP-responsive element-binding protein	Rat brain	Trypsin	TOF, ESI, LC/MS/MS, Glycopeptide	GlcNAc	First demonstration of glycosylation by GlcNAc in this peptide	(Lamarre-Vincent & Hsieh-Wilson, 2003)
Decay-accelerating Factor (CD55)	Recombinant in <i>E. coli</i>	Hydrazine	R-TOF (DHB), Glycans (2-AB)	Core-1	Structural identification	(Lukacik, <i>et al.</i> , 2004)
Granulocyte-macrophage colony-stimulating factor	Human recombinant in CHO cells	Glu-C	TOF/TOF (CHCA Sinapinic acid), ESI MS/MS, Glycopeptides	Ser-5,9, Thr-10, core 1	Structural identification	(Forno, <i>et al.</i> , 2004)
IgA (secretory)	Human colostrum	Hydrazine	R-TOF (DHB), ESI, LC/MS/MS, Glycans (2-AB)	Core II	Structural identification, mainly by HPLC	(Royle, <i>et al.</i> , 2003)

(Continued)

TABLE 4. (Continued)

Glycoprotein	Type	Cleavage/ release	Instrument (Matrix) Other technique Sample (derivative)	Glycans	Notes	Reference
IgA1	Human cell lines	-	R-TOF (+ve) (CHCA), Glycopeptide	GalNAc	O-Glycosylation of hinge region initiated by single enzyme	(Iwasaki, <i>et al.</i> , 2003)
IgA1	Human tonsillar lymphocytes	Trypsin	TOF (s-DHB), Glycopeptides	(GalNAc) _x , (Gal) _y , (Neu5Ac) _z	Under-O-glycosylation in IgA nephropathy	(Horie, <i>et al.</i> , 2003a)
IgA1	Human serum	Trypsin	R-TOF (THAP/NH ₄ citrate, CHCA, s-DHB), Glycopeptides	GalNAc, Gal, Neu5Ac	Identification of glycans. Computer program to interpret data	(Pouria, <i>et al.</i> , 2004)
IgA1	Human serum	Trypsin	R-TOF (THAP/NH ₄ citrate), Glycopeptides	Thr-225, Thr-228, Ser-230, Ser-232, Thr-236, GalNAc, Gal, Neu5Ac	Structural identification. Up to six O-glycans	(Tarelli, <i>et al.</i> , 2004)
Selenoprotein P	Rat	Glu-C Trypsin	L-TOF (CHCA), Glycopeptides	Thr-346, Hex, HexNAc, Neu5Ac	Structural identification	(Ma, <i>et al.</i> , 2003a)
Zona pellucida 3	Human and mouse	β-Elimination	R-TOF (DHB), Glycans (Per-Me)	Gal, GalNAc, Neu5Ac, Neu5Gc	Proteins from both sources have same glycans	(Dell, <i>et al.</i> , 2003)

2-aminoacridone (AMAC, **1/58**) or 8-aminonaphthalene-1,3,6-trisulphonic acid (ANTS, **34**). They were then separated by gel electrophoresis, the bands containing the glycans were excised and the derivatized glycans were extracted for analysis by MALDI and electrospray MS. Comparisons between the electrophoresis profiles from the AMAC (uncharged) and ANTS (charged) derivatives provided an indication of the charge state of the glycan. The method was designed mainly for profiling mucin glycans and was applied to porcine gastric mucin and bovine submaxillary mucin.

34. 8-aminonaphthalene-1,3,6-trisulphonic acid (ANTS)

A popular method for the independent study of both *N*- and *O*-glycosylation is to remove the *N*-linked glycans with, for example, PNGase F and then examine the *O*-glycosylation, either by further release using β-elimination or by measurements on the glycopeptides obtained from the *O*-linked region (Forno *et al.*, 2004; Trimble *et al.*, 2004). The well-established differential hydrazinolysis method was used by Gervais *et al.* (2003) to study *N*- and *O*-linked glycosylation of human recombinant chorionic gonadotropin allowing *O*-linked glycans to be identified for first time.

5. Function of O-Linked Glycans

With the recent sequencing of several genomes, attention has become focused on how the complexity of higher organisms can be encoded by such a small number of genes. Post-translational modifications and, in particular, glycosylation have emerged as important determinants of the control of biological systems. Lamarre-Vincent and Hsieh-Wilson (2003) have studied glycosylation of cyclic AMP-responsive element-binding protein, a transcription factor essential for long-term memory and have shown the first link between *O*-GlcNAc and information storage in the brain. *O*-GlcNAc was detected by specific enzymatic radiolabeling with galactose from [³H]UDP-galactose to the 4-position of *O*-GlcNAc. MALDI-TOF analysis of the tryptic peptides was used to show that the enzyme was modified with two *O*-GlcNAc residues.

Most organisms synthesize α-linked-polyglucans, such as glycogen, as an energy source when other reduced carbon compounds are insufficiently available but, to date, the method for the initiation of glycogen synthesis in prokaryotes has not been determined. In an attempt to rectify this gap in our knowledge, Albrecht *et al.* (2004) have produced the eukaryotic enzyme yeast glycogenin (Glg2p), a known initiator in fungi and animals, in *E. coli* and found it to be autocatalytically glucosylated at tyrosine residues 232 and 230 with from 4 to 25 glucose residues, as determined by MALDI-TOF and MS/MS analysis. Further incubation with UDPglucose resulted in transfer of more than 36 additional glucose residues over 20 min as detected by MALDI-TOF. In another study, the

TABLE 5. Use of MALDI MS for examination of *O*-glycans from intact organisms or tissues

Organism	Type	Release	Instrument (Matrix) Other technique Sample (derivative)	Glycans	Notes	Reference
<i>Bombina bombina</i> and <i>B. variegata</i>	Egg mucin	β -Elimination	TOF (DHB), Glycans	Neutral and acidic	Comparison of glycans in related species.	(Coppin, <i>et al.</i> , 2003)
<i>Caenorhabditis elegans</i>	Total glycoproteins	β -Elimination	TOF, Q-TOF (DHB), Glycans	GlcA, Gal, GalNAc	Srf-3 Mutant, resistant to bacterial infection is deficient in glycoconjugates	(Cipollo, <i>et al.</i> , 2004b)
Hepatitis B virus	Surface glycoproteins	Trypsin, <i>O</i> -glycosidase	TOF (ATT), Glycopeptides	GalNAc-Gal-Neu5Ac	Structural identification	(Schmitt, <i>et al.</i> , 2004a)
Human	Airway mucin	β -Elimination	TOF, Glycans	Core-2, core-4	Biosynthesis of airway mucins related to bronchial disease	(Degroote, <i>et al.</i> , 2003)
Human	Intestinal mucins	β -Elimination	TOF (DHB), GC/MS, Glycans	Core-5	Evidence for region-specific glycosylation dependent on pH	(Robbe, <i>et al.</i> , 2003b)
Human	Tonsillar lymphocytes	Trypsin	TOF, glycopeptides	GalNAc, Gal, Neu5Ac	Asialo- <i>O</i> -glycans deposited in IgA nephropathy	(Hiki, <i>et al.</i> , 2004)
Mouse	Intestinal mucins	β -Elimination	TOF, GC/MS, Glycans (<i>N</i> -Me-amide)	Core-1, core-2	Alterations in intestinal mucins caused by parasite	(Holmén, <i>et al.</i> , 2002)
<i>Xenopus laevis</i>	Egg mucin	β -Elimination	FT-ICR (DHB (+ve) 2,5-DHA (-)), CID, Glycans	Neutral, sulfated, core-2	Structural determination. Catalog library approach	(Zhang, <i>et al.</i> , 2004b)
Ox	Submaxillary gland mucin	β -Elimination	TOF (DHB, 3-AQ), ESI, MS/MS, Glycans (AMAC, ANTS)	Neutral, acidic, unspecified core	Non-reductive β -elimination with NH_4OH allows labeling	(Robbe, <i>et al.</i> , 2003a)
Pig	Gastric mucin	β -Elimination	TOF (DHB, 3-AQ), ESI, MS/MS, Glycans (AMAC, ANTS)	Neutral, acidic, unspecified core	Non-reductive β -elimination with NH_4OH allows labeling	(Robbe, <i>et al.</i> , 2003a)
Rat	Skeletal muscle	Trypsin	TOF (DHB), Glycopeptides	GalNAc	GalNAc identified in 14 skeletal muscle glycoproteins suggesting regulatory role	(Cieniewski-Bernard, <i>et al.</i> , 2004)
<i>Xenopus tropicalis</i>	Egg mucin	β -Elimination	FT-ICR (DHB), CID, Glycans	Neutral, sulfated, sialylated, core-2	Structural determination. Exoglycosidase digestion	(Zhang, <i>et al.</i> , 2004a)

N-terminal segment of potato X virus has been found to be glycosylated at the N-terminus with galactose or fucose and that the presence of the sugar mediates the formation of a bound water shell on the virion surface (Baratova et al., 2004).

6. Glycoproteins and Disease

Glycan profiling by MALDI-TOF MS for the detection of disease is gaining ground. For example, a new method has been developed for the extraction of the acute-phase glycoprotein, α 1-acid glycoprotein from human serum and its *N*-glycans have been compared in patients with inflammation or cancer and healthy controls. The disease states produced an increase in both the degree of branching and in the amount of fucosylation (Kremmer et al., 2004; Szölloosi et al., 2004). The amounts of fucosylated bi-, tri-, and tetra-antennary glycans were also found to increase at the expense of unfucosylated triantennary glycans in this glycoprotein in patients with acute inflammation (Higai et al., 2003a). Sialylated glycans were stabilized for MALDI-TOF analysis by formation of methyl esters. The same stabilization method was used by Flahaut et al. (2003) in a study on the effect of ethanol on transferrin glycosylation in chronic alcoholics has revealed patient-dependent differences in both the level of sialylation and in the number of glycans attached to the molecule, possibly accounting for the controversial use of transferrin glycosylation as a marker for chronic ethanol consumption.

a. Detection of cancer biomarkers. *N*-glycans from normal human pancreatic cells consist mainly of complex biantennary structures with small amounts of tri- and tetra-antennary compounds and larger compounds with poly-*N*-acetylactosamine extensions and extensive fucosylation (up to five fucose residues and eight Gal-GlcNAc units as detected by MALDI-TOF MS). Glycans from ribonuclease extracted from pancreatic adenocarcinoma tumor cells, on the other hand, contain fucosylated hybrid structures and biantennary glycans whose antennae terminated in GalNAc, thus providing a potential diagnostic test for the adenocarcinoma (Peracaula et al., 2003a). MALDI-TOF analysis gave much better resolution of the large glycans than could be obtained by HPLC. Altered glycosylation has also been found associated with prostate cancer; glycans from prostate-specific antigen (PSA) contained increased amounts of fucose and GalNAc in the antennae, again indicating a possible diagnostic test (Peracaula et al., 2003b). Another study found increased amounts of α -(2 $\rightarrow$ 3)-linked sialic acid in human polysialic acid (PSA) suggesting that differential binding to *Maackia amurensis* agglutinin lectin could be used to differentiate prostate cancer from benign prostate hypertrophy (Ohyama et al., 2004). Masses of some sialylated glycans were reported from linear MALDI-TOF spectra but most measurements were made on neutral glycans following linkage-specific removal of sialic acid. Increased fucosylation has also been found in *N*-linked glycans from human hepatoma (Higai et al., 2003b) and melanoma (Ciolczyk-Wierzbicka et al., 2004) cell lines and a reduction in the concentration of fucosylated glycoproteins has been found in dogs undergoing treatment for lymphosarcoma (Xiong, Andrews, & Regnier, 2003a). An extensive study of the *N*- and

O-glycosylation of the human ovarian tumor marker CA125 has been reported. The bi-, tri-, and tetra-antennary glycans were identified by MALDI-TOF, MS/MS and FAB mass spectrometry as permethylated derivatives coupled with exoglycosidase digestion (Wong et al., 2003). Highly processed glycans have also been found on the breast tumor marker protein, gross cystic disease fluid protein-15, than on the same protein from healthy controls (Caputo et al., 2003). Glycans were again examined as permethylated derivatives but, this time, by surface-enhanced laser desorption/ionization (SELDI) mass spectrometry. MUC1 glycoprotein is overexpressed in breast cancer and *O*-Glycans have been shown to control proteolysis by preventing proteolysis of the Thr3-Ser4 bond if either amino acid is glycosylated (Hanisch et al., 2003). Tetrasialylated-tetra-antennary glycans carrying a β 1 $\rightarrow$ 6 antenna appear to be associated with the development of cancer metastases as demonstrated by studies on integrins from metastatic and non-metastatic human melanoma cell lines (Pochec et al., 2003). MALDI-TOF profiles were obtained following in-gel glycan release using the method described by Küster et al. (1997) as modified by Hoja-Lukowicz et al. (2000). Bi- and tri-antennary glycans have been identified attached to human chorionic gonadotropin produced in a choriocarcinoma cell line (JEG-3); the biantennary glycans, which were useful markers, were unusual in having a branched 3-antenna (35) rather than carrying both unbranched 3- and 6-antenna (Takamatsu et al., 2004). The structure was confirmed by MALDI-TOF analysis of exoglycosidase digestion products with an α -mannosidase digestion following β -galactosidase digestion to confirm the presence of the exposed mannose residue on the 6-antenna. The study illustrates the danger of assigning structures directly by matching masses with those from a database or by using only the usual exoglycosidase sequence of β -galactosidase followed by *N*-acetylhexosaminidase which would incorrectly have suggested a normal biantennary structure such as 23.

35. Unusual biantennary glycan detected in a choriocarcinoma cell line
Glycan symbols as described above.

b. Congenital diseases of glycosylation. Matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) analysis of both the *N*-glycans released from tryptic peptides and of the peptides themselves has shown that the asparagine residues of α 1-antitrypsin in patients suffering from congenital disorder of glycosylation (CDG) type I (deficiency in the initial protein glycosylation by Glc₃Man₉GlcNAc₂, 36) are preferentially glycosylated in the order Asn-46 > 247 > 83 (Mills et al., 2003a). Asparagine occupation was measured by observing the extent to which it was converted to aspartic acid during glycan removal by the enzyme PNGase F. In a newly characterized disorder, CDG-1i, MALDI-TOF and HPLC were used to show that some of the *N*-glycans on fibroblasts consisted only of Man₁GlcNAc₂ and Man₂GlcNAc₂ (Thiel et al., 2003). Analysis

by MALDI-TOF and HPLC of serum glycoproteins from several patients with CDG type II syndromes (defective glycan processing) has been used to identify several enzyme defects. One patient was characterized with a mannosidase-III deficiency that prevented biosynthesis of complex glycans as the result of failure to remove residual mannose residues from the 6-antenna. The predominant hybrid glycan **37** was characterized by CID using a MALDI-Q-TOF instrument (Butler et al., 2003). α -Mannosidase deficiency causes accumulation of mannose-containing oligosaccharides (released from *N*-glycans) in various tissues but the disease has been successfully treated by enzyme replacement therapy. Residual glycans were monitored in this study by MALDI-TOF MS (Roces et al., 2004). A GlcNAc-transferase-II deficiency in another patient in the study by Butler et al. prevented elaboration of the 6-antenna and resulted in the same biantennary glycans with a branched 3-antenna (**35**) as observed in the choriocarcinoma cell line (JEG-3) discussed above. In this case, however, the glycan was identified by negative ion MS/MS. Mass spectrometric methods for elucidating the pathogenesis of CDG type IIx have been reviewed with examples of transferrin and α 1-antitrypsin glycosylation (Mills et al., 2003b).

36. High-mannose glycan $\text{Glc}_3\text{Man}_9\text{GlcNAc}_2$

37. Complex glycan from a CDG-III patient

c. Other diseases. The hinge region of IgA1 has been shown by MALDI-TOF MS to be under-*O*-glycosylated in patients with IgA neuropathy (Horie et al., 2003a). As upper respiratory tract infection often precedes hematuria and proteinuria in these patients, Horie et al. (2003b) have investigated the effect of tonsillectomy and steroid treatment and found that they produce increases in the extent of IgA *O*-glycosylation. Again, analysis was by MALDI-TOF with s-DHB as the matrix. A method has been reported for detection of the biomarker, serum amyloid component, in human plasma or urine. Extraction was by monoclonal antibody binding and analysis by MALDI-TOF MS from sinapinic acid. Several new, desialylated compounds were detected from three individuals (Kiernan et al., 2004).

Krokhin et al. (2003) have identified, in a preliminary study, several high-mannose and complex *N*-glycans from the spike protein of the SARS virus taken directly from patients. The compounds were identified mainly with the aid of MS/MS on a Q-TOF instrument.

C. Glycated Proteins (Non-Enzymatic Attachment of Sugars)

Work with these compounds usually involves analysis of intact proteins that are modified with chemically attached glycan residues and are ideal subjects for the high mass resolving ability of MALDI-TOF instruments. Analysis of β -lactoglobulin glycation products formed from lactose and galactose has revealed lysine as the main binding site with additional binding at the amino-terminal leucine residue and at Arg-124. MALDI-TOF analysis was found to be more efficient than LC/ESI-MS for locating the binding sites (Fenaille et al., 2004). MALDI-TOF has, for the first time, enabled kinetic data to be obtained on defined glycation products from specific sites of a glycated protein. Thus, lysozyme was incubated with D-glucose for 1, 4, 8, or 16 weeks in phosphate-buffered saline, digested with endoproteinase Glu-C and the C- and N-terminal peptides were used for the relative quantification of the initial Amadori product, *N*^ε-(carboxymethyl)lysine (**38**) and an imidazolone (**39**) formed from arginine (Kislinger et al., 2003). The preferred glycation sites in human serum albumin (HSA) have been determined as lysines 233, 276, 378, 525, and 545 following incubation of HSA with glucose, enzymatic digestion of the protein with either trypsin or endoproteinase Lys-C, and examination of the resulting glycated peptides by LC/MS/MS and MALDI-TOF from CHCA (Lapolla et al., 2004a). Glycation of casein by glucose, fructose or ribose has been characterized and shown not to affect its protective effects on human intestinal Caco-2 cells (Jing & Kitts, 2004). A study of 20 Type 2 diabetic patients and 10 controls have shown that complications of diabetes are associated with a higher concentration of glyco-oxidized variants of globin than observed in controls or patients without complications (Lapolla et al., 2004b).

38. *N*^ε-(carboxymethyl)lysine

39. Imidazolone advanced glycan end-product

Several other studies on advanced glycation end products (AGEs) have been reported. Yamada et al. (2004) have synthesized a collagen model peptide and subjected it to glycation with glucose, ribose and glyoxal (**40**). A dimeric peptide linked glyoxal-lysine dimer (**41**) was isolated and identified by MALDI-TOF as the first example of an intact glycation-derived dimer. The same technique has been used to show that methylglyoxyl, produced during the Maillard reaction binds to Arg-184 and Arg-185 located within the binding site of bovine glutathione peroxidase and to deactivate the enzyme, resulting in an increase in the intracellular peroxidases responsible for oxidative cellular damage (Park et al., 2003b). AGEs from ribose were formed more rapidly when incubated with bovine serum albumin (BSA) than those from glucose or fructose as measured by binding to AGE receptors (Valencia et al., 2004). The use of in-house-developed PERL script software has been used to identify and localize AGEs on β_2 -microglobulin (Cocklin et al., 2003; Zhang et al., 2003d). The program identifies masses, measured by MALDI-TOF MS that correspond to those of peptides with an AGE modification. In other studies, the AGEs N^{ϵ} -(carboxymethyl)-lysine, imidazolone A (**42**) and B (**43**), pyrraline (**44**) and 1-alkyl-2-formyl-3,4-glycosyl pyrrole (**45**) have been found on the type 2 ryanodine receptor calcium-release channel, accounting for decreased cardiac contractility in diabetes (Bidasee et al., 2003). The soluble AGE receptor (sRAGE), a member of the immunoglobulin superfamily, has been characterized from mouse and its *N*-glycosylation identified by MALDI-TOF MS (Hanford et al., 2004). In another study, measurements of protein molecular weights have added support to the conclusion that Amadori decomposition pathways are constrained in the presence of metal ion chelators and radical traps (Culbertson et al., 2003).

