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Structure Analysis of Trivalent Glycoclusters by Post-source Decay Matrix-assisted Laser Desorption/Ionization Mass Spectrometry

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Post-source decay (PSD) matrix-assisted laser desorption/ionization time-of-flight mass spectra were found to be useful for the structural elucidation of a series of tris[2-(glycosylthiourylene)