42. Imidazolone A

43. Imidazolone B

Although protein glycation is usually associated with pathology, as in the secondary effects of diabetes, the reaction is also involved in cooking where it changes the color (browning) and flavor of the food. Fay and Brevard (2004) have recently reviewed the use of mass spectrometry in the study of the involved Maillard reaction.

D. Peptidoglycans

One protein encoded by the 13 *Drosophila* peptidoglycan recognition proteins has been shown to possess enzymatic activity. MALDI-TOF analysis of the degradation products demonstrated that the enzyme hydrolyses the lactylamide bond between the glycan chain and the cross-linking peptides. The enzyme was, thus, classified as an *N*-acetylmuramoyl-L-alanine amidase (Mellroth, Karlsson, & Steiner, 2003). HPLC and MALDI-PSD were used by Antignac et al. (2003) to characterize the peptidoglycan from *Neisseria meningitidis*. The peptidoglycan was hydrolyzed with muramidase from *Streptomyces globisporus*. Fourteen of the 28 muropeptides separated by HPLC were found to be *O*-acetylated to different degrees. Proteoglycan-like glyconectins from three sponges, *Microciona prolifera*, *Halichondria panicea*, and *Cliona celata* have been examined by NMR and mass spectrometry. The structures were complicated and contained distinct acid-resistant and acid-sensitive domains. Some sugars were sulfated and others pyruvylated (Guerardel et al., 2004). Muropeptides derived from *Escherichia coli* peptidoglycan have been labeled with the fluorescent dyes ANTS, 7-aminonaphthalene-1,3-disulfonic acid (ANDA, **46**) or 4-aminonaphthalene-1-sulfonic acid (ANSA, **47**) and examined by fluorophore-assisted carbohydrate electrophoresis (FACE). Results compared favorably, both qualitatively and quantitatively with earlier HPLC-based methods. MALDI-TOF MS was used to confirm the identity of the FACE separated glycans (Li, Hölte, & Young, 2004d).

46. 7-aminonaphthalene-1,3-disulfonic acid (ANDA)

47. 4-aminonaphthalene-1-sulfonic acid
(ANSA)

E. Glycoconjugates from Bacteria

Some small anaerobic bacteria such as treponemes contain glycoconjugates that are different from the normal glycolipids. One of these glycoconjugates has been isolated from the oral spirochete, *Treponema medium* by phenol/water extraction and the carbohydrate removed enzymatically for structural identification by NMR and MALDI-TOF MS. Fragmentation was performed with a TOF/TOF instrument and revealed the glycan sequence of a tetrasaccharide repeat unit containing the two amino acids ornithine (**48**) and Asp. The repeat unit ($\rightarrow 4$)- β -D-GlcpNAc3NAcA-(1 $\rightarrow$ 4)- β -D-ManpNAc3NAOrn-(1 $\rightarrow$ 3)- β -D-GlcpNAc-(1 $\rightarrow$ 3)- α -D-Fucp4NAcp-(1 $\rightarrow$) contained mannose that was amide-linked to L-ornithine and fucose linked to D-aspartic acid (Hashimoto et al., 2003a).

48. Ornithine

F. Glycolipids

1. Lipo-Poly- (LPS) and -Oligosaccharides (LOS)

Many examples of the application of MALDI-based methods to analysis of these compounds have been published; these are summarized in Table 6 and only a few representative studies and some with more unusual features will be discussed here. Extractions are normally carried out with hot aqueous phenol. These compounds often contain many more monosaccharide constituents than are found in the glycoproteins, requiring additional techniques such as combined gas chromatography/mass spectrometry (GC/MS) and NMR for their analysis. Their generally acidic nature, conferred by Kdo glycans and phosphate groups, makes them ideal candidates for examination in negative ion mode.

a. Intact LOS. Although small lipooligosaccharides can be examined directly, most have to be modified by hydrolysis of the large *O*-glycan chain or by removal of much of the lipid component of the lipid A (**1/18**) moiety. Thus, for example, the backbone structures of the LOS from *V. parahaemolyticus* strains have been obtained following *O*- and *N*-deacylation with

hydrazine and dephosphorylation with TFA (Hashii et al., 2003a,b). Laser-induced decomposition can be a problem or, in some cases, can yield useful structural information. Thus, the negative ion MALDI-TOF spectrum from DHB of the short-chain LOS from a strain of *V. parahaemolyticus* (3 kDa) following deacylation showed successive losses of phosphoethanolamine (**49**), hexuronic acid and phosphate (Hashii et al., 2003b). LOS from *M. catarrhalis* has been deacylated with hydrazine and examined in negative linear mode from DHB/hydroxy-*iso*-quinoline (HIQ). The spectrum showed several peaks produced by in-source cleavage at the labile Kdo-lipid A glycosidic bond (Luke et al., 2003). The lipid A molecule itself, examined in reflectron mode, was found to contain seven acyl groups and varying amounts of ethanolamine esterified to the two phosphate groups. Both MALDI-TOF and nanospray MS/MS analysis of the de-*O*-acylated (hydrazine) LOS from *Pseudoalteromonas haloplanktis* TAC 125 grown at 15°C has shown the presence of three phosphate groups, two on the lipid A and one on the core oligosaccharide, whereas at 25°C, an additional phosphate was added to the core (Ummarino et al., 2003). The LOS from *Pseudoalteromonas issachenkonii* KMM 3549^T has been found to contain what is thought to be the first example of a 4,7-disubstituted heptose as determined mainly by NMR; MALDI-TOF MS was used to delineate the lipid A acylation pattern (Silipo et al., 2004b). Two sets of ions were obtained as the result of cleavage of the labile Kdo-lipid A bond.

49. Phosphoethanolamine

Haemophilus ducreyi has been shown to be capable of incorporating sialic acids with *N*-acyl groups larger than acetyl. However, the extent of sialylation dropped with increasing chain length, becoming non-existent for sialic acid with chains above C8 (Goon et al., 2003). The LOS was deacylated with hydrazine and examined by MALDI-TOF from DHB/HIQ in negative ion mode; positive ion MS/MS spectra were obtained with electrospray ionization.

b. Lipid A. Mild acid hydrolysis of the labile bond between Kdo and the lipid A moiety is the normal method for releasing lipid A from these glycolipids. A method combining thin-layer chromatography (TLC) with both MALDI-TOF and ESI-MSⁿ (up to MS⁴) has been applied to the analysis of lipid A from *E. coli*. The predominant fragmentation was loss of acyl groups but, whereas this appeared to occur by charge-remote processes in the CID spectra, charge-driven processes appeared to predominate in the MALDI spectra (Lee et al., 2004a).

Several novel lipid As have been discovered during the review period. The first example of a neutral lipid A has been isolated from the predatory bacterium *Bdellovibrio bacteriovorus* (Schwudke et al., 2003). The molecule consisted of a β -(1 $\rightarrow$ 6)-linked 2,3-diamino-2,3-dideoxy-D-glucopyranose disaccharide carrying six hydroxylated fatty acids. α -D-mannopyranose residues occupied the two positions normally occupied by phosphates. A similar 2,3-diamino-2,3-dideoxy-D-glucopyranose

TABLE 6. Use of MALDI MS for examination of bacterial glycolipids

Species	Type	Instrumentation	Acyl chains	Glycans	Notes	Reference
<i>Aeromonas salmonicida</i>	Core	Q-TOF (DHB), ESI, CID	-	α -D-GlcN, α -D-Glc, α -D-GalNAc, α -D-Gal, α -D-Hep, Kdo	Structural determination of wild strain	(Banoub, <i>et al.</i> , 2004)
<i>Agrobacterium radiobacter</i>	O-Chain	R-TOF (DHB), SEC, NMR, GC/MS	-	α -L-Rhap, α -D-Manp, α -D-Fucp	Structural determination	(De Castro, <i>et al.</i> , 2004a)
<i>Agrobacterium tumefaciens</i>	Lipid A	TOF (-ve) (DHB), THAP, NMR, GC/MS	5	GlcN	First full structural characterization of lipid A	(Silipo, <i>et al.</i> , 2004a)
<i>Bartonella henselae</i>	LPS Lipid A	L-TOF (THAP), ESI, GC/MS, FTICR NMR	4	Di-NH ₂ -Glc, Glc, Kdo	Structural determination and biological activity	(Zähringer, <i>et al.</i> , 2004)
<i>Bdellovibrio bacteriovorus</i>	Intact + Lipid A	L-, R-TOF (DHB), NMR, ESI, GC/MS	6	Di-NH ₂ -Glc, α -D-Man, Kdo, Glc, GlcN, Hep	Identification of lipid A that contains α -D-mannose in place of phosphate	(Schwudke, <i>et al.</i> , 2003)
<i>Bordetella avium</i> ATCC 5086	O-Chain	TOF (-ve) (DHB), NMR, GLC	-	Substituted GlcAN ₂	Structural determination	(Larocque, <i>et al.</i> , 2003)
<i>Bordetella bronchiseptica</i>	Lipid A	TOF (DHB), TLC, GLC, GC/MS	5, 6	β -D-GlcN	Structural ident. Mutant shows 6 th C16:0 chain necessary for persistent infection	(Preston, <i>et al.</i> , 2003)
<i>Burkholderia caryophylli</i>	Lipid A	TOF (DHB), NMR, GLC, GC/MS	5	β -D-GlcN, Ara4N	Structural characterization. First demo. of Ara4N at reducing end of lipid A	(Molinaro, <i>et al.</i> , 2003)
<i>Burkholderia cepacia</i>	EPS	L-TOF (+ve, -ve) (DHB), NMR	-	α -D-Gal, β -D-Gal, Kdo	Structure. Growth in mannitol-enriched medium gave $\rightarrow$ 6)- β -D-Fru-(2 $\rightarrow$ (levan)	(Cescutti, <i>et al.</i> , 2003)
<i>Butyrivibrio fibrisolvens</i> H10b	EPS	R-TOF (+ve) (DHB), NMR, CD, GC/MS	-	β -D-Glc, α -D-Gal, carboxyethyl, OAc.	Structural determination. Growth under anaerobic conditions with Glc as source	(Andersson, <i>et al.</i> , 2003)
<i>Chlamydomphila psittaci</i> 6BC	LPS Lipid A	L-TOF (THAP + 0.1% TFA), NMR	5	α -GlcN, β -GlcN, Kdo	Structure. LPS less active than typical endotoxins in cytokine induction.	(Heine, <i>et al.</i> , 2003)
<i>Chlamydia trachomatis</i> E, L ₂	LPS Lipid A	L-TOF (THAP + 0.1% TFA), NMR	5	α -GlcN, β -GlcN, Kdo	Structure. LPS less active than typical endotoxins in cytokine induction.	(Heine, <i>et al.</i> , 2003)
<i>Coxiella burnetii</i>	Lipid A	L-TOF (THAP), GC/MS, FTIR, X-ray	4	GlcN	Structural determination	(Toman, <i>et al.</i> , 2004)
<i>Escherichia coli</i> O157:H7:K	Lipid A	R-TOF (-ve) (DHB), ESI, MS ⁿ , NMR	6	GlcN	Structural determination	(Lee, <i>et al.</i> , 2004a)
<i>Escherichia coli</i> mutant	LPS Core	L- + R-TOF (-ve) (DHB), ESI, NMR	-	Gal, Glc, Hep, Kdo, GlcN, PPETn	Overexpression of <i>waaZ</i> gene leads to modification of inner core	(Fridrich, <i>et al.</i> , 2003)
<i>Escherichia coli</i>	LPS	TOF (DHB)	6	GlcN	Study of central effects in rats	(Singh & Jiang, 2004)
<i>Escherichia coli</i>	Lipid A	TOF (THAP)	4, 5, 6	GlcN	Lipid As with different degrees of Ac more potent than fully acylated	(Mueller, <i>et al.</i> , 2004)
<i>Francisella novicida</i> in <i>E. coli</i>	Short-LPS	L-TOF (ATT)	6	GlcN, Kdo	Lipid A 1-de-phosphorylation occurs on periplasmic surface of inner membrane	(Wang, <i>et al.</i> , 2004c)
<i>Haemophilus ducreyi</i>	LPS	TOF (DHB/HIQ)	-	Neu5Ac homologues, β -D-Gal, β -D-GlcNAc	<i>H. ducreyi</i> can incorporate unnatural sialic acids into LPS	(Goon, <i>et al.</i> , 2003)

Species	Type	Instrumentation	Acyl chains	Glycans	Notes	Reference
<i>Haemophilus influenzae</i> 2019	LOS from biofilm	TOF (DHB)	-	Gal, Glc, Hep, Kdo, Neu5Ac	Structures may mimic O-linked glycans	(Greiner, <i>et al.</i> , 2004)
<i>Halomonas magadiensis</i>	Lipid A	R-TOF (THAP), NMR, GLC, GC/MS	6	GlcP _N	Structural ident. from organism living at high pH. Heterogeneous fatty-acids	(Silipo, <i>et al.</i> , 2004e)
<i>Helicobacter pylori</i>	Lipid A	TOF (ATT, NH ₄ ⁺ -citrate)	3, 4	GlcP _N	Modification of 1-phosphate requires two steps	(Tran, <i>et al.</i> , 2004)
<i>Klebsiella pneumoniae</i>	LPS	R-TOF (DHB)	-	GalA, Glc, GlcN, Hep, Kdo, Lipid A	<i>wabG</i> gene involved in attachment of D-GalA to 3-position of α -L-Hepp	(Izquierdo, <i>et al.</i> , 2003)
<i>Klebsiella pneumoniae</i>	LPS	R-TOF (DHB), PSD	-	GalA, Glc, GlcN, Hep, Kdo, Lipid A	uge gene (UDP-GalA 4 epimerase) essential for LPS synthesis	(Regué, <i>et al.</i> , 2004)
<i>Leptospira interrogans</i>	Lipid A	TOF (+ve, -ve) (ATT/NH ₄ ⁺ citrate), NMR, GC/MS	6	Di-NH ₂ -Glc	Structural determination. Contains methylated phosphate and four amide-linked acyl groups	(Que-Gewirth, <i>et al.</i> , 2004)
<i>Mesorhizobium huakuii</i>	Lipid A	TOF (DHB), FAB, GC/MS, ESI, NMR	4, 5, 6	Di-NH ₂ -Glc, GlcA	Structural determination of cyclic- β -glucan	(Choma & Sowinski, 2004)
<i>Methylobacterium</i> sp.	EPS	R-TOF (DHB/HIQ), NMR, IR	-	α -D-Galp	Highly pyruvated galactan EPS from Finnish paper machine bacterium	(Verhoef, <i>et al.</i> , 2003)
<i>Moraxella catarrhalis</i> 7169	LOS, Lipid A	L-TOF (-ve) (DHB/HIQ)	De-acyl 7	Hex, Kdo, GlcNR	Structural determination. LOS important for integrity of outer membrane and to avoid bactericidal activity of human sera	(Luke, <i>et al.</i> , 2003)
<i>Moraxella catarrhalis</i>	LOS, Lipid A	R-TOF, GC/MS	7	GlcP _N	Elimination of gene encoding Kdo-transferase gives LOS with only Lipid A	(Peng, <i>et al.</i> , 2004)
<i>Neisseria meningitidis</i> mutant	LPS	TOF (DHB), ESI, NMR	-	GlcNAc, Hep, Kdo, GlcN	Structural characterization of mutant aimed at vaccine production	(Monteiro, <i>et al.</i> , 2003)
<i>Neisseria meningitidis</i> mutant	Lipid A, LOS	TOF (DHB), ESI, MS/MS, GC/MS	4, 5, 6	GlcN, PEA	Structure. New pathway for regulation of LOS inner-core structure	(Tzeng, <i>et al.</i> , 2004)
<i>Neisseria meningitidis</i> NMB	Inner core	TOF (THAP), GLC, GC/MS, NMR	-	Kdo, α -Hepp, β -GlcP, α -GlcNAcP	Structural determination	(Kahler, <i>et al.</i> , 2004)
<i>Porphyromonas gingivalis</i>	Lipid A	TOF, GC/MS	4	GlcP _N	Structural determination of species that interact with Toll-like receptors 2 and 4	(Darveau, <i>et al.</i> , 2004)
<i>Prevotella intermedia</i> ATCC 25611	Lipid A	R-TOF, TOF/TOF (DHB), TLC, NMR	4	β -D-GlcN	Structural determination and immunobiological activity.	(Hashimoto, <i>et al.</i> , 2003b)
<i>Pseudoalteromonas haloplanktis</i> TAC 125	LPS	TOF (DHB), ESI, MS/MS	-	GlcN, Kdo, Gal, GlcNAc	Determination of phosphorylation sites in bacteria grown at different temps.	(Ummarino, <i>et al.</i> , 2003)
<i>Pseudoalteromonas issachenkonii</i> KMM 3549 ^T	LPS, Lipid A	R-TOF (-ve) (THAP), NMR	3, 4, 5	GlcN, α -Gal, β -Gal, Hep, Kdo	Structural determination	(Silipo, <i>et al.</i> , 2004b)
<i>Pseudomonas aeruginosa</i>	Core	TOF (DHB), FTICR	-	α -D-GalN, L- α -D-Hep, Kdo, β -D-Glc, β -D-Glc, α -L-Rha,	Structure of highly phosphorylated core	(Kooistra, <i>et al.</i> , 2003)

(Continued)

TABLE 6. (Continued)

Species	Type	Instrumentation	Acyl chains	Glycans	Notes	Reference
<i>Pseudomonas aeruginosa</i>	Lipid A	TOF	6	GlcN, AraN	Bacterium adds AraN to PO ₄ of lipid A as resistance to antimicrobial peptides	(Moskowitz, Ernst & Miller, 2004)
<i>Pseudomonas cichorii</i>	Core	TOF (THAP), NMR, GC/MS	-	α -L-Rha, β -GlcNAc, Kdo, α -GalNAc, β -GlcN4P, α -GlcN1P	Structural characterization. Lacks heptose and phosphate	(De Castro, <i>et al.</i> , 2004b)
<i>Pseudomonas corrugata</i>	Lipid A	L-TOF (DHB), ESI, MS/MS	5	GlcN	Structural characterization	(Corsaro, <i>et al.</i> , 2004)
<i>Pseudomonas stutzeri</i> OX1	Core	TOF (-ve) (THAP), NMR	-	GlcN, GalN, Hep, Kdo, Glc, pyruvic acid	Structural determination of novel highly negatively charged LPS	(Leone, <i>et al.</i> , 2004a)
<i>Pseudomonas stutzeri</i> OX1	LPS	R-TOF (THAP), GLC, GC/MS, NMR	-	GlcN1P, GlcN4P, α -Kdo, α -Hep2P4P, α -Hep6P, α -GalN, β -GlcN, β -Glc	Structural determination of minor oligosaccharide after deacylation	(Leone, <i>et al.</i> , 2004b)
<i>Pseudomonas syringae</i>	EPS	TOF (Sinapinic acid), FTIR, HPTLC	-	Glc, GlcA (Mannose in strain affecting tomato)	Glycan composition in strains that infect different plants	(Datta, <i>et al.</i> , 2004)
<i>Pseudomonas tolaasii</i>	Core-lipid A	TOF (THAP), GLC, GC/MS, NMR	-	GlcNp, GlcNp1P, Kdo, Hep, α -D-GalNp, α -D-GlcNp, β -GlcN, L-Rha, β -QuiNp	Structural determination	(Silipo, <i>et al.</i> , 2004c)
<i>Rhizobium leguminosarum</i>	Lipid A	L-TOF (DHB), TLC	5	β -D-GlcN, 2-NH ₂ -gluconate, GalA	Structural determination. First report of outer membrane oxidase.	(Que-Gewirth, <i>et al.</i> , 2003)
<i>Salmonella enterica</i>	Lipid A	R-TOF (DHB)	-	GlcN	Genetics of PEA modification conferring polymyxin resistance	(Lee, <i>et al.</i> , 2004b)
<i>Sinorhizobium</i> sp. NGR234	Lipid A	TOF (THAP), GC/MS, TLC, NMR	3, 4, 5	β -D-GlcN	Structural characterization.	(Gudlavalleti & Forsberg, 2003)
<i>Sinorhizobium meliloti</i>	LPS	TOF	5	Kdo, GlcNp	Role of LPS core in symbiosis of rhizobiaceae with plants	(Kanipes, <i>et al.</i> , 2003a)
<i>Streptococcus</i> group B	CPS	TOF (THAP), LC/MS	-	β -D-Galp, β -D-GlcNAc, β -D-GlcN, Neu5Ac	Some sialic acid is O-acetylated at C7, 8 or 9	(Lewis, Nizet & Varki, 2004)
<i>Treponema medium</i> ATCC 700293	Glyco-conj.	TOF, TOF/TOF (DHB), NMR	-	β -D-GlcNac3NAcA, β -D-ManpNAc3NA, β -D-GlcNac, α -D-Fucp4N	Structural characterization. Glycoconjugate showed weak endotoxic properties, L-Orn, D-Asp	(Hashimoto, <i>et al.</i> , 2003a)
<i>Vibrio fischeri</i> strain HMK	LPS	TOF(DHB)	-	-	SDS-PAGE sepn. depends on variations in oligosaccharide region and PO ₄ conc.	(Pupo, <i>et al.</i> , 2004)
<i>Vibrio parahaemolyticus</i> O2	LPS	R-TOF (-ve) (DHB), FAB, GC/MS, NMR	-	D-Gal, D-Glc, D-GlcN, Kdo, Non5Ac7Ala, D-GlcA, Hep	Structural characterization.	(Hashii, <i>et al.</i> , 2003a)
<i>Vibrio parahaemolyticus</i> KX-V212	LPS	R-TOF (-ve) (DHB), NMR, GLC, GC/MS	-	D-Gal, D-Glc, D-GlcN, Kdo, Non5Ac7Ala, D-GlcA, Hep	Structural characterization.	(Hashii, <i>et al.</i> , 2003b)
<i>Xanthomonas campestris</i> pv. <i>pruni</i>	Lipid A	L-, R-TOF (THAP)	6	GlcNAc, phosphate	Presence of methyl-branched acyl chains	(Silipo, <i>et al.</i> , 2004d)
<i>Yersinia pseudotuberculosis</i>	Lipid A	L-TOF	6, 7	GlcN, AraN	Bacterium adds AraN to PO ₄ of lipid A as resistance to antimicrobial peptides	(Marceau, <i>et al.</i> , 2004)

disaccharide carrying six hydroxylated fatty acids was found in the lipid A from *Leptospira interrogans* which causes a hemorrhagic fever known as Weil's disease. The molecule carried only one phosphate group which was mono-methylated. The two hydroxy-acyl groups attached to the diamino-glucose residue I were esterified with unsaturated ω -6-12:1 and 14:1 fatty acids (Que-Gewirth et al., 2004). 2,3-Diamino-2,3-dideoxy-D-glucopyranose has also been found in lipid A from *Bartonella henselae*, a Gram-negative bacterium that causes cat-scratch disease (Zähringer et al., 2004) and in that from *Mesorhizobium huakuii*. In the latter compound, the phosphate group normally attached to the di-NH₂-Glc moiety II was replaced by D-glucuronic acid (Choma & Sowinski, 2004). A 4-amino-4-deoxy-L-arabinopyranose-1-phosphate has been found at the reducing end of the lipid A from *Burkholderia caryophylli* (Molinaro et al., 2003). The negative ion MALDI-TOF (DHB) spectrum showed considerable heterogeneity due to differing numbers of acyl groups. The proximal (reducing end) GlcN residue of lipid A from *Rhizobium leguminosarum* lacks a phosphate group and can be oxidized to 2-amino-2-deoxy gluconate by an enzyme recently identified in the outer membrane of the bacterium (Que-Gewirth et al., 2003). The first example of lipid A from an *Agrobacterium* species (*A. tumefaciens* strain C58, a plant pathogen) has revealed the presence of a normal bis-phosphorylated glucosamine disaccharide backbone with several acyl variants. The main species was a penta-acyl derivative bearing two 3-OH-14:0 fatty acids in ester linkage and two 3-OH-16:0 acids in amide linkage. The 3-OH-16:0 acid on GlcN II was esterified with 27-OH-28:0 that, in turn was esterified with a 3-OH-butyryl residue (Silipo et al., 2004a). Other lipid A molecules to have been analyzed in the review period are listed in Table 6.

c. Core oligosaccharide. Mild acid hydrolysis is also the method normally used to obtain core oligosaccharides from LPS. Ethanolamine diphosphate has been identified for the first time and its position of substitution established in the LPS core of a rough-type mutant of *Pseudomonas aeruginosa* (PAO1 Δ algC). In addition, the paper reported a reinvestigation of the structure of the complete LPS core of wild-type *P. aeruginosa* PAO1 and a reassignment of the phosphorylation sites (Kooistra et al., 2003). The LPS was complex because of the presence of esterified fatty acids that were removed by mild hydrazinolysis prior to analysis by ESI and MALDI. Phosphoethanolamine has also been found in strains of *N. meningitidis* (Tzeng et al., 2004) and 4,6-*O*-(1-carboxy)-ethylidene residues derived from pyruvic acid have been identified in the core of LOS from *Pseudomonas stutzeri* OX1 (Leone et al., 2004a).

d. O-chain. The O-chain from *Bordetella avium* LPS, obtained by mild acid hydrolysis to remove the lipid A followed by hydrofluorolysis to remove the core oligosaccharide, was found to consist only of a chain of the substituted 1 $\rightarrow$ 4 linked monosaccharide 2-acetamido-3-(3-hydroxybutanamido)-2,3-dideoxy- β -D-glucopyranosyluronic acid (**50**). The negative ion MALDI-TOF spectrum from DHB showed a series of peaks from 1 to 3.8 kDa (Larocque et al., 2003). Other examples of O-chain analysis are cited in Table 6.

50. 1 $\rightarrow$ 4 linked monosaccharide 2-acetamido-3-(3-hydroxybutanamido)-2,3-dideoxy- β -D-glucopyranosyluronic acid

e. Extracellular polysaccharides (EPS). Several examples of recent analyses of EPS are cited in Table 6 and only two representative examples will be expanded here. The structure of the repeating unit of the EPS from *Butyrivibrio fibrisolvens* strain H10b is a hexasaccharide carrying *O*-acetyl and 1-carboxyethyl groups and has been determined with a variety of techniques including NMR and circular dichroism (CD) following extraction with phenol. MALDI-TOF MS of fractions from a partial acid hydrolysate was used to determine the carbohydrate sequence (Andersson, Cotta, & Kenne, 2003). *B. cepacia*, a bacterium causing lung infections was obtained from a cystic fibrosis patient and cultured on agar plates. Phenol was added to the harvested cells, stirred for 5 hr at 4°C and the EPS was precipitated with isopropanol. MALDI-TOF and NMR were used to determine the structure of the repeating unit as $\rightarrow$ 5)- β -Kdop-(2 $\rightarrow$ 3)- β -D-Galp2Ac-(1 $\rightarrow$ 4)- α -D-Galp-(1 $\rightarrow$ 3)- β -D-Galp-(1 $\rightarrow$). When grown on a mannitol-rich medium, conditions that induce the formation of EPS, the bacteria produced a second compound with the structure ($\rightarrow$ 6)- β -D-Fruf-(2 $\rightarrow$) (Cescutti et al., 2003).

2. Glycosphingolipids (GSL)

a. Studies on the glycan moiety following removal of the ceramide. Glycosphingolipids can be analyzed directly by MALDI-TOF but the spectra are usually complicated because of heterogeneity in the ceramide portion of the molecule. Thus, many investigators use an enzyme such as ceramide glycanase to remove the ceramide. A recent example is a study of the effect of *N*-alkyl analogues of deoxynojirimycin (DNJ), inhibitors of ceramide glucosyltransferase, on GSL structure. GSLs were cleaved with ceramide glycanase and labeled with 2-AB (**1/56**) for analysis by HPLC and MALDI-TOF (Mellor et al., 2004a). Carbohydrates substituted with phosphocholine (PC) and phosphoethanolamine (PE) have been released with endoglycosamidase from glycosphingolipids of the pig parasitic nematode *Ascaris suum* and identified by MALDI, ESI, GC/MS (methylation analysis) and NMR (Friedl et al., 2003).

A rapid method for splitting glycosphingolipids into their three components (sugar, sphingosine and fatty acid) by use of a domestic microwave oven has been reported by Itonori et al. (2004b). The glycosphingolipids were heated with 0.1 M NaOH/MeOH for 2 min followed by 1 M HCl/MeOH for 45 sec. The alkaline methanolysis step produced the intermediate lysoglycosphingolipids virtually free of by-products such as the *O*-methyl ethers that are usually seen but the *N*-acetyl amino-sugars needed re-acetylation by reaction with acetic anhydride/pyridine at room temperature.

b. Studies by TLC and MALDI. Glycosphingolipids are frequently separated by TLC. Ivleva et al. (2004) have published a method for attaching TLC plates to a MALDI target and have obtained spectra of even fragile compounds such as gangliosides by using an FTICR instrument fitted with a vibrationally cooled MALDI ion source. Twenty matrices were investigated and the soft matrices, DHB, ATT and anthranilic acid (**1/57**) were found to produce the least sialic acid loss from gangliosides. TLC resolution was best preserved by using matrices in rapidly evaporating organic solvents.

c. Applications to the structural elucidation of glycosphingolipids. A novel GlcNAc- α -1 $\rightarrow$ HPO₃ $\rightarrow$ 6-Gal-(1 $\rightarrow$ 1)-ceramide (**51**) has been identified from the liver fluke, *Fasciola hepatica*, by MALDI-TOF and electrospray-MS/MS. HF treatment released the GlcNAc residue, confirming the GlcNAc-phosphate link (Wuhrer et al., 2003b). Mammalian type globo- and iso-globotriaosylceramides have also been found in this species obtained from infected sheep. MALDI-TOF spectra were obtained from both the intact glycosphingolipids and the 2-AP derivatives of their released glycans using ATT as the matrix, and the presence of antigenic terminal α -galactose was demonstrated by on-target incubation with α -galactosidase (from chicken) (Wuhrer et al., 2004a). Ceramides carrying β -Gal-(1 $\rightarrow$ 6)- β -Gal-(1 $\rightarrow$ 1)-Cer and larger oligosaccharides with from one to three additional β 1 $\rightarrow$ 6-linked mannose residues represent a departure from the common glycosphingolipids that contain inositol phosphate found in many fungi and may account for the resistance of this species to the fungicide, Aureobadin A (Aoki et al., 2004b). Measurements by SELDIMS of tetanus neurotoxin (53.7 kDa) incubated with the ganglioside GT1b, and observation of the intact protein/ganglioside complex, has shown that two carbohydrate binding sites are required for toxicity (Rummel et al., 2003).

d. Miscellaneous studies. Two photoaffinity probes (ganglioside GM2 labeled on the acyl chain with a *p*-(3-trifluoromethyl)diaziriny-phenyl group) (**52**), were used to probe the active site of GM2-activator protein. After binding, and trypsinization, the tryptic peptides were examined by MALDI-TOF and ESI-MS and found to be bound to the most flexible region of the protein (Wendeler et al., 2004a). γ -Radiolysis of glucosylceramide and galactosyl diglyceride resulted in the release of the sugars as shown by MALDI-TOF MS (Shadyro et al., 2004). Other studies and details of the above work are summarized in Table 7.

52. *p*-(3-trifluoromethyl)diaziriny-phenyl photoaffinity labels

3. Glycoinositolphosphosphingolipids (GIPSL)

Novel zwitterionic glycosphingolipids having the sugar chain capped with phospholcholine have been reported from the

mycelia of filamentous fungi of *Acremonium* species (Aoki et al., 2004a). Structures were of the type PC $\rightarrow$ 6-(Man-(α 1 $\rightarrow$ 6)-Man-(α 1 $\rightarrow$ 6)-GlcN-(α 1 $\rightarrow$ 2)-Ins1-P-1-Cer. A related compound without the attached glycan chain has been identified from the red alga *Gracilaria verrucosa* (Khotimchenko & Vas'kovsky, 2004).

4. Glycoglycerolipids

The glycoglycerolipid α -D-Glcp-(1 $\rightarrow$ 3)- α -D-Glcp-(1 $\rightarrow$ 1)-lipid where the lipid is glycerol containing a C₁₅ acyl group at C-2 and a C₁₅ ether at C3 has been separated from the propionibacterium *Propionibacterium propionicum* by TLC and its structure determined by MALDI-TOF, NMR, ESI/MS, and GC/MS. Related compounds acylated at C6 of the outer glucose residue and compounds lacking either this glucose or one fatty acid residue were also found (Pasciak et al., 2003). Glycoglycerolipids from the thermophilic bacterium *Meiothermus taiwanensis* have been shown to have the structure α -Galp-(1 $\rightarrow$ 6)- β -Galp-(1 $\rightarrow$ 6)- β -GalNAcy-(1 $\rightarrow$ 2)- α -Glc-(1 $\rightarrow$ 1)-Gro where Acyl = 17:0 or hydroxy-17:0 (Yang et al., 2004). A similar compound from the thermophilic bacterium *Thermus oshimai* NTU-063 has the structure β -Glcp-(1 $\rightarrow$ 6)- β -Glcp-(1 $\rightarrow$ 6)- β -GlcpNAcy-(1 $\rightarrow$ 2)- α -Glc-(1 $\rightarrow$ 1)-glycerol diester where the *N*-acyl group is a 15:0 or 17:0 fatty acid and the glycerol-attached fatty acids have chain lengths from 15:0 to 18:0. The MALDI-TOF spectrum contained a range of peaks from *m/z* 1,300 to 1,550 (Lu et al., 2004a). Di-mannosyl-acyl monoglyceride in which the mannose residue adjacent to the glycerol contained an anteiso-branched 15-carbon acid at the 6-position has been characterized from *Rothia mucilaginosa*, a pathogen responsible for several opportunistic infections. Unusually the acyl group attached to the glycerol was at C2 rather than the more usual C3 (Pasciak et al., 2004).

5. Lipomannans, Lipoarabinomannans, and Arabinomannans

The structure of a novel mannose-capped lipoarabinomannan from *Amycolatopsis sulphurea* has been investigated with a range of techniques of which MALDI-TOF (DHB, negative ion) showed an average molecular mass of around 10 kDa but with a broad, unresolved envelope of peaks with a half-height width of approximately 5 kDa (Gibson et al., 2003). The lipoarabinomannan from the mycobacterium-like organism *Tsukamurella paurometabola* has produced a MALDI-TOF spectrum from s-DHB with a broad peak with a mass range from 9 to 16 kDa. The structure was mainly determined by NMR. It lacked some of the features such as branching arabinan chains that are found in arabinomannans from mycobacteria but, nevertheless the molecule was claimed to be the most elaborated non-mycobacterial arabinomannan identified to date (Gibson et al., 2004). Lipomannan and lipoarabinomannan from *Mycobacterium kansasii* has been shown to possess a manno-oligosaccharide cap but to lack the phosphoinositol cap found in lipoarabinomannans from *Mycobacterium smegmatis* (Guérardel et al.,

TABLE 7. Use of MALDI MS for examination of glycosphingolipids

Organism	Type	Instrument (Matrix) Other technique Sample (derivative)	Sugars	Sphingosine	Acyl group	Notes	Reference
<i>Abidia corymbifera</i> F-965 (fungus)	Total	TOF (+ve) (DHB), TLC, IR, NMR	D-Glc	9-Me-Δ ⁸ -C ₁₈ - sphingosine	2-OH-16:0	Structural determination	(Batrakov, <i>et al.</i> , 2004)
<i>Ascaris suum</i>	Total	R-TOF (+ve) (s-DHB), ESI, MS ⁿ , GC/MS (Per- Me), NMR	α-Gal, β- GalNAc, β- GlcNAc, β- Man, α-Fuc, Glc	Not analysed	Not analysed	Structural identification of zwitterionic glycans containing phosphocholine and phosphoethanolamine	(Friedl, <i>et al.</i> , 2003)
Cow and human	Milk	TOF (DHB), GLC	Gal, GlcNAc, Glc, Neu5Ac	D18:1	Sat. 12:0- 25:0, Unsat., 16, 17, 18, 20, 22, 24	Milk gangliosides in the two species differ in their fatty acyl composition	(Bode, <i>et al.</i> , 2004)
<i>Fasciola hepatica</i> (liver fluke)	Total	TOF (ATT), ESI, MS ⁿ , NMR, TLC	GlcNAc, Gal	C ₁₈ - and C ₂₀ - phytosphingosine	2-OH-16:0	Structural identification. Novel GlcNAc1-HPO ₃ -6-Gal-1-Cer	(Wuhrer, <i>et al.</i> , 2003b)
<i>Fasciola hepatica</i> (liver fluke)	Total, from sheep	TOF (ATT), ESI, MS/MS, (Glycans 2- AP)	Mammalian globbo- and iso- globbo- types	C ₂₀ - phytosphingosine	α-OH-18:0, 24:1	Structural determination. Mammalian-type glycolipids. α- Gal by exoglycosidase digestion	(Wuhrer, <i>et al.</i> , 2004a)
Human	Vitreous body of eye	TOF (DHB), TLC	Glc	d18:1	16:0, 18:0, 18:1, 22:0, 24:1, 24:0	Accumulation of ceramide monohexoside in eyes of patients with Gaucher disease	(Fujiwaki, <i>et al.</i> , 2004)
Human and rat	Intestine	TOF (DHB), ESI, MS/MS, NMR	β-Gal, α-Fuc, β-GlcNAc, Glc, Cer	d18:1, t18:0	OH-16:0, OH-22:0, OH-24:0, n20:0, n22:0	Identification of default biosynthetic pathway	(Ångström, <i>et al.</i> , 2004)
<i>Lingula unguis</i> (Lamp shell)	Soft tissues	L-TOF (7-NH ₂ -4-Me- coumarin), GC/MS, TLC	β-Man, α- Man, β-Glc	d18:1, d18:3, t18:0	16:0-24:0, OH-16:0- OH-18:0	Structural identification. Classification into new molu- series	(Aoki, <i>et al.</i> , 2002)
<i>Mucor hiemalis</i>	Total	L-TOF (7-NH ₂ -4-Me- coumarin), GC/MS, NMR	α-Gal, β-Gal	Phytosphingosine	24:0, 25:0, OH-26:0	Ident. of a novel GSLs. Proposal that biosynthesis in <i>Zygomycetes</i> different from that in other fungi	(Aoki, <i>et al.</i> , 2004b)
<i>Plasmodium falciparum</i>	Intra- erythrocyte stage	L-, R-TOF (DHB, <i>nor</i> - harmene), TLC	Gal, Glc, GalNAc	d18:0, d20:0	OH-10:0, OH-12:0, OH-14:0	Structural determination	(Couto, <i>et al.</i> , 2004)

53. Hexamannoside from *Mycobacterium* species

2003). Mechanistic studies on the biosynthesis of lipoarabinomannan from *M. smegmatis* have been conducted with the help of MALDI-TOF analysis (Korduláková et al., 2003; Zhang et al., 2003c).

6. Other Glycolipids

Mass spectrometric methods for analysis of flavonoids have been reviewed (Prasain, Wang, & Barnes, 2004). Three glycosyltransferases (Pérez et al., 2004b) and two methyltransferases (Pérez et al., 2004a) involved in the biosynthesis of phenolic glycolipids from *Mycobacterium tuberculosis* have been characterized. MALDI-TOF spectra of the glycolipids showed a range of products with masses of around 1.5 kDa resulting from acyl heterogeneity. A new mannitol teichoic acid has been reported from the cell wall of the bacterium *Brevibacterium* sp. VKM Ac-2118 isolated from a frozen late Pliocene layer (1.8–3 Myr) of Kolyma lowland, Russia. The structure consisted of 1,6-poly(mannitol phosphate) with the majority of mannose residues carrying additional phosphate groups. Positive ion MALDI spectra from DHB gave peaks to 6 kDa (Potekhina et al., 2003). The structures of hexamannosides from mycobacterial species have been elucidated and found to contain up to four long-chain acyl groups (Gilleron, Quesniaux, & Puzo, 2003). A typical structure is 53. Studies with *M. smegmatis* have shown that mannose metabolism, as revealed by studies on phosphoinositol mannosides, is required for growth (Patterson et al., 2003). Mycoglycolipids have been isolated from the edible fungus *Hypsizygus marmoreus* and identified by GC/MS and MALDI-TOF MS (Itonori, Aoki, & Sugita, 2004a). The biosynthesis of mycobacterial phosphatidyl mannosides has been revised with the help of MALDI-TOF analysis (Morita et al., 2004). PIM₄ (four mannose residues and two palmitate chains) present in mycobacterial phosphatidyl manno-oligosaccharide, identified by PSD from CHCA, has been shown to be a natural antigen for CD1d-restricted T cells (Fischer et al., 2004).

G. Glycosides

Although MALDI-TOF MS is used quite extensively in this field, FAB still appears to be the most commonly encountered ionization technique. Glycosides identified by MALDI during the review period are listed in Table 8. Glucosylated heteropolyflavans (e.g., 54) from *Sorghum bicolor* have been examined by Krueger, Vestling, and Reed (2003); polymers with from three to seven flavan units and up to seven glucose residues were found. To overcome problems with estimation of the number of hydroxyl groups present due to the coincidence in mass with the $[M + K]^+$ ion, samples were analyzed as cesium adducts by addition of cesium trifluoroacetate to the sample/matrix solution. Silver trifluoroacetate was also investigated but problems were

54. Glucosylated heteropolyflavan from *Sorghum bicolor*

TABLE 8. Use of MALDI MS for examination of glycosides

Species	Glycoside type	Instrumentation	Sugars	Reference
<i>Ajuga salicifolia</i>	1 st reported ionone glycoside	FT-ICR (DHB), NMR, HPLC	Glucose	(Akabay, <i>et al.</i> , 2003)
<i>Ardisia mamillata</i>	Triterpenoid saponins	TOF, FAB, ORD, NMR, LC	β -D-Glcp, α -L-Rhap, α -L-Arap	(Huang, <i>et al.</i> , 2003)
<i>Borrelia burgdorferi</i>	Cholesteryl galactoside	L-TOF, NMR, FAB, GLC	β -D-Gal, Agent for Lyme disease	(Ben-Menachem, <i>et al.</i> , 2003)
<i>Borrelia burgdorferi</i>	Acylated galactosyl-glycerol	L-TOF, NMR, FAB, GLC	α -D-Gal	(Ben-Menachem, <i>et al.</i> , 2003)
<i>Borrelia burgdorferi</i>	Acylated cholesteryl galactoside	TOF, TLC, GC/MS, NMR	α -D-Gal 16:0, 18:0, 18:1, 18:2 Ac	(Schröder, <i>et al.</i> , 2003)
<i>Cheilanthes glauca</i>	Flavonol glycosides	TOF	Rha	(Pastene, <i>et al.</i> , 2001)
<i>Clitoria ternatea</i>	Malonated flavonol glycosides	TOF, ESI, NMR, HPLC	α -Rhap, β -D-Glcp	(Kazuma, Noda & Suzuki, 2003a)
<i>Clitoria ternatea</i>	Flavonol glycosides	TOF, ESI, NMR, HPLC	α -Rhap, β -D-Glcp	(Kazuma, Noda & Suzuki, 2003b)
<i>Hedysarum setigerum</i>	Flavonol glycosides	MALDI	D-Glc, L-Rha	(Neretina, <i>et al.</i> , 2002)
<i>Henricia sanguinolenta</i> and <i>H. leviuscula leviuscula</i>	Steroidol polyols. Cholesterol	TOF (CHCA), HPLC, TLC, NMR	Mono-, di- and tri-OMe- β -Xylp (sanguinolenta C from starfish)	(Levina, <i>et al.</i> , 2003)
<i>Henricia sanguinolenta</i> and <i>H. leviuscula leviuscula</i>	Steroidol glycosides (Cholestanes, cholestenes)	TOF (CHCA), NMR, HPLC, TLC	2-O-Me-Xylp	(Kalinovskii, <i>et al.</i> , 2004)
<i>Hippasteria phrygiana</i> (Starfish)	Steroidol glycosides (Cholestanes, cholestenes)	TOF (CHCA), NMR, HPLC, TLC	β -D-Xylp, α -L-Araf	(Levina, <i>et al.</i> , 2004)
<i>Hordeum sp.</i> (Barley)	Flavone glycosides	TOF (DHB), NMR	β -D-Glc	(Norbæk, <i>et al.</i> , 2003)
<i>Lupinus oreophilus</i>	Ten triterpenoid saponins	FT-ICR, ESI, NMR, HPLC	α -L-Ara, β -D-Gal, β -D-Glc, α -L-Rha	(Woldemichael, Montenegro & Timmermann, 2003)
<i>Marrubium velutinum</i>	Acetylated glycosides	FT-ICR (DHB) LC TLC NMR	β -D-Glcp	(Karioti, <i>et al.</i> , 2003)
<i>Mycale laxissima</i>	Steroidol glycosides	TOF, NMR, GC/MS	D-Ara, D-Glc, D-Gal	(Antonov, <i>et al.</i> , 2003)
<i>Mycobacterium tuberculosis</i> , <i>M. bovis</i>	Phosphatidyl- <i>myo</i> -inositol mannoside	R-TOF (-ve) (HABA), NMR	β -D-Man	(Gilleron, <i>et al.</i> , 2003)
<i>Phlomis physocalyx</i> Hub.-Mor.	Phenylethanoid tetraglycoside (physocalyxoside)	FT-ICR (DHB), MNR, OR, IR, UV, TLC	α -L-Rha, β -D-Glc, Api	(Ersöz, <i>et al.</i> , 2003)
<i>Plantago major</i> L. (Greater plantain)	Phenolic glycoside, (Verbascoside, 56)	TOF, NMR, HPLC	Glc, Rha	(Egorov, <i>et al.</i> , 2004)
<i>Pseudostichopus trachus</i>	Triterpene glycoside	TOF (CHCA), NMR	Xyl, Qui, OMe-Glc	(Silchenko, <i>et al.</i> , 2004)
<i>Quillaja saponaria</i>	Triterpenoid saponins (28 compounds)	R-TOF (DHB), NMR	β -Gal, β -GlcA, α -L-Rha, β -Fuc, α -L-Ara, β -Xyl, β -Glc, β -D-Api	(Nyberg, <i>et al.</i> , 2003)
<i>Rhizophora mangle</i> L.	Flavonol glycosides from tannin	MALDI	Rha, Glc, (Mangrove) Glc	(Kandil, <i>et al.</i> , 2004)
<i>Sorghum bicolor</i> (L.) Moench	Heteropolysaccharides	L+R-TOF (+ve) (3-IAA),		(Krueger, <i>et al.</i> , 2003)
<i>Solenostemma argel</i> (Asclepiadaceae)	Oxo-pregnane glycosides (stemmosides C and D)	R-TOF (CHCA), NMR, ESI	Linear pentasaccharide, β -D-Glcp β -D-Olep, β -D-Canz, β -D-Cymp	(Plaza, <i>et al.</i> , 2004)
<i>Stichopus mollis</i>	Triterpenoid saponins	TOF (CHCA), NMR, GC/MS	β -D-OMe-Glcp, β -Xyl, β -D-Quip, β -4-SO ₃ Na-Xyl	(Moraes, <i>et al.</i> , 2004)
<i>Thalictrum orientale</i>	Phenyl glycosides	FT-ICR, UV, NMR	β -D-Glcp, β -D-Xylp	(Erdemgil, <i>et al.</i> , 2003)

encountered with cluster formation and the two mass unit difference between the silver isotopes. Over 28 triterpene saponins, 8 of them new, have been identified in a fraction, named QH-B from the South American tree *Quillaja saponaria*. The 28 saponins formed 14 equilibrium pairs by the migration of an *O*-acyl group between two adjacent positions on a fucosyl group (Nyberg, Baumann, & Kenne, 2003). The triterpene glycoside from the sea cucumber *Stichopus mollis* (Stichopodidae) was found by Moraes et al. (2004) to differ in structure from the corresponding glycosides from all other known stichopodids in possessing a sulfate group in the carbohydrate chain, prompting the authors to propose a reclassification to a new genus, *Australostichopus* Levin. Both MALDI and ESI in negative ion mode have been demonstrated to be capable of detecting carminic acid (**55**), the red dye isolated from cochineal, in paintings applied to canvas. Binding agents such as linseed oil did not interfere with the detection (Maier, Parera, & Seldes, 2004). MALDI-TOF has also been used to study the biotransformation of ginsenoside Rb₁ by 49 microbial strains (Dong et al., 2003).

55. Carminic acid

56. Verbascoside

H. Osmoregulated Periplasmic Glucans

These compounds are mixtures of cyclic, branched, and linear polymers of glucose containing various species-different substituents. They accumulate in the periplasmic space of gram-negative bacteria in response to low osmolarity. Lequette et al. (2004) have identified the gene that encodes for the protein that controls the backbone structure of these carbohydrates. Associated analysis of the carbohydrates with masses up to 3.5 kDa was performed by MALDI-TOF from DHB or 3-AQ.

IX. GLYCOSYLATION AND OTHER REACTION MECHANISMS

Matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS) has been used by many

laboratories to monitor the products of enzyme reactions aimed at deducing mechanisms of action or characterization of newly discovered enzymes. Much of this work is summarized in Table 9. Desorption/ionization on silicon mass spectrometry (DIOS-MS) or laser desorption without a matrix has been shown to be a viable alternative to MALDI for analysis of small molecules and carbohydrates and has been used to monitor the activity of $\alpha 2 \rightarrow 6$ -sialyltransferase on the disaccharide Gal- β -(1 $\rightarrow$ 4)-GlcNAc (Shen et al., 2004). Jobron et al. (2003) have immobilized *N*-acetyllactosamine on a cellulose membrane and used it as an acceptor for assaying four glycosyltransferases. The trisaccharide produced by $\alpha 1 \rightarrow 3$ -galactosyltransferase was identified by MALDI and the method was proposed as the basis of a high-throughput technique for enzyme screening. A method for detecting the presence of *O*-glycanase, an enzyme that cleaves the Gal- β -(1 $\rightarrow$ 3)-GalNAc- α -(1 $\rightarrow$) link to serine or threonine, uses MALDI to detect the $[M + Na]^+$ ion at *m/z* 406 corresponding to the released sugar (Akai et al., 2003).

X. INDUSTRIAL APPLICATIONS

A. Food Industry

A MALDI-TOF method for the simultaneous detection and measurement of sugars, ascorbic acid (**57**), citric acid (**58**), and sodium benzoate has been developed by Ayorinde, Bezabeh, and Delves (2003). The matrix was *meso-tetrakis*-(pentafluorophenyl)porphyrin (**59**), a compound with a molecular weight of 974 which is well above that of the target molecules. Sodium or potassium hydroxides were used as dopants. The method was particularly good at detecting the low molecular weight organic acids. MALDI-TOF MS, from DHB also provides a method for detecting the presence of starch hydrolysates as adulterants in fruit juices (Cabálková et al., 2004). The action of the enzyme preparation, Olivex, used in the preparation of olive (*Olea europaea*) oil has been investigated. The preparation is mainly a pectinase that hydrolyses (1 $\rightarrow$ 4)-linked galactan chains from the cell wall. However, it is unclear how this reaction improves the quality of the product (Vierhuis et al., 2003). A comparative study of five *Rhizobium* strains from different cropping areas of China has concluded that differences in the efficiency of nitrogen fixation is not related to differences in Nod factor structure (identified by MALDI-TOF and ESI-Q-TOF MS) (Thomas-Oates et al., 2003).

57. Ascorbic acid

58. Citric acid

59. *meso*-tetrakis(pentafluorophenyl)porphyrin

B. Biopharmaceutical Industry

Production of humanized antibodies and other biopharmaceuticals in plants and insect cell lines, as a safe and economically feasible alternative to production in animals, requires re-engineering of the *N*-glycan synthesis machinery to eliminate potentially antigenic $\alpha 1 \rightarrow 3$ -fucose- and $\beta 1 \rightarrow 2$ -xylose-containing glycans. MALDI-TOF analysis is ideally placed for analysis of the resulting glycans and many examples are listed in Table 10. An analysis of human transferrin expressed in *Lymantria dispar* (gypsy moth), however, has shown the natural absence of core $\alpha 1 \rightarrow 3$ -fucosylation, a normally common motif in insects (Choi et al., 2003b). To study the specificity of IgG and IgE antibodies against the $\alpha 1 \rightarrow 3$ -fucose and $\beta 1 \rightarrow 2$ -xylose epitope, Bencúrová et al. (2004) have modified the relatively simple biantennary glycans on human transferrin to incorporate these epitopes. Products were monitored by MALDI-TOF MS and antibody binding was reported to be greatest for the fucosylated glycans. Maeda et al. (2004) have replaced all of the asparagine residues of the *N*-glycosylation sites in procaryotepeptidase Y by alanine and expressed the protein in *P. pastoris*. The resulting protein lacked *N*-glycosylation but contained residual glucose, thought to be *O*-linked, as detected by an increase in the MALDI mass from that expected from the amino acid sequence. Analysis of erythropoietins (EPO) with varying glycosylation has shown the fully sialylated tetra-antennary glycan, identified by HPLC and MALDI-TOF MS as its 4-AA derivative, to be the major determinant of biological activity (Yuen et al., 2003). Sanz-Nebot et al. (2003) have recorded MALDI spectra from intact human recombinant EPO and noted a drop in measured mass with increasing laser power which they attributed to loss of sialic acid within the ion source. MALDI-TOF has been used to identify *O*-glycans from a von Willebrand factor C domain produced in *P. pastoris* (O'Leary et al., 2004). Methods for their removal were investigated.

A method for monitoring glycosylation in pharmaceuticals based on capillary electrophoreses of 3-AA derivatives with structural confirmation by MALDI-TOF MS has been published by Kamoda et al. (2004). MALDI and ion exchange chromatography have proved useful for checking the batch-to-batch variation in glycosylation of three recombinant gonadotropins. As an example, excellent consistency (3.5) was found for the degree of sialylation of 120 batches of recombinant thyroid stimulating hormone over a 3-year period (Gervais et al., 2003).

Several reviews on the production of biopharmaceuticals and containing reference to MALDI-TOF analysis have been published. These include production of human therapeutic proteins in yeasts (Bretthauer, 2003), yeasts and filamentous fungi (Gerngross, 2004) and lepidopteran cell lines (Tomiya et al., 2004). Methods for characterizing biological products after manufacturing changes have also been reviewed (Chirino & Mire-Sluis, 2004).

C. Paper Industry

Slime deposits in paper mills, termed biofouling, is a problem in the paper industry and has prompted studies on the composition of their glycolipids. The EPS from *Methylobacterium* strains, responsible for "pink slime" have been shown by Verhoef et al. (2003) to consist of the repeating unit: $(\rightarrow 3)\text{-[4,6-}O\text{-(1-carboxyethylidene)]-}\alpha\text{-D-Galp-(1}\rightarrow 3\text{)[4,6-}O\text{-(1-carboxyethylidene)]-}\alpha\text{-D-Galp-(1}\rightarrow 3\text{)-}\alpha\text{-D-Galp-(1}\rightarrow)$.

XI. CARBOHYDRATE SYNTHESIS

Sadly, many chemical papers appear to ignore details of the equipment and conditions used to obtain mass spectra of carbohydrates (and other compounds) even though conditions such as the choice of MALDI matrix are often essential in getting the compounds to "fly." Although NMR experiments and equipment are usually described in great detail, information on mass spectrometry is frequently reduced to uninformative acronyms such as HRMALDIMS with no further details. Without knowledge of the equipment used to record the spectra it is difficult to judge if HR is indeed meaningful. The relegation of essential methods to "supplementary information" is also to be regretted. The absence of essential information such as the matrix from many publications is reflected in Table 11 (with apologies to authors from whose papers this information has been missed). "MALDI" is used for papers omitting to cite the type of instrument used to record the spectra. Most of the publications in this area relate to routine monitoring of reaction products and are summarized in Table 11. In addition to chemical reactions, enzymes are frequently used in this area because of their stereospecificity. Both transferases and glycosidases such as PNGase F used in their reversed mode to catalyze transglycosylations (Park, Lee, & Park, 2003a) can be used; however, evidence has been found to suggest that enzymes used for reverse hydrolysis reactions can have their activity reduced by Maillard glycation (Maitin & Rastall, 2004). As with the previous review,

(text continued on page 330)

TABLE 9. Use of MALDI to study the products of enzyme action on carbohydrates

Enzyme/Source	Notes	Instrumentation	Reference
GlcNAc transferases			
Human, Gn T-VB	Transfers GlcNAc to 6-position of $\alpha 1 \rightarrow 6$ -linked Man of <i>N</i> -glycans. Substrate specificity with PNGaseF-released 2-AP-labeled <i>N</i> -glycans from thermolysin, fibroinogen + fetuin.	TOF, HPLC	(Kaneko, <i>et al.</i> , 2003)
Human, Gn T-IX	Transfers GlcNAc to the 6-antenna of <i>N</i> -glycans. Also transfers GlcNAc to the 6-position of the 3-antenna to produce a novel structure. Enzymes uniquely expressed in brain.	R-TOF (DHB), NMR	(Inamori, <i>et al.</i> , 2003)
Human, Gn T-IX	Enzyme acts on the GlcNAc- β -(1 $\rightarrow$ 2)-Man- α -1-Ser/Thr moiety forming a 2,6-branched structure in brain <i>O</i> -mannosyl glycan.	R-TOF (DHB), NMR	(Inamori, <i>et al.</i> , 2004)
pp α -GlcNAc-T2 (<i>Dictyostelium</i>)	Attaches GlcNAc to numerous secretory proteins.	TOF (CHCA)	(Wang, <i>et al.</i> , 2003a)
Rat intestinal $\beta 1$ -6GnT	Substrate specificity of a newly purified rat intestinal $\beta 1,6N$ -acetylglucosaminyltransferase ($\beta 1$ -6GnT) responsible for the formation of $\beta 1 \rightarrow 6$ -branched poly- <i>N</i> -acetylglucosamines	TOF (DHB), ESI, NMR	(Korekane, <i>et al.</i> , 2003)
<i>Saccharomyces cerevisia</i>	A strain of this yeast is unusually shown to be able to add a single GlcNAc residue to a variety of high-mannose glycans. In-gel removal of glycans with PNGase F.	TOF (DHB)	(Yoko-o, <i>et al.</i> , 2003)
GalNAc transferases			
GalNAc-transferase 2	Modification of the glycosylation signal of the herpes simplex virus type 1 glycopeptide gC-1 by mutation, increased the content of <i>O</i> -linked glycans but little effect on <i>N</i> -glycans.	TOF	(Mårdberg, <i>et al.</i> , 2004)
Human CSGalNAcT-1 and CSGalNAcT-2	CSGalNAcT-1 is involved in the initiation of chondroitin sulfate synthesis. CSGalNAcT-2 participates mainly in elongation.	TOF	(Sato, <i>et al.</i> , 2003b)
Human, GalNAc-T1, -T2, -T3, -T4, -T6, -T9	Enzymes have different substrate specificities but GalNAc-T2 was found to transfer GalNAc residues to the same five positions of the IgA1 hinge region in B cells.	R-TOF (CHCA)	(Iwasaki, <i>et al.</i> , 2003)
Human, pp-GalNAc-T13 (neuroblastoma cell)	Transfers GalNAc to several mucin-derived peptides including Muc5Ac and Muc7.	TOF (DHB), glycopeptides	(Zhang, <i>et al.</i> , 2003e)
Human, pp-GalNAc-T14	Transfers GalNAc to Muc2, Muc5Ac, Muc7 and Muc13. May be involved in <i>O</i> -glycosylation in the kidney	TOF (CHCA)	(Wang, <i>et al.</i> , 2003c)
Human, $\beta 4$ GalNAc-T3	Transfers GalNAc to the 4-position of GlcNAc in <i>N</i> - and <i>O</i> -linked glycans.	TOF (DHB)	(Sato, <i>et al.</i> , 2003c)
Human, GalNAc-TIV	Similar enzyme to human, $\beta 4$ GalNAc-T3. Transfers GalNAc in $\beta 1 \rightarrow 4$ -linkage	TOF, NMR	(Gotoh, <i>et al.</i> , 2004)
Human, $\beta 1,3$ - <i>N</i> -GalNAc-II	Synthesises a unique carbohydrate structure, GalNAc- β -(1 $\rightarrow$ 3)-GlcNAc.	TOF, NMR	(Hiruma, <i>et al.</i> , 2004)
Human, pp-GalNAc-T15	Transfers up to seven GalNAc moieties onto the Muc5AC peptide whereas the T2 enzyme only transfers five.	TOF (CHCA), LC, glycopeptides	(Cheng, <i>et al.</i> , 2004)
Other transferases			
α -(1 $\rightarrow$ 2)-L-Gal-T from <i>Helix pomatia</i>	Transfers an L-fucose residue to terminal D-galactopyranosyl residues, has been found to be specific for galactose with β -linkages.	R-TOF (DHB), HPAEC	(Scheppokat, <i>et al.</i> , 2003b)
B4-Gal-T, CHO Lec19 cells	Resistance to plant lectins such as ricin and abrin that bind galactose shown, in these cells, to be due to a reduced ability of the cells to transfer galactose to the <i>N</i> -linked glycans.	TOF (DHB, neutral, THAP, acidic)	(Lee, <i>et al.</i> , 2003c)
LpcC (<i>Rhizobium leguminosarum</i>)	Adds a mannose unit to the inner Kdo moiety of the LPS precursor, Kdo2-lipid IVA.	TOF (ATT, ammonium citrate)	(Kanipes, <i>et al.</i> , 2003b)
PimF (<i>Mycobacterium marinum</i>)	First analysis of a Man-T involved in the later stages of phosphatidylinositol mannoside synthesis.	R-TOF, PSD	(Alexander, <i>et al.</i> , 2004)
Membrane bound Gal-T, <i>Glycine Max</i> pectin	Gal-T involved in biosynthesis of β -(1 $\rightarrow$ 4)-galactan chains of pectin. Gal transfer to unmodified pectic galactan (Mr>150,000) low but increased after partial acid hydrolysis.	TOF (DHB) HPLC, GC/MS	(Konishi, <i>et al.</i> , 2004)

Enzyme/Source	Notes	Instrumentation	Reference
AknK (L-2-deoxyfucosyltransferase)	Identified as the enzyme that adds L-2-deoxyfucose as the second sugar in the carbohydrate of the antitumor drug aclacinomycin A produced by <i>Streptomyces galilaeus</i> .	TOF, LC/MS, NMR	(Lu, <i>et al.</i> , 2004b)
Dermatan sulfate biosynthesis	Clarification of controversy over whether 4-sulfation in dermatan sulfate occurs on GalNAc before epimerization of neighbouring GlcA to iduronic acid or afterwards	L-TOF (DHB), HPLC	(Mikami, <i>et al.</i> , 2003)
Glycosidases			
β -N-Acetylhexosaminidases	Enzymes hydrolyse and transglycosylate β -GlcNAc and β -GalNAc moieties suggesting that they are single enzymes having affinity for both substrates. Studies in about 60 fungi	TOF	(Weignerová, <i>et al.</i> , 2003)
α -Amylase (<i>Bacillus licheniformis</i> and human saliva)	Characterization of binding site	TOF (THAP)	(Kandra, 2003)
<i>Aspergillus niger</i> pectinase	An isoenzyme that cleaves both GlcNAc-GlcN and GlcN-GlcN linkages thus offering a replacement for expensive chitinase and chitosanase currently used for this purpose.	TOF (DHB)	(Kittur, <i>et al.</i> , 2003)
Endo- β -N-GlcNAc-ase from <i>Mucor hiemalis</i> (endo-M)	Enzyme expressed in <i>Candida boidinii</i> can transglycosylate a disialo-biantennary glycan in media containing 30% acetone, DMSO or MeOH in higher yield than aqueous solution.	TOF (THAP), HPLC	(Akaike, <i>et al.</i> , 2004)
Endo- β -N-GlcNAc-ase from <i>Mucor hiemalis</i>	A variety of sugar derivatives modified at C-1 or C-2 can be used as acceptors for this enzyme.	TOF (DHB, THAP), HPLC	(Yamanoi, <i>et al.</i> , 2004)
Endo- β -Gal-ase from <i>Bacteroides fragilis</i>	Glycolysis of the Tamm-Horsfall glycoprotein releases two sugars, measurement of which in identical twins indicates closely-controlled glycosylation in the two individuals.	TOF (CHCA), glycans	(Rohfritsch, <i>et al.</i> , 2004)
Polygalacturonases from <i>Trichoderma reesei</i>	Study of substrate specificity; the random pattern for pectin cleavage showed that the enzymes were exo-polygalacturonases.	TOF (THAP/nitro-cellulose)	(Mohamed, <i>et al.</i> , 2003)
Xyloglucanases	Xyloglucanases from <i>Aspergillus japonicus</i> , <i>Chrysosporium lucknowense</i> , <i>Trichoderma reesei</i> . <i>A. japonicus</i> displayed endo-type attack whereas other two enzymes exo-type.	TOF	(Grishutin, <i>et al.</i> , 2004)
Other enzymes acting on carbohydrates			
Chitinases, Chit33 and Chit42	Purified from the filamentous fungus <i>Trichoderma harzianum</i> and their hydrolysis products characterized	TOF (DHB)	(Boer, <i>et al.</i> , 2004)
GDP-6-deoxy-D-talose synthetase	Identification of the enzyme that converts GDP-4-keto-6-deoxy-D-mannose into GDP-6-deoxy-D-talose in the Gram-negative bacterium <i>Actinobacillus actinomycetemcomitans</i> .	R-TOF (THAP, NH ₄ citrate)	(Mäki, <i>et al.</i> , 2003)
Heparin/Heparan 2-O-sulfatase	Enzyme from <i>Flavobacterium heparinum</i> active site and saccharide substrate specificity	MALDI (CHCA)	(Raman, <i>et al.</i> , 2003)
Lipid A 1-phosphatase from <i>Rhizobium leguminosarum</i>	Expression, cloning and biochemical characterization	L-TOF	(Karbarz, <i>et al.</i> , 2003)
LpxA (<i>Mesorhizobium loti</i> and <i>Leptospira interrogans</i>)	Mechanism of action in formation of lipid A with four <i>N</i> -linked acyl groups. Enzyme does not acylate UDP-GlcNAc but requires UDP-GlcNAc ₃ N	TOF	(Sweet, <i>et al.</i> , 2004)
Pectin methylesterase from <i>Aspergillus niger</i>	Enzyme prefers oligogalacturonides with one or two non-esterified galacturonic acids in its active cleft rather than fully methylated acids.	TOF (THAP/nitrocell)	(van Alebeek, <i>et al.</i> , 2003)
Potato D enzyme	Catalyzes the cyclization of amylose to produce cycloamylose	TOF	(Takaha, <i>et al.</i> , 2003)
Quinone-dependent pyranose dehydrogenase	Products formed by enzyme extracted from basidiomycete fungi studied with lactose as substrate. The 2-oxo-product characterized as its <i>N,N</i> -diphenylhydrazone derivative.	MALDI-TOF, NMR.	(Volec, <i>et al.</i> , 2004)
Enzyme inhibitors			
XEGIP (Tomato)	Inhibits activity of xyloglucan-specific endoglucanase from <i>Aspergillus aculeatus</i>	TOF	(Qin, <i>et al.</i> , 2003)

TABLE 10. Use of MALDI analysis to monitor *N*-glycosylation in biopharmaceuticals

Biopharmaceutical	Expression system	Release	Instrumentation	Notes	Reference
Alcohol oxidase (K3 domain)	<i>Pichia pastoris</i>	PNGase F	R-TOF (s-DHB), Glycans	<i>P. pastoris</i> engineered to produce complex glycans	(Bobrowicz, <i>et al.</i> , 2004)
Angiogenic vascular growth factor VEGF ₁₂₁ (Human)	<i>Physcomitrella patens</i> (moss)	Pepsin, PNGase A	TOF (DHB)	Genes encoding the enzymes for (1→3)-fucose and 1→2-xylose disrupted. Did not affect plan to be produced without the immunogenic sugars.	(Koprivova, <i>et al.</i> , 2004)
Anti-rabies monoclonal antibody	Tobacco	PNGase (In gel)	R-TOF (DHB), Ion-trap/TOF, Glycans	Structural characterization (high-mannose, complex). Glycosylation did not affect efficacy	(Ko, <i>et al.</i> , 2003)
Avidin (chicken)	Maize	PNGase A	R-TOF (DHB), Glycans (Reduced, Per-Me)	For detection of immunogenic core (1→3)-fucose and core xylose	(Bardor, <i>et al.</i> , 2003a)
Bile salt-stimulated lipase	<i>Pichia pastoris</i>	Endo H	TOF (DHB), PSD, NMR	<i>O</i> -linked glycans have α - and β -(1→2)-linked Man chains with up to 6 Man residues. Trace amount of novel, branched (Man) ₄ chain and Man phosphate.	(Trimble, <i>et al.</i> , 2004)
C1 Inhibitor (Human)	Rabbit mammary gland	PNGase F	R-TOF (DHB (+ve), THAP/NH ₄ citrate (-ve)), Glycans	Structural determination of glycans expressed in milk	(Koles, <i>et al.</i> , 2004a, Koles, <i>et al.</i> , 2004b)
C5-1 Antibody	Alfalfa	PNGase A	R-TOF, PSD (DHB, CHCA), glycans (Per-Me)	Structural determination, Plant-type, complex	(Bardor, <i>et al.</i> , 2003b)
C5-1 Antibody (Human)	Alfalfa	PNGase A Pepsin	R-TOF (DHB, CHCA), PSD, (glycopeptides), Glycans (Reduced, per-Me)	Alfalfa plants able to synthesise IgG with human-type glycans	(Bardor, <i>et al.</i> , 2003b)
Chorionic gonadotrophin	CHO cells	Hydrazine	R-TOF(DHB, CMBT), ESI, Ion trap, Glycans (2-AB)	Structural determination of complex glycans and method development.	(Gervais, <i>et al.</i> , 2003)
Dac g 5 and proDer p 1	<i>Pichia pastoris</i>	Trypsin	TOF (CHCA)	Presence of Man can have an adverse effect on the specificity of diagnostic tests for autoimmune and allergic diseases that are based on immunoassays.	(van Oort, <i>et al.</i> , 2004)
Endogenous glycoproteins	<i>Arabidopsis thaliana</i>	Pepsin, PNGase A	TOF (DHB)	Enzymes adding potentially antigenic β -(1→2)-Xylose and α -(1→3)-fucose removed	(Strasser, <i>et al.</i> , 2004b)
Erythropoietin	CHO cells	PNGase F	TOF (DHB), Glycans (4-AA)	Structural determination under different culture conditions. Fully sialylated tetra-antennary glycan is the major determinant of biological activity	(Yuen, <i>et al.</i> , 2003)
Erythropoietin	CHO cells	-	L-TOF (Sinapinic acid, DHB, HPA, ferulic acid), ESI, CE, glycoproteins	Characterization of intact glycoprotein by several methods	(Sanz-Nebot, <i>et al.</i> , 2003)
Follicle stimulating hormone	CHO cells	Hydrazine	R-TOF (DHB, CMBT), ESI, Ion trap, Glycans (2-AB)	Structural determination of complex glycans and method development	(Gervais, <i>et al.</i> , 2003)

Biopharmaceutical	Expression system	Release	Instrumentation	Notes	Reference
Gastric lipase (Dog)	Tobacco	Glycopeptides	L-TOF, (DHB, Sinapinic acid), ANTS label and tryptic glycopeptides	Site-specific glycan analysis. Three sites contained complex and high mannose glycans, the fourth contained only high-mannose.	(Mokrzycki-Issartel, <i>et al.</i> , 2003)
Glycosyltransferases	<i>Pichia pastoris</i>	Pronase	TOF	β -(1→2)-Xyl-T and α -(1→3)-Fuc-T incorporated into yeast. Pronase glycotetrapeptide from bovine fibrin to assay enzyme activity.	(Bencúrová, <i>et al.</i> , 2003)
GM2-activator protein	<i>Pichia pastoris</i>	PNGase F	TOF (DHB), ESI-TOF, NMR	Structural determination	(Wendeler, <i>et al.</i> , 2004b)
Hyaluronan synthase (human)	<i>E. coli</i>	-	L-TOF (DHB)	Expression of catalytic region of human hyaluronan synthase (HAS2) in <i>E. coli</i> shown to synthesise a mixture of HA oligomers from 8- to 16-mer.	(Hoshi, <i>et al.</i> , 2004)
IgG (Rituximab, Trastuzumab)	Commercial	PNGase F	TOF (3-AA), CE	Method for monitoring glycosylation in pharmaceuticals based on capillary electrophoresis with structural confirmation by MALDI-TOF MS	(Kamoda, <i>et al.</i> , 2004)
IgG ₁ (Mouse/Human)	Plants	PNGase A	R-TOF (DHB)	IgG modified with KDEL sequence expressed in tobacco plants. Complex glycans lack α -(1→3)-fucose and xylose	(Sriraman, <i>et al.</i> , 2004)
IgG ₁ (Human)	Rat hybridoma or CHO	Hydrazine	MALDI, HPLC, Glycans (2-AP),	Absence of fucose but not presence of galactose or bisect enhances cellular toxicity	(Shinkawa, <i>et al.</i> , 2003)
IgG ₃	Mouse	Hydrazine, PNGase F	R-TOF (+ve) (DHB), ESI, MS/MS, Glycans	Effect of buffer and pH on production.	(Müthing, <i>et al.</i> , 2003)
IgG ₄ (Human)	CHO-S cells	Trypsin	TOF (CHCA), Glycopeptides	Glycosylation (high-mannose, truncated complex) not significantly affected by anti-mitotic agent	(Tait, <i>et al.</i> , 2004)
KDEL-tagged cPIPP antibody	Tobacco and wild cherry	Trypsin, PNGase F, pepsin, PNGase A	TOF (DHB)	Mouse/human chimeric IgG1 antibody binding to β -subunit of human chorionic gonadotropin (hCG), fused to C-terminal KDEL sequence. Eliminated N-glycans with β -(1→2)-Xyl and α -(1→3)-Fuc	(Sriraman, <i>et al.</i> , 2004)
Lactoferrin (Human)	Maize, tobacco	PNase A, F Trypsin	R-TOF (DHB), ESI, CID, Glycans, Glycopeptides	Determination of site-specific glycosylation (Asn-79)	(Samyn-Petit, <i>et al.</i> , 2003)
Lactoferrin (Human)	Rice	Hydrazine	TOF, Glycans (2-AP)	Structural characterization	(Fujiyama, <i>et al.</i> , 2004)
Lecithin:cholesterol acyltransferase	Human lung cell line	PNase F trypsin	MALDI, Glycans (per-Me)	Characterization of recombinant enzyme. Complex glycans (composition only)	(Lane, <i>et al.</i> , 2004)
Luteinizing hormone (Human)	CHO cells	Hydrazine	R-TOF (DHB, CMBT), ESI, Ion trap, Glycans (2-AB)	Structural determination of complex glycans and method development	(Gervais, <i>et al.</i> , 2003)
MFE-CP	<i>Pichia pastoris</i>	Trypsin	R-TOF, LC/MS, CID, PSD, Glycopeptides	Structural characterization of glycoprotein (high-mannose, Asn-442, 484) used to target cancer cells	(Medzihradsky, <i>et al.</i> , 2004)

(Continued)

TABLE 10. (Continued)

Biopharmaceutical	Expression system	Release	Instrumentation	Notes	Reference
Onconase	<i>Pichia pastoris</i>	Endo-H	TOF, glycoprotein	Cancer chemotherapeutic agent. Structures of high mannose glycans (Man ₆ , ₁₆) from mass of glycoprotein	(Kim, <i>et al.</i> , 2004)
Ovotransferrin (Chicken)	<i>Pichia pastoris</i>	Endo H	TOF (Sinapinic acid), Glycoprotein, Glycan mass by difference	Structural characterization	(Mizutani, <i>et al.</i> , 2004)
Placental alkaline phosphatase (Human)	<i>Trichoplusia ni</i> insect cell lines	PNGase F	TOF (DHB), Glycans (2-AB)	Presence of silkworm hemolymph improves <i>N</i> -glycosylation, High mannose	(Joosten, Park & Shuler, 2003a)
Plasminogen Kringle 3 (Human)	<i>Pichia pastoris</i> (Several strains)	PNGase F	TOF (s-DHB)	Glycosylation machinery re-engineered to perform sequential glycosylation reactions that mimic <i>N</i> -glycan processing in humans.	(Choi, <i>et al.</i> , 2003a)
Plasminogen Kringle 3 (Human)	<i>Pichia pastoris</i>	PNGase F	TOF	Yeast engineered to produce GlcNAc ₂ Man ₃ GlcNAc ₂	(Hamilton, <i>et al.</i> , 2003)
Plasminogen Kringle 3 (Human)	<i>Pichia pastoris</i>	PNGase F	TOF	<i>Pp4L G3</i> gene that encodes for Dol-P-Man:-Man ₅ GlcNAc ₂ -PP-Dol Man-T deleted to prevent further mannose addition. Incorporation of a β-(1→4)-Gal-T gave human-type glycans.	(Bobrowicz, <i>et al.</i> , 2004)
Procarboxypeptidase Y	<i>Pichia pastoris</i>	-	TOF (Sinapinic acid), Glycoprotein	Replacement of all Asn of <i>N</i> -glycosylation sites by Ala removed <i>N</i> -glycosylation. Residual glucose thought to be <i>O</i> -linked.	(Maeda, <i>et al.</i> , 2004)
Soluble human complement receptor type 1 (sCR1)	CHO Cells	PNGase F	TOF (DHB), Glycans (2-AA)	Glycan remodelling using the soluble glycosyltransferases, α-(2→3)-silyltransferase to introduce silyl Lewis X moieties.	(Thomas, <i>et al.</i> , 2004a)
Transferrin	Insect cell line	PNGase A Trypsin + Chymotrypsin	TOF (DHB), Glycans (2-AP)	<i>Trichoplusia ni</i> insect cells engineered to produce biantennary glycans	(Tomiya, <i>et al.</i> , 2003)
Transferrin (Human)	<i>Lymantria dispar</i> (gypsy moth)	PNGase A	L-TOF (DHB), Glycans (2-AP)	Attempt to find a better expression system than those currently used. Absence of core (1→3)-fucosylation, a normally common motif in insects.	(Choi, <i>et al.</i> , 2003b)
Transferrin (human)	<i>Pichia pastoris</i>	Pepsin, PNGase A	L-TOF, Q-TOF (CHCA), Glycans, glycopeptides	To study specificity of IgG and IgE antibodies against (1→3)-fucose and (1→2)-xylose epitopes. Binding greatest for fucosylated glycans.	(Bencúrová, <i>et al.</i> , 2004)
Vascular growth factor VEGF ₁₂₁ (Human)	<i>Physcomitrella patens</i>	Pepsin, PNGase F	TOF (DHB), Glycans	Recombinant in the moss lacking (1→3)-fucose and (1→2)-xylose enzymes	(Koprivova, <i>et al.</i> , 2004, Koprivova, 2003 #2924)

TABLE 11. Use of MALDI MS for monitoring products of synthetic reactions

Carbohydrate	Synthetic methods and Comments	Instrumentation	Reference
Monosaccharides			
2-Chloro-4-nitrophenyl- α -L-fucopyranoside	As substrate for α -L-fucosidase	TOF (DHB)	(Gu, <i>et al.</i> , 2003)
Glucose-based glycoprobes	Ident. of binding sites in Na/D-glucose co-transporter protein	L-TOF (Sinapinic)	(Tyagi & Kinne, 2003)
Monosaccharide amides	Ru(III)-Promoted amide formation from azides and thioacids, which do not form amides at room temp. in the absence of Ru	FT-ICR	(Fazio & Wong, 2003)
Quaternary ammonium salts of ribitol	Salts from five aromatic amines	TOF (CHCA)	(Skorupa, <i>et al.</i> , 2004)
Oligosaccharides			
N-Acetylglucosaminobioses	By reverse hydrolysis using fungal N-acetylhexosaminidases	TOF	(Rauvolfová, <i>et al.</i> , 2004b)
Arabinogalactosyl nonasaccharides	β -(1 $\rightarrow$ 6)-D-Galp backbone, α -(1 $\rightarrow$ 3)-linked L-Araf side-chain	TOF (DHB)	(Li & Kong, 2004)
Aza-C-disaccharides	Sugar mimics with pyrrolidine as aza-sugar	MALDI-FTMS	(Argyropoulos & Sarli, 2004)
Biotinylated saccharides	As part of avidin/biotinylated drug delivery system	TOF	(Ouchi, <i>et al.</i> , 2004)
6-Branched cyclo-(1 $\rightarrow$ 3)-glucosaxose	Cyclisation of branched glucosaxose	TOF	(Wu & Kong, 2004b)
2,6-Branched galacto-oligosaccharides	Synth. of tetra- and hexa-saccharides related to arabinogalactans	TOF	(Ning, Yi & Yao, 2003b)
Carbohydrate-modified poly(di-Me-siloxane)s	Linear dimethylsiloxanes capped with various monosaccharides	TOF	(Henkensmeier, <i>et al.</i> , 2004)
6-O-carboxymethylated chitotetraose	Selective glycosidation with chitinase catalysts	TOF	(Ochiai, Ohmae & Kobayashi, 2004b)
Chitobiose analogues	Sugar oxazolines glycosylated with chitinase from <i>Bacillus</i> sp.	TOF (DHB) on Nafion plate	(Ochiai, Ohmae & Kobayashi, 2004a)
Chitoooligosaccharides	As standards for diffusion-ordered spectroscopy	TOF	(Groves, <i>et al.</i> , 2004)
Chitoooligosaccharides	Synthesis from chitosan by enzymatic hydrolysis with chitinase	TOF	(Lee, <i>et al.</i> , 2003a)
Chitoooligosaccharides DP 2-6	Use of pectinase from <i>Aspergillus niger</i>	TOF, FAB	(Kittur, <i>et al.</i> , 2003)
Chitriose nicotinic amide	For conformational studies. Inhibitor of chitinase hevinamine	MALDI	(Germer, <i>et al.</i> , 2003)
C-Linked disaccharides	Synthesised using Carbon-Ferrier reaction	TOF	(Gemmell, Meo & Osborn, 2003)
Conformationally constrained oligosaccharides	N-CO-CH ₂ Cl to form ring with adjacent monosaccharide	TOF (DHB)	(Galan, <i>et al.</i> , 2003)
Cyclosophoraoses of <i>Rhizobium meliloti</i> 2011	Enzymatic synthesis. As additive for enantioseparation in CE	TOF	(Lee & Jung, 2003)
Deoxy phospho sugar-sugar disaccharides	Phospho analogues of normal sugar disaccharides	TOF (CHCA)	(Haritha, <i>et al.</i> , 2004)
Fucosyl-lactoses	Specific Fuc addn. after lipase-catalysed regioselective acylation	TOF	(Rencurosi, <i>et al.</i> , 2003)
Fucosyl pentasaccharide (sulfated fucan repeat)	Convergent "2+3" synthetic strategy	TOF (DHB)	(Hua, <i>et al.</i> , 2004a)
Fucosylated trisaccharides	Study of the specificity of the α -(1 $\rightarrow$ 2)-L-galactosyltransferase from <i>Helix pomatia</i> . Specific for D-Galp β -linkages	TOF (DHB)	(Scheppokat, <i>et al.</i> , 2003b)
Fucosylated trisaccharides	Synthesis with α -(1 $\rightarrow$ 2)-L-galactosyltransferase from <i>Helix pomatia</i> .	TOF (DHB)	(Scheppokat, Bretting & Thiem, 2003a)
Fucosylated trisaccharides (lactose)	Random fucosylation to construct library	TOF	(Meng, <i>et al.</i> , 2004)
Gal- β -(1-3)-GlcNAc	Use of an anomeric fluorosilyl protecting group	TOF	(Manzoni, 2003)
Galactans	Biosynthesis by use of galactosyltransferase from radish (<i>Raphanus sativus</i> L.) seedlings	TOF (ABEE derivs.)	(Kato, <i>et al.</i> , 2003)
Galactose-containing oligosaccharides	β -Gal-T from bovine testes to synth. novel food components	TOF (DHB)	(Schröder, <i>et al.</i> , 2004)
β -(1 $\rightarrow$ 6)-D-Galactosylated oligosaccharides	Use of β -(1 $\rightarrow$ 6)-D-Gal-transferase from <i>Helix pomatia</i>	TOF	(Scheppokat, <i>et al.</i> , 2004)
Galactosylated tri- and tetrasaccharides	Regioselective synth. β -galactosidase from <i>Bacillus circulans</i>	TOF (DHB)	(Farkas, <i>et al.</i> , 2003)

(Continued)

TABLE 11. (Continued)

Carbohydrate	Synthetic methods and Comments	Instrumentation	Reference
α -galactose-containing pentasaccharide	Four-stage, one-pot synthesis	TOF	(Wang, <i>et al.</i> , 2004d)
β -D-Glucosamine-containing nonasaccharide	Chemical synthesis with isopropyl thioglycosides	TOF (CHCA)	(Yang & Du, 2003)
β -D-Glucosamine-containing oligosaccharides	Chemical synthesis with isopropyl thioglycosides	TOF (CHCA)	(Yang, Hua & Du, 2003)
Glucosylated isokestose and nystose	Enzymatic (kijibiose phosphorylase from <i>Thermoanaerobacter brockii</i> to synthesise five novel oligosaccharides	TOF	(Okada, <i>et al.</i> , 2003)
Glycosaminotrioses	With GalNAc or ManNAc termini. To study binding to hevein from <i>Hevea brasiliensis</i>	TOF (CHCA)	(Aboitiz, <i>et al.</i> , 2004)
Imino sugar scaffolds	For the generation of glycosidase inhibitor libraries	TOF (DHB)	(La Ferla, <i>et al.</i> , 2004)
Kanamycin analogues	Chemical synthesis and antibacterial evaluation	MALDI	(Li, <i>et al.</i> , 2004b)
Lacto- <i>N</i> -neotetraose	Dendrimetric polyethylene glycol supported synthesis	TOF	(Renaudie, <i>et al.</i> , 2004)
Laminarin hexasaccharide	4,6- <i>O</i> -benzylidenated acceptor to avoid generation of α -isomer in attempted β -D-(1 $\rightarrow$ 3) glycosylation under standard condits.	TOF (CHCA)	(He, Gu & Du, 2003)
Lepidimide	Unsaturated disaccharide (4-deoxy- β -L- <i>threo</i> -hex-4-enopyranosyl-(1 $\rightarrow$ 2)-L-Rhap) from okra mucilage	TOF (Co powder)	(Hirose, <i>et al.</i> , 2004)
Lewis ^x glycocluster	Chemical (PEG scaffold)	TOF (-) (DHB)	(Majumdar, Zhu & Boons, 2004)
Lewis ^a trisaccharide	Chemical. Use of anomeric fluorosul silyl protecting group	TOF	(Manzoni & Castelli, 2004)
Maltooligosyl fructofuranosides	Immobilized cyclodextrin glucosyltransferase catalyst	TOF (DHB)	(Martin, <i>et al.</i> , 2004)
Maltoyl-erythritol	Transglycosylation product of erythritol by maltogenic amylase	TOF (CHCA)	(Yoon, <i>et al.</i> , 2003)
α -(1 $\rightarrow$ 3)-linked manno-hexaose and -octaose	α -(1-3)-linked di- and tetra-saccharide donor + tetrasaccharide	TOF	(Chen & Kong, 2002)
Mannosides	Synthesis by double differential glycosylation by lanthanide triflates	TOF	(Jayaprakash & Fraser-Reid, 2004)
Mannobioside mimic	Dicarboxy-cyclohexyl reducing-terminal ring	TOF	(Mari, <i>et al.</i> , 2004)
4- <i>O</i> -Me-maltosyl fluoride plus gluconohydroximinoo-1,5-lactam	<i>In situ</i> method for identification of novel α -amylase inhibitors	R-TOF	(Numao, <i>et al.</i> , 2004)
<i>N</i> -(methyl-2,3,4-tri- <i>O</i> -acetyl-6-deoxy- α - and - β -D-glucopyranoside-6-yl)-ammonium salts	From Me-D-glucopyranosides	TOF (CHCA)	(Pellowska-Januszek, <i>et al.</i> , 2004)
4-Nitrophenyl 1-thio- β -D-maltosides	Transglycosylation with mutant of human salivary α -amylase	TOF	(Remenyik, <i>et al.</i> , 2003)
Oligosaccharides (di-, tri- and tetrasaccharides)	Solid-phase synthesis with azidoglucose as a glycosyl acceptor	TOF (DHB)	(Wu & Schmidt, 2004)
Oligosaccharides (general)	Polymer - resin hybrid capture-release strategy for rapid oligosaccharide construction	TOF	(Hanashima, Manabe & Ito, 2003)
Oligosaccharides (general)	Reiterative intramolecular glycosylation by use of a rigid spacer	TOF (DHB)	(Paul, Müller & Schmidt, 2003)
Oligosaccharides (rhamnan backbone)	Synthesis of oligosaccharides consisting of α -(1 $\rightarrow$ 2)- and α -(1 $\rightarrow$ 3)-linked rhamnan backbones and GlcNAc side chains	TOF	(Zhang & Kong, 2003d)
<i>n</i> -Pentenyl arabinofuranosyl donors	Synthesis of little-studied donors for furanosides	TOF	(Lu & Fraser-Reid, 2004)
N(OCH ₃)-linked disaccharide analogues	Carbohydrate mimics resistant to hydrolysis by glycosidases	TOF (DHB)	(Peri, <i>et al.</i> , 2004)
Non-reducing oligosaccharides	By reverse hydrolysis using fungal <i>N</i> -acetylhexosaminidases	TOF	(Rauvolfová, <i>et al.</i> , 2004a)
Silyl Lewis ^x and silyl Lewis ^a precursors	Determination of recombinant (2 $\rightarrow$ 3)- α -silyltransferase	TOF (DHB)	(Ivannikova, <i>et al.</i> , 2003)
Sucrose laurate	Enzymatic (alkaline protease from <i>Bacillus pseudofirmus</i> AL-89	TOF (CHCA)	(Pedersen, <i>et al.</i> , 2003)
6-Sulfo-GlcNAc- β -(1 $\rightarrow$ 3)-Gal- β -(1 $\rightarrow$ 4)-Glc	Product of 6-sulfo-transferase produced in baculovirus system	TOF (DHB)	(El-Fasakhany, <i>et al.</i> , 2003)

Carbohydrate	Synthetic methods and Comments	Instrumentation	Reference
Ureido-linked disaccharides	Glycosyl isothiocyanates plus glycosylamine. 2-, 4-, 6-positions	TOF	(Prosperi, <i>et al.</i> , 2004)
Various oligosaccharides	Automated solid-phase synthesiser. 20 times faster than solution	TOF (DHB)	(Palmacci, <i>et al.</i> , 2003)
Various oligosaccharides	Formylacetal (CH ₂) as novel linker on soluble-polymer support	TOF	(Oikawa, <i>et al.</i> , 2004)
Various oligosaccharides	Use of a novel hexakisfluorobutanol support	TOF (CHCA), ESI	(Goto <i>et al.</i> , 2004)
Various oligosaccharides	Use of fluoros-tagged saccharide primers	TOF (DHB)	(Kasuya, <i>et al.</i> , 2004)
Various oligosaccharides	Acetyl esterase from <i>Trichoderma reesei</i> RUT-C30 found able to transglycosylate carbohydrates in organic solvents	TOF (DHB)	(Kremnický, Mastihuba & Côté, 2004)
Polysaccharides			
Arabinan from <i>Mycobacterium tuberculosis</i>	Chemical (<i>n</i> -pentenyl-arabinofuranosyl donor)	TOF	(Lu & Fraser-Reid, 2004)
Arabinogalactans from <i>Echinacea purpurea</i>	Synthesis of double-branched isomeric nonasaccharides	TOF	(Csávas, <i>et al.</i> , 2003)
C-oligosaccharides	Carbon-linked bacterial cell wall analogue	TOF	(Dondoni & Marra, 2003)
(1→3,1→4)-β-D-Glucan	With a mutated (1→3,1→4)-β-D-glucanase from <i>Bacillus</i>	TOF	(Fajies, <i>et al.</i> , 2004)
Lepidimic acid from <i>Hibiscus esculentus</i>	Chemical degradation of okra	TOF (Co powder)	(Hirose, <i>et al.</i> , 2003)
Maltobi- and -triose 2,4-dinitrophenyl-derivs.	Substrates for human pancreatic α-amylase. Kinetic analysis	R-TOF	(Damager, <i>et al.</i> , 2004)
Mannan of <i>Candida kefyr</i> IFO 0586, cell wall	First synthesis of penta- and deca-saccharide repeats	TOF (CHCA)	(Xing & Ning, 2003)
Pectic oligogalacturonides	Enzymatic synthesis on solid support	TOF (Sinapinic)	(Guillaumie, <i>et al.</i> , 2003)
Glycosaminoglycans and related compounds			
Chondroitin sulfate E hexasaccharide	Synthesis from β-D-GalNAc-(1→4)-β-D-GlcA	TOF	(Tamura & Tokuyoshi, 2004)
Heparan sulfate fragments	Use of 6 orthogonal protecting groups to synth. 20 disaccharides	TOF (-) (DHB)	(Prabhu, Venot & Boons, 2003)
Heparin-like fragments	Synthesis of hexa- and octasaccharides on a solid support	TOF	(Ojeda, <i>et al.</i> , 2004)
Heparin fragments	Chemical synthesis <i>via</i> regio- and stereo-selective glycosylation	FTMS	(Yu, <i>et al.</i> , 2004)
Heparin-like hexasaccharides	Synthesis by convergent block strategy	TOF (DHB)	(Lucas, <i>et al.</i> , 2003)
Isotope-labelled hyaluronan oligomers	¹⁵ N-, ¹³ C-labels for NMR studies	TOF (DHB)	(Blundell, <i>et al.</i> , 2004)
Monodisperse hyaluronan oligosaccharides	Chemoenzymatic synthesis with immobilized mutant enzymes	R-TOF (ATT) (-)	(DeAngelis, Oatman & Gay, 2003)
Carbohydrates from algae			
Fucoidan repeat from brown algae	Chemical synthesis. Good antitumor activity	TOF (DHB)	(Hua, Gu & Du, 2004b)
Carbohydrates from bacteria			
Disaccharide fragments of LPS from several species	Jones oxidation of allyl glycosides	TOF (CHCA)	(Madaj, Jankowska & Wisniewski, 2004)
Lipid A from <i>Chlamydia trachomatis</i>	Chemical synthesis and purity assessment	TOF (DHB)	(Zamyatina, <i>et al.</i> , 2004)
<i>Mycobacterium tuberculosis</i> phosphoinoside intermediate	Use of regioselective mannosylations	MALDI	(Jayaprakash, Lu & Fraser-Reid, 2004)
O-Chain from <i>Campylobacter jejuni</i>	Trisacch. from GlcNPhth-(1→3)-Gal + allyl 6-deoxy- <i>altr</i> oHep	TOF (2,4-DHB)	(Yoon, <i>et al.</i> , 2004b)
O-Chain repeat from <i>Helicobacter pylori</i>	Chemical synthesis of unique trisaccharide from Danish strains	TOF	(Kwon, <i>et al.</i> , 2004)
O-Chain repeat from <i>Shigella flexneri</i>	Chemical synthesis of tetra- and two pentasaccharide fragments	-	(Mulard & Guerreiro, 2004)
Capsular polysaccharide from <i>Streptococcus pneumoniae</i> 9A&L, 9N, 9A	Preparation by consecutive α-galactosylations with ethyl thioglycosides as donors.	R-TOF (THAP)	(Alpe & Oscarson, 2003)
Capsular polysaccharide from <i>E. Coli</i> O7:K98:H6	Chemical synthesis	TOF	(Zeng & Kong, 2004)

(Continued)

TABLE 11. (Continued)

Carbohydrate	Synthetic methods and Comments	Instrumentation	Reference
Carbohydrates from fungi			
Capsular polysaccharide from <i>Cryptococcus neoformans</i>	Synthesis and assembly of di- and tri- thioglycoside donor blocks	TOF (THAP)	(Alpe, Oscarson & Svahnberg, 2003)
EPS repeat from <i>Cryptococcus neoformans</i>	Chemical synthesis of repeating unit	TOF	(Zhang & Kong, 2003a)
Repeat of <i>O</i> -deacylated exopolysaccharide from <i>Cryptococcus neoformans</i> serotype A	β -D-GlcA-(1 $\rightarrow$ 2)- α -D-Manp-(1 $\rightarrow$ 3)-[β -D-Xylp-(1 $\rightarrow$ 2)]- α -D-Manp-(1 $\rightarrow$ 3)-[β -D-Xylp-(1 $\rightarrow$ 2)]- α -D-Manp. Synth as glucoside	TOF	(Zhang & Kong, 2003c)
Repeat of the exopolysaccharide from <i>Cryptococcus neoformans</i> serotype A	β -D-Xylp-(1 $\rightarrow$ 2)- α -D-Manp-(1 $\rightarrow$ 3)-[β -D-Xylp-(1 $\rightarrow$ 2)]- α -D-Manp-(1 $\rightarrow$ 3)-[β -D-GlcA-(1 $\rightarrow$ 2)]- α -D-Manp and above glycan	TOF	(Zhang & Kong, 2003b)
Lipoarabinomannan from <i>Rhodococcus equi</i>	Chemical synth of pentasaccharide repeat from equine pathogen	TOF (CHCA)	(Ma, Zhang & Kong, 2004)
N-linked glycans			
Biantennary sialyl/lactosaminoglycan	Chemical plus enzymatic synthesis	TOF (DHB)	(Furuike, <i>et al.</i> , 2003)
Biotinylated, sialylated biantennary glycan	Enzymatic synthesis with endo-M	TOF (DHB)	(Mori, <i>et al.</i> , 2004)
High-mannose type attached to ethylene glycol	As microarrays for gp-120 (from HIV) interaction studies	TOF	(Adams, <i>et al.</i> , 2004)
Hybrid-type, bisected glycan	Transglycosylation with endo- β -N-acetylglucosaminidase	TOF (THAP)	(Osumi, <i>et al.</i> , 2004)
α -D-Man-(1 $\rightarrow$ 4)- β -D-GlcNAc-(1 $\rightarrow$ 4)-D-GlcNAc-dolichyl pyrophosphate	Synthesis of unnatural α -D-mannose isomer	TOF (DHB)	(Nakahara, <i>et al.</i> , 2003)
α -(2 $\rightarrow$ 3)-sialic acid-containing N-glycans	Use of α -(2 $\rightarrow$ 3)-sialyltransferase	TOF	(Fukae, <i>et al.</i> , 2004)
Sialylated biantennary-glycopeptide	Thiol link to cysteine	TOF	(Yamamoto, Sakakibara & Kajihara, 2004)
Trisaccharide component of N-glycans	Chemical synthesis as methyl-glycosides	TOF (CHCA)	(Ma, Zhang & Kong, 2003c)
O-linked glycans			
Neoglycopeptides	As models to study the binding properties of <i>Amaranthus leucarpus</i> syn. <i>hypocondriacus</i> lectin	TOF	(Hernández, <i>et al.</i> , 2004)
Glycopeptides/Glycoproteins			
Amino acid conjugates	Synthesis by reductive amination	TOF (DHB)	(Weingarten & Thiem, 2003)
C-linked α -Gal-containing glycopeptide library	Generation and screening of C-linked glycopeptide library	TOF, ESI	(Xian, <i>et al.</i> , 2004)
Complex N-glycans-glycopeptides	Chemoenzymic synth. of sugar-Asn. solid-phase synth. of g-pep	TOF	(Kajihara, <i>et al.</i> , 2004)
Core II <i>O</i> -linked glycopeptide	Solid phase plus enzymatic synthesis	TOF (DHB)	(Takano, <i>et al.</i> , 2003)
Core II <i>O</i> -linked glycopeptide	Solid-phase synthesis plus enzymatic sialylation	TOF	(Takano, <i>et al.</i> , 2004)
Core II sialyl Lewis-X <i>O</i> -glycopeptide from Human (MUC1a' mucin)	Enzymatic synthesis	L-TOF (CHCA)	(Gallego, <i>et al.</i> , 2003)
Cyclic glycopeptides	Macrolactamization of glycosylated peptide thioesters	MALDI	(Lin, <i>et al.</i> , 2004)
Eel calcitonin plus N-glycans	Transglycosylation with GlcNAc-ase from <i>Mucor hiemalis</i>	TOF (CHCA)	(Haneda, <i>et al.</i> , 2004)
Emmprin with N-linked core pentasaccharide	Chemical, solid phase. N-glycosylated Ig domain	TOF	(Hojo, <i>et al.</i> , 2003)
Epidermal growth factor-like domain of blood coagulation factor IX carrying Xyl-Glc	Solid-phase synthesis. Mild conditions enabled protecting groups on the sugar to be omitted	TOF	(Kitamura, <i>et al.</i> , 2004)
Epithelial cadherin glycopeptides	Solid-phase synthesis. Fmoc chemistry	TOF	(Reipen & Kunz, 2003)
Epithelial mucin MUC4	Synthesis of tumor-associated glycopeptide by solid-phase synthesis	TOF	(Brocke & Kunz, 2003, Brocke & Kunz, 2004)
Galactosylated 5-hydroxylysine mimetics	For incorporation into peptides such as type II collagen	TOF	(Marin, <i>et al.</i> , 2004)

Carbohydrate	Synthetic methods and Comments	Instrumentation	Reference
GalNAc-tyrosine	Conjugate addition of phenols to 2-nitrogalactal	TOF (DHB)	(Khodair, Winterfeld & Schmidt, 2003)
Glycoinsulin	Sialic acid attached to lactose-insulin to increase solubility	TOF	(Sato, <i>et al.</i> , 2004b)
Glycopeptides (of cell-surface glycoproteins)	Use of (2-Ph-2-TMS)ethyl-(PTMSEL) as a fluoride-sensitive anchor for solid-phase synthesis	TOF	(Wagner, Dziadek & Kunz, 2003)
Glycopeptides	Study of glycosylation catalysed by PNGase F in reverse m.	R-TOF (CHCA)	(Jeong, Lee & Park, 2004)
Glycopeptide mimetics	Incorporation of unnatural (keto) amino acids in peptide chain	FTICR	(Liu, <i>et al.</i> , 2003a)
Glycopeptide from collagen	Chemical synthesis. Helical models incorporating collagen sequences for receptor binding studies	TOF (DHB, sinapinic acid)	(Lauer-Fields, <i>et al.</i> , 2003)
Gly-Pro-Thr(β -Gal) ₁₀	Carbohydrate stabilizes triple helix of collagen by H-bonds	TOF	(Bann, Bächinger & Peyton, 2003)
Heterocyclic-tethered C-glycosyl amino acids	Monosaccharide - heterocycle - amino acid	TOF (CHCA)	(Dondoni, Giovannini & Massi, 2004)
IgG -Fc neoglycoprotein	Site-specific glycosylation	TOF (Sinapinic)	(Watt, <i>et al.</i> , 2003)
Interleukin-1 α plus Neu5Ac-Gal β -Lactoglobulin	Coupling by acyl azide, 2.5 mols/mol of protein	TOF	(Ootsubo, <i>et al.</i> , 2004)
Mucin-type O-glycan	Glycosylation by heating with Glc or Fru for thermal stability	TOF (CHCA)	(Broersen, <i>et al.</i> , 2004)
N-Glycan core carbohydrates linked to cysteine	By 2-nitrogalactal concatenation	TOF (DHB)	(Winterfeld, Khodair & Schmidt, 2003)
S-Neoglycopeptides	Cysteine-containing peptides linked -S-S- to glycan	TOF (DHB)	(Watt & Boons, 2004)
Ribonuclease A	Chemical synthesis with glycosylthiomethyl azides	MALDI	(Zhu, Pachamuthu & Schmidt, 2004d)
Sialopeptide libraries	Use of PNGase F in reverse reaction	TOF	(Park, <i>et al.</i> , 2003a)
S-Linked glycopeptides	Chemical synth. High affinity sialopeptide ligands	TOF	(Halkes, <i>et al.</i> , 2003)
S-Linked glycopeptide derived from human Tamm-Horsfall glycoprotein	Wo-phase system to avoid use of strong base	MALDI	(Zhu & Schmidt, 2003)
Sperm CD52	Direct base-catalyzed S-glycosylation of cysteine and Homocysteine-containing peptide with O-Ac protected bromides	MALDI	(Zhu, Haag & Schmidt, 2004c)
	Fmoc chemistry to build peptide chain with glycan-Asn	TOF	(Shao, Xue & Guo, 2004)
Glycosides			
Acyated ginsenosides	Regioselective acylation in organic solvents with vinyl acetate and <i>Candida antarctica</i> lipase	TOF (CHCA)	(Teng, <i>et al.</i> , 2003, Teng, <i>et al.</i> , 2004)
1-Adamantylmethyl glycosides	Single-step synthesis from per-Ac-monosaccharides. Binding to cyclodextrins depends on pyrenose stereochemistry	TOF	(Charbonnier & Penadés, 2004)
β -D-Aminoglycosides	Use of 2-nitro-thioglycoside donors	TOF (DHB)	(Barroca & Schmidt, 2004)
Diadzein glycosides	Transglycosylation with <i>Thermotoga maritima</i> maltosyltransferase to increase solubility	L-TOF (DHB)	(Li, <i>et al.</i> , 2004a)
Dihydrodiosgenin glycosides	As mimetics of bidesmosidic steroidal saponins	R-TOF (DHB)	(Suhr, <i>et al.</i> , 2003a)
Diosgenyl glycoside	Synthesis with partially protected donors	TOF	(Du, <i>et al.</i> , 2003)
D-Galp- β -(1 $\rightarrow$ 4)-Glc pNAc- α -(1 $\rightarrow$ 2)-Manp-(1 $\rightarrow$ O)(CH ₂) ₇ CH ₃ mimics	Chemical synthesis. To explore the substrate specificity of sialyltransferases and <i>trans</i> -sialidases	TOF (DHB)	(Joosten, <i>et al.</i> , 2003b)
Glycosyl glycerol	Transglycosylation by cyclodextrin glucanotransferase	TOF (CHCA)	(Nakano, <i>et al.</i> , 2003a)

(Continued)

TABLE 11. (Continued)

Carbohydrate	Synthetic methods and Comments	Instrumentation	Reference
Iminosugar-C-glycosides	Organometallic addition to <i>N</i> -Bz- <i>N</i> -glycosylhydroxylamines	TOF (CHCA)	(Dondoni & Perrone, 2003)
Lipo-chitooligosaccharide nodulation factors	Two-step chemical and biotechnology synthesis	TOF	(Rasmussen, <i>et al.</i> , 2004)
<i>p</i> -Methoxyphenyl β -lactosides with 3'-suphonic acid	To find carbohydrate ligand(s) which can inhibit adhesion of <i>Helicobacter pylori</i> and gastrointestinal epithelial cells.	MALDI	(Borbás, <i>et al.</i> , 2004)
OSW-1 Analogues (cholestanes saponins)	Mod. of steroid side-chain. No change in anti-tumor action.	MALDI	(Deng, <i>et al.</i> , 2004)
Rhamnosylated diosgenyl glucosides	As mimetics of cytostatic steroidal saponins from <i>Ornithogalum saundersiae</i> and <i>Galtonia candicans</i>	R-TOF (DHB)	(Suhr, <i>et al.</i> , 2003b)
Salicin analogues	Enzymatic synthesis from salicyl-OH	TOF (DHB)	(Yoon, Fulton & Robyt, 2004a)
Sialoside-biotin complexes	To study binding to siglees	FT-ICR	(Blixt, <i>et al.</i> , 2003)
3'-Substituted <i>p</i> -methoxyphenyl β -lactosides	To find ligand(s) which can inhibit the adhesion between <i>Helicobacter pylori</i> and gastrointestinal epithelial cells.	MALDI	(Borbás, <i>et al.</i> , 2004)
Glycosphingolipids			
GSLs from <i>Echinococcus multilocularis</i>	Reaction of thioglycosides with benzoyl ceramide	TOF	(Yamamura, <i>et al.</i> , 2004)
GPI Anchors			
Mannobiose-linked phosphoethanolamine	Chemical synthesis of GPI anchor intermediate	TOF (DHB)	(Tanaka, <i>et al.</i> , 2003)
Glycolipids			
Dolichylphosphomannose analogues	Potential inhibitors of Man-T in endoplasmic reticulum	MALDI (DHB)	(Kulesza, <i>et al.</i> , 2004)
Glycolipids (general)	Ag triflate as alternative to TMS-triflate for sensitive ms.	TOF (CHCA)	(Wei, Gu & Du, 2003)
Glycolipids with partially F-alkyl chains	For study of lipid monolayers	TOF (DHB)	(Gege, <i>et al.</i> , 2004)
O-Glycosyl lactates	As glycopeptide mimics	TOF (DHB)	(Khodair, Pachamuthu & Schmidt, 2004)
Lipoteichoic acid	Synthesis of first fully active acid	TOF (THAP, DHB, NO ₃ -aniline)	(Stadelmaier, <i>et al.</i> , 2003)
Maltosyl erythritol	Transglycosylation with amylase (<i>Bacillus stearothermophilus</i>)	TOF (CHCA)	(Yoon, <i>et al.</i> , 2003)
6-O-Sulfo Lewis ^x neo-glycolipid	Containing lactamized neuraminic acid	TOF (DHB)	(Yamaguchi, <i>et al.</i> , 2004)
Cyclodextrins			
Acetylated α , β and γ cyclodextrins	Chemical synthesis. Properties in dense CO ₂	TOF	(Potluri, <i>et al.</i> , 2003)
Allylated cyclodextrin	(6-NH-(CH ₂) ₃ -Si(OEt) ₃) ₇ substituents. As chiral HPLC phase.	TOF (DHB)	(Lai & Ng, 2004)
Amphiphilic cyclodextrins	Novel monosaccharide carriers through bulk liquid membranes	TOF	(Kida, <i>et al.</i> , 2004)
Amphiphilic CDs fully subst. on primary face	Chemical and enzymatic synthesis. 6-Substituted CDs	TOF	(Sallas, Niikura & Nishimura, 2004)
Bis- β -Cyclodextrin/pyromellitic acid 2,5-diamide	Chemical. Improved binding of guest dyestuff result of double cavity.	TOF	(Liu, <i>et al.</i> , 2003b)
Cationic cyclodextrins	6-NHPy, 6-Me-imidazole, 6-Bu-imidazole, 6-NH-C ₂ H ₄ OCH ₃ , NH ₂ . Plasmid complexes for transfection	MALDI	(Cryan, <i>et al.</i> , 2004)
6-N-Carboxyalkyl	(-NH-CO(CH ₂) _{4.5 or 10} -COOH) ₇ substituents, Enzyme promoter	MALDI	(Fernández, <i>et al.</i> , 2004)
6-O-Carboxymethyl (Per-Me)	(CH ₂ COOH) ₇ , (Me) ₁₄ substituents. CEC	R-TOF (+) (THAP)	(Culha, <i>et al.</i> , 2004)
Cyclodextrin dimer	For transport of saccharides through a liquid membrane	TOF	(Ikeda, Matsuhisa & Ueno, 2003)
Cyclodextrins with acetone bridge	Epoxidation catalysts. Ozone, catalysed epoxidation of alkenes	TOF (CHCA)	(Rousseau, <i>et al.</i> , 2004)

TABLE 11. (Continued)

Carbohydrate	Synthetic methods and Comments	Instrumentation	Reference
Glycocluster thioureamethyl cavitands	Determination of binding constants	TOF (DHB, dithranol)	(Oshovsky, <i>et al.</i> , 2004)
Glycoclusters from silsesquioxanes	Prepared <i>via</i> thiol-radical addition reaction	TOF	(Gao, <i>et al.</i> , 2004)
Glycoinsulins	Gave prolonged blood-sugar lowering activity	TOF	(Sato, <i>et al.</i> , 2004a)
Glyco-self-assembled monolayers	Mannose and fucose, peptide-alkane coupling. Trivalent	TOF (<i>nor</i> -harmane)	(Kleinert, Röckendorf & Lindhorst, 2004)
GM1 ganglioside mimics	3,5-Di-(2-aminoethoxy)-benzoic acid branching unit. To study cholera toxin binding	TOF	(Arosio, <i>et al.</i> , 2004)
Lewis ^x glycocluster	Chemical (trimesic acid scaffold)	TOF	(Nishida, <i>et al.</i> , 2003)
Lactoside glycodendrimers	Di- and trivalent	FTMS (DHB)	(Nelson & Stoddart, 2004)
Mannodendrimers	Convergent Synthesis of "Fully-carbohydrate" Man-dendrimers	TOF	(Backinowsky, <i>et al.</i> , 2002)
Mannose/cyclodextrin	Sonogashira coupling to give seven, fourteen or twenty one Man-O-Ph-C≡C-CH ₂ - groups to β-cyclodextrin	TOF	(Ortega-Caballero, <i>et al.</i> , 2003)
Mannose/hydroxyl-functionalized dendrimers	Lectin binding with variously Man-loaded PAMAM dendrimers	TOF	(Woller, <i>et al.</i> , 2003)
Multivalent ligands for HIV-1 gp120	Up to 64 galactose residues	TOF (THAP, IAA)	(Kensinger, <i>et al.</i> , 2004)
Peptide dendrimers based on β-cyclodextrin	Thiol link to 6-position of glucose residues	TOF	(Muhanna, <i>et al.</i> , 2003)
Poly(<i>n</i> -butyl-2-cyanoacrylate)/dextran nanoparticles	Comparison of methods for nanoparticle characterization	TOF (DHB)	(Weyermann, <i>et al.</i> , 2004)
Polyphenylene-based glycodendrimers	Synthesis of glycodendrimers with sugars within scaffold	TOF	(Sakamoto & Müllen, 2004)
Porphyryin-containing glycodendrimers	With four and twelve derivatized glucose residues	TOF (CHCA)	(Ballardini, <i>et al.</i> , 2003)
Schizophyllan with β-lactoside and α-mannoside appendages	NaIO ₄ oxidation followed by reductive amination with aminoethyl-sugars	TOF	(Hasegawa, <i>et al.</i> , 2004)
Spherical and hemispherical poly(ornithine) glycodendrimers	Maltose or lactose. Up to 32 arms	TOF (DHB)	(Baigude, <i>et al.</i> , 2004a)
TEMPO-functionalized PAMAM dendrimers	With increasing mannose loadings	TOF (3-IAA)	(Baigude, <i>et al.</i> , 2003)
Thioglycosylated cationic porphyrins	Potential photodynamic drugs for colorectal adenocarcinoma	MALDI	(Samuelson, <i>et al.</i> , 2004)
tri(<i>meta</i> -O-β-glucopyranosyloxyphenyl)chlorin	Study of stability	TOF	(Ahmed, <i>et al.</i> , 2004)
Trivalent β-(1→6)-branched (1→3)-glucotriose	Linked to glycerol with (C ₂ H ₄ O) ₂ chains	TOF (CHCA)	(Laville, <i>et al.</i> , 2004)
Trival. amino acid-cyclotrimeratrylene scaffold	Library, oligoethylenglycol spacers, mono- or di-saccharides	TOF	(Wu & Kong, 2004a)
Carbohydrate-protein conjugates			
Bisected biantennary glycans plus BSA	hemoenzymatic synth. Bisect modulates ligand properties	TOF (DHB)	(André, <i>et al.</i> , 2004)
Core of <i>E. coli</i> R4 plus tetanus toxoid	Synthesis <i>via</i> reductive amination	TOF (THAP)	(Lukasiewicz, <i>et al.</i> , 2003)
Fluorescent saccharide biosensors plus Con-A	Photoaffinity labelling to concanavalin A	TOF (DHB, CHCA, sinapinic)	(Nagase, <i>et al.</i> , 2003)
GD ₃ , GQ _{1b} and GM ₂ epitopes plus BSA	For oligosaccharide-specific immunoadsorption therapy of Guillain-Barré syndrome	TOF	(Andersen, <i>et al.</i> , 2004)
Glycopeptides from Group A <i>Streptococcus</i> and <i>Shigella flexneri</i> Y plus BSA or TT	Diethyl squarate coupling	TOF (DHB, sinapinic acid)	(Hossany, <i>et al.</i> , 2004)
LDNT, LDNFP and LNFPIII plus BSA	Study of targets for immune intervention in schistosomiasis	TOF	(Nyame, <i>et al.</i> , 2003)
Lewis ^b hexasaccharide plus HSA	Linked with disuccinimidyl suberate spacer	TOF (THAP)	(Lahmann, <i>et al.</i> , 2004)

Carbohydrate	Synthetic methods and Comments	Instrumentation	Reference
Lewis ^x tetrasaccharide plus KLH	Binding studies show that IgG response can be directed at linker	TOF (DHB)	(Buskas, Li & Boons, 2004)
Mannose plus BSA	Comparison of seven linkers.	TOF (sinapinic)	(Izumi, <i>et al.</i> , 2003)
Neoglycoproteins plus BSA	To study binding of human galectin-1	TOF	(Stowell, <i>et al.</i> , 2004)
Oligogalacturonates plus BSA	Linked by reductive amination	TOF (CHCA)	(Clausen & Madsen, 2004)
Saikosaponin a plus BSA	To develop monoclonal antibodies for assay	TOF (sinapinic)	(Zhu, <i>et al.</i> , 2004b)
O-Specific polysaccharides from <i>Vibrio cholerae</i> plus BSA	Di- to penta-saccharides linked <i>via</i> squaric acid diester chemistry	SELDI-TOF	(Saksena, <i>et al.</i> , 2003) (Ma, <i>et al.</i> , 2003b)
<i>Schistosoma mansoni</i> glycan fragments + BSA	Squaric acid chemistry. For studies on antibody binding	TOF	(de Souza, <i>et al.</i> , 2004)
Trisaccharides from <i>Toxocara</i> sp. plus BSA	Trisaccharides bound to BSA to act as immunoreagents	TOF (+ve) (DHB)	(Amer, Hofinger & Kosma, 2003)
Truncated complex glycans plus BSA	Ninhydrin coupling of pronase glycopeptides for binding studies	MALDI	(van Remoortere, <i>et al.</i> , 2003)
Polymers			
Allylurea-carbohydrate polymers	Synthesis of biodegradable material similar to thermoplastic	TOF (DHB)	(Olivier, <i>et al.</i> , 2003)
(1,2,3,4)-di- <i>O</i> -isopropylidene-6- <i>O</i> -(2-vinylxyethyl)-D-galactopyrenose polymer	Use of hexamethylenediamine or Fmoc end caps to characterize polymer by MALDI and NMR	TOF (dithranol)	(D'Agosto, <i>et al.</i> , 2004)
Glucan with large intervals in MW distribution	Ring-opening polymerization of per-methylated cyclodextrins	TOF (DHB)	(Suzuki & Shimazaki, 2003)
Hyaluronan neoglycopolymer	Disaccharide, pentylenyl link to norbornene. Ring-open metathesis	TOF	(Iyer, <i>et al.</i> , 2003)
Polyacetyl containing trehalose	Reaction of α , α -D-trehalose with terephthalaldehyde. MW 8 kDa	TOF (CHCA)	(Teramoto, <i>et al.</i> , 2004)
Polymers by ring-opening metathesis	From norbornene-attached acetyl-protected carbohydrates	TOF (IAA)	(Nomura, <i>et al.</i> , 2004)
Schizophyllans with β -lactoside and α -Man	Synthesis by NaIO ₄ oxidation followed by reductive amination	TOF	(Hasegawa, <i>et al.</i> , 2004)
Antibiotics/Anticancer			
Aminoglycoside antibiotics	For RNA binding studies	FTMS	(Bryan & Wong, 2004)
Carbohydrate-mod cyclic peptide antibiotics	Chemoenzymatic synthesis	TOF	(Lin & Walsh, 2004)
Chromomycin A ₃	Modifications of deoxysugars during biosynthesis	R-TOF (DHB)	(Menéndez, <i>et al.</i> , 2004)
Kanamycin A-cholesterol conjugates	Aminoglycoside-derived cationic lipids for gene transfection	R-TOF (DHB)	(Sainlos, <i>et al.</i> , 2003)
Landomycin E clusters	New compounds synthesised by biosynthetic and LndGT4 gene manipulation	TOF	(Ostash, <i>et al.</i> , 2004)
Squaric acid amides of anthracycline antibiotics	For covalent binding to biomolecules	TOF	(Tevyashova, <i>et al.</i> , 2004)
Vancomycin analogues	One-pot synth. of modified carbohydrate after glycan removal	MALDI	(Ritter, <i>et al.</i> , 2003)
Miscellaneous			
Azido-glycoside on a sensor chip	Mannose-C ₁₂ glycoside attached by two methods	TOF	(Sato, <i>et al.</i> , 2004c)
Ferrocene-carbohydrate complexes	Two methods to attach mono- and di-saccharides to one or both cyclopentadienyl rings. Electrochemical behaviour studied.	TOF	(Casas-Solvas, <i>et al.</i> , 2004)
Glycoprobes	Chemical. Based on digoxin-Lys	TOF (ATT)	(Lauc, <i>et al.</i> , 2003)
Oligosaccharide microarrays	Assembly of sugars on polystyrene plates	FT-ICR	(Fazio, <i>et al.</i> , 2004)

the two areas that are particularly relevant for monitoring are those of glycodendrimers and glycoprotein conjugates.

A. Synthesis of Multivalent Carbohydrates, Dendrimers and Glycoclusters

Many polyfunctional compounds have been used to build the basic scaffold of these molecules. Dendrimers built on such scaffolds include those on *tris*-(2-aminoethyl)-amine (**60**) (Li et al., 2004e), trimesic acid (**61**) (Nishida et al., 2003),

carbosilanes (**62**) (Boysen & Lindhorst, 2003), silsesquioxanes (**63**) (Gao et al., 2004), cyclotrimeratrylene (**64**) (van Ameijde & Liskamp, 2003), tetra-aryl porphyrins (**65**) (Ballardini et al., 2003; Ahmed et al., 2004; Laville et al., 2004) and fullerene derivatives (Isobe et al., 2003). Further examples are in Table 11. Poly-hydroxy compounds such as carbohydrates themselves have been used as the core for dendrimers. Thus, Wu and Kong (2004a) used ethylene glycol and glycerol to produce di- and trivalent (**66**) first order dendrimers with trisaccharides attached to each arm and Köhn et al. (2004) used glucose to produce pentavalent dendrimers containing either mono- or

60. Glycodendrimer built on *tris*-(2-aminoethyl)-amine

61. Glycodendrimer built around trimesic acid

62. Glycodendrimer built around a carbosilane

63. Glycodendrimer built around a silsesquioxanes

64. Glycodendrimer built around cyclotrimeratrylene

65. Glycodendrimer built around a tetra-aryl porphyrin

66. First order glycodendrimer built on glycerol

trisaccharides. Three first-order dendrimers based on a cyclodextrin core containing fourteen Val, Phe or Val-Phe residues have been synthesized. The amino acids were linked to the terminal residues of 3,3'-iminobispropanolamine which was coupled through the central nitrogen to the 6-iodo derivative of β -cyclodextrin (Muhanna et al., 2003). First-order dendrimers have also been synthesized by coupling 7, 14, or 21 Man-O-Ph-C $\equiv$ C-CH₂- groups to β -cyclodextrin (Ortega-Caballero, Giménez-Martínez, & Vargas-Berenguel, 2003) (**67**) giving glycodendrimers with carbohydrate moieties both at the core and periphery. Wu and Kong (2004a) have also synthesized related compounds by cyclization of nona- and dodecasaccharides to give dendrimers containing hexa- and octa-saccharide rings. Schizophyllan ([$\rightarrow$ Glc- β -(1 $\rightarrow$ 3)-(Glc- β -(1 $\rightarrow$ 6)Glc- β -(1 $\rightarrow$ 3)-Glc- β -(1 $\rightarrow$ 3)-]_n) has also been used as a backbone;

β -glucoside appendages were oxidized to give aldehyde groups which were coupled to aminoethyl- β -lactoside or α -mannoside by reductive elimination (Hasegawa et al., 2004). There is also a report of a glycodendrimer, based on a polyphenylene scaffold with a ring of monosaccharides between the center and the periphery (Sakamoto & Müllen, 2004) (**68**).

Some of the largest multivalent glycodendrimers are based around a scaffold consisting of amino acids or polyamidoamine (PAMAM). In one of the latter cases, MALDI-TOF analysis of the products gave broad peaks with masses less than those predicted and attributed to incomplete synthesis of the PAMAM scaffold. Masses ranged from 10,000 to 132,000 Da (178 mannose residues) (Woller et al., 2003). Choudhury, Kitaoka, and Hayashi (2003) have used MALDI-TOF for monitoring PAMAM glycodendrimers containing cellobiose. The dendrimer

67. Glycodendrimer built around β -cyclodextrin

68 . Glycodendrimer based on a polyphenylene scaffold

with 32 sugar units produced a spectrum (m/z 19,132) but the next generation glycodendrimer (64 sugar units, predicted mass for $C_{1518}H_{2656}O_{892}N_{250} = 38,661$) failed to give a signal. Synthesis of large, fifth generation glycodendrimers with potentially 64 outer sugar residues (**69**) and sulfated analogues has been monitored by MALDI-TOF MS from THAP (positive ion) and IAA (negative ion) to reveal that approximately 50 of the potential 64 sugars were actually incorporated (Kensinger et al., 2004). Sharp MALDI-TOF peaks from spherical oligosaccharide- β -alanine-poly(lysine) dendrimers containing 32 cellobiose (13,418 Da), maltose (13,472 Da), and lactose (13,507 Da) groups respectively, synthesized by Baigude et al. (2003), showed complete substitution of the amino groups by carbohydrate. Differences were found between the measured and calculated masses of larger maltose-containing glycodendrimers

(13 kDa) based of a poly(ornithine) scaffold with the higher experimental masses being attributed to instrumental factors. The MALDI-TOF spectrum from CHCA of a third generation maltose-proline-poly(lysine) dendrimer (4.4 kDa) synthesized by Baigude et al. (2004b) gave three peaks attributed to the $[M + H]^+$, $[M + Na]^+$, and $[M + K]^+$ ions. Attempted addition of a succinyl group to each maltose residue produced a MALDI spectrum with multiple peaks attributed to different numbers of attached succinyl groups and giving an average number of 13 attached groups. Finally a 24-mer peptide was attached to produce a potential AIDS vaccine; MALDI-TOF analysis indicated the addition of one and two such units. Self-assembling dendrimers carrying four or eight GalNAc residues have been constructed on a bipyridyl core; addition of $Cu(II)SO_4$ linked two bipyridyl units to give the dendrimer. It was characterized by

69. Fifth generation glycodendrimer

MALDI-TOF MS from DHB and THAP as the intact Cu(II)-linked molecule (**70**) (Roy & Kim, 2003).

B. Synthesis of Carbohydrate–Protein Conjugates

Matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) analysis is used in this area mainly to estimate the number of carbohydrate molecules bound to the protein. Conjugation of the glycans is frequently by squaric acid chemistry or by reductive amination. However, Lewis b hexasaccharide has been linked to human serum albumin (HSA) with a disuccinimidyl suberate spacer. The authors found that this spacer had a similar linking efficiency to squaric acid but

had the advantage of a fast incorporation of the receptor saccharide to the carrier protein (Lahmann et al., 2004). Saksena, Ma, and Kováč (2003) have used SELDI to monitor the conjugation to BSA of di- through penta-saccharides that mimic the upstream terminus of the O-specific polysaccharide of *Vibrio cholerae* O:1, serotype Ogawa using squaric diester chemistry. SELDI monitoring of the reaction allowed the extent of conjugation to be monitored and stopped when the desired molar hapten-BSA ratio had been reached. The accuracy of the mass measurement could be increased by using the carrier protein as the internal standard. Tri- and hexa-galacturonates have been synthesized and coupled to BSA by reductive amination; MALDI analysis gave a molecular weight of 70.996 corresponding to 3.8 mol of ligand/mol of BSA (Clausen & Madsen, 2004). To

70. Cu(II)-Containing glycodendrimer

develop antibodies to Saikosaponin A, a triterpenoid saponin from the root of *Bupleuri radix*, a plant used in Eastern medicine, Zhu et al. (2004b) have conjugated the saponins to BSA and determined the hapten number as 11 by MALDI from sinapinic acid. Concanavalin A has been labeled with a fluorescent probe at the active site by first binding a photolabile linker attached to mannose to the active site. The probe was then fixed to the protein by photoaffinity labeling, the mannose was removed by reduction of the disulfide link and the resulting thiol group used to attach various fluorescent reagents (Nagase et al., 2003). MALDI from sinapinic acid was used to monitor various stages of the reaction and to demonstrate that the final addition of the fluorescent group occurred to the extent of 70–90%. Nyame et al. (2003) have synthesized di- and trisaccharides bearing the LDN and LDNF epitopes and conjugated these, together with one carrying the Lewis^x epitope, to BSA. MALDI-TOF analysis showed that three to four molecules were coupled per mole of BSA. These and additional examples are listed in Table 11.

C. Carbohydrates as Synthetic Reagents

A cyclosophoraose (cyclic-(1 → 2)-β-D-glucan) named Cys, produced by soil micro-organisms of the genus *Rhizobium*, has been found to act as a catalyst for the methanolysis of several compounds such as phosphatidylcholine (Lee & Jung, 2004). MALDI analysis of several of the reaction intermediates showed acylated cyclosophoraoses in the mass range 3,000–4,500. Carboxymethylated Cys from *R. leguminosarum* biovar *trifolii* could be used to complex and, consequently increase the aqueous solubility of hydrobenzoin and *N*-acetyltryptophan (Lee et al., 2004c).

XII. MISCELLANEOUS

A few additional papers documenting the use of MALDI analysis for carbohydrate analysis that do not fit the above categories, have

been published. Thus, Microsin E492 (MccE492), an 84-amino acid antimicrobial peptide from *Klebsiella pneumoniae* has been purified in a post-translationally modified form from *E. coli* VCS257 harboring the pJAM229 plasmid or *K. pneumoniae* RCY492. The 831 Da modification was characterized by MS and NMR and found to consist of a trimer of *N*-(2,3-dihydroxybenzoyl)-L-serine linked *via* a C-glycosidic bond to β -D-glucose that was O-linked to the MccE492 (Thomas et al., 2004b). It has been proposed that tunicamycin, an inhibitor of D-HexNAc-1-P-translocases, exerts its action by coordinating the divalent metal cofactor at the active site. MALDI spectra of tunicamycin in the presence of several divalent metals contained ions with compositions of [Tun + Metal] $_2^+$ showing that one of the metal atoms was present as a metal chelate (Xu et al., 2004). The C-terminus loop 13 of the Na $^+$ glucose co-transporter (SGLT1) has been shown to contain a binding site for inhibitory alkyl glucosides following photoaffinity labeling and MALDI-TOF analysis (Raja, Kipp, & Kinne, 2004). Three hydroxyproline-rich peptides of 15, 18, and 20 amino acids, containing several pentose residues have been identified as defense signaling peptides released at wound sites in tomato plants (Pearce & Ryan, 2003).

XIII. CONCLUSIONS

From the above, it can be seen that MALDI and particularly MALDI-TOF is still a major analytical method for carbohydrate analysis. Even though electrospray ionization, particularly when coupled with CID, is being increasingly used, MALDI-TOF usually provides a superior profile of constituent sugars because of the tendency of the technique to produce only singly charged ions. However, MALDI-TOF MS of native acidic glycans is less satisfactory because of problems with prompt fragmentation. Electrospray causes less fragmentation of these compounds but tends to produce ions in different charge states from glycans with several acidic groups, thus giving a profile that is not representative of the glycan content. However, this problem, and the instability of acidic carbohydrates under MALDI conditions, can be overcome by derivatization of the carboxylic acid group of sialic acids but sulfates must be protected by other techniques such as ion pairing. Thus, both MALDI and electrospray have advantages and disadvantages for carbohydrate work and the best technique to use will be dictated by the problem to be solved. The work during the review period has shown that although MALDI is now relatively “mature,” it is expected that future editions of this review series will be able to report many improved methods for performing these analyses.

XIV. ABBREVIATIONS

2-AA	2-aminobenzoic acid
2-AB	2-aminobenzamide
ABEE	aminobenzoic acid ethyl ester
ABOE	aminobenzoic acid octyl ester
A/D	analogue to digital
AGE	advanced glycation end products
AGP	α 1-acid glycoprotein

AIDS	acquired immunodeficiency syndrome
AMAC	aminoacridone
AMP	adenosine monophosphate
AMT	5-amino-2-mercapto-1,3,4-thiadiazole
ANDA	7-aminonaphthalene-1,3-disulfonic acid
ANSA	4-aminonaphthalene-1-sulfonic acid
ANTS	8-aminonaphthalene-1,3,6-trisulfonic acid
AP	aminopyridine
AP-MALDI	atmospheric pressure MALDI
Api	apiose
AQ	aminoquinoline
Ara	arabinose
Ara4N	4-amino-4-deoxy-L-arabinopyranose
Arg	arginine
Asp	aspartic acid
Asn	asparagine
BSA	bovine serum albumin
CAM	cell adhesion molecule
CD	cyclodextrin or circular dichroism
CE	capillary electrophoresis
Cer	ceramide
CDG	congenital disorder of glycosylation
CHCA	α -cyano-4-hydroxycinnamic acid
CHO	Chinese hamster ovary
CID	collision-induced dissociation (decomposition)
CMBT	5-chloro-2-mercaptobenzothiazole
CPS	capsular polysaccharide
Cym	cymarose
Cys	cysteine
Da	Dalton
DHB	dihydroxybenzoic acid (normally the 2,5-isomer)
DHBB	dihydroxybenzoic acid/butylamine
DIOS	desorption/ionization on silicon
DMSO	dimethylsulfoxide
DNJ	deoxynojirimycin
Dol	dolichol
DP	degree of polymerization
DTT	dithiothreitol
ECD	electron capture dissociation
Endo-H	endoglycosidase-H
EPO	erythropoietin
EPS	extracellular polysaccharide
ESI	electrospray ionization
FAB	fast atom bombardment
FACE	fluorophore-assisted carbohydrate electrophoresis
Fru	fructose
FT	Fourier transform
Fuc	fucose
FucNAc	<i>N</i> -acetyl fucoseamine
GAGS	glycosaminoglycans
Gal	galactose
GalA	galacturonic acid
GalNAc	<i>N</i> -acetyl galactosamine
Gal-T	galactosyltransferase
GC/MS	gas chromatography/mass spectrometry
GDP	guanosine-5'-diphosphate

GIPSL	glycoinositolphosphosphingolipid
Glc	glucose
GLC	gas–liquid chromatography
GlcA	glucuronic acid
GlcN	glucosamine
GlcNAc	<i>N</i> -acetylglucosamine
Glu	glutamic acid
Gly	glycine
GnT	GlcNAc transferase
Gro	glycerol
GSL	glycosphingolipid
HABA	2-(4′hydroxyphenyl)azobenzoic acid
Hep	heptose
HepP	heptose phosphate
Hex	hexose
HexA	hexuronic acid
HexNAc	<i>N</i> -acetylhexosamine
HIQ	hydroxy- <i>iso</i> -quinoline
HPAEC	high-performance anion exchange chromatography
HPLC	high-performance liquid chromatography
HR	high resolution
HSA	human serum albumin
IAA	indoleacrylic acid
ICR	ion cyclotron resonance
IgA (E, G, or M)	immunoglobulin A (E, G, or M)
Ins	inositol
IR	infrared
IRIS	infrared ionization from solution
IRMPD	infrared multiphoton dissociation
IUPAC	International Union of Pure and Applied Chemistry
Kdo	3-deoxy-D- <i>manno</i> -oct-2-ulosonic acid
LC	liquid chromatography
LOS	lipooligosaccharide
LPS	lipopolysaccharide
L-TOF	linear-TOF
MALDI	matrix-assisted laser desorption/ionization mass spectrometry
Man	mannose
ManNAc	<i>N</i> -acetylmannosamine
MS	mass spectrometry
<i>m/z</i>	mass to charge ratio
MUC	mucin
NB-DNJ	<i>n</i> -butyl deoxynojirimycin
N-CAM	neural cell adhesion molecule
Neu5Ac	<i>N</i> -acetylneuraminic (sialic) acid
Neu5Gc	<i>N</i> -glycoylneuraminic acid
NMR	nuclear magnetic resonance
Orn	ornithine
PAD	pulsed amperometric detection
PAGE	polyacrylamide gel electrophoresis
PAMAM	polyamidoamine
PC	phosphatidyl choline or phosphoryl choline
PE	phosphoethanolamine
PEG	polyethylene glycol
PEA	phosphoethanolamine
Phe	phenylalanine
PNGase	protein- <i>N</i> -glycosidase

PSA	prostate-specific antigen or polysialic acid
PSD	post-source decay
Q	quadrupole
QIT	quadrupole ion trap
Qui	quinovose
Rha	rhamnose
R-TOF	reflectron-TOF
SARS	severe acute respiratory syndrome
SDS	sodium dodecyl sulfate
SEC	size-exclusion chromatography
SELDI	surface-enhanced laser desorption/ionization
Ser	serine
s-DHB	super-DHB
SL	sialyl-lactose
sRAGE	soluble receptor for advanced glycation end products
TFA	trifluoroacetic acid
THAP	trihydroxyacetophenone
Thr	threonine
TLC	thin-layer chromatography
TOF, Tof	time-of-flight
TT	tetanus toxoid
UDP	uridine diphosph(ate)(o)
UV	ultraviolet
Val	valine
X	xylose
Xxx	any amino acid except proline
Xyl	xylose
YAG	yttrium aluminum garnet

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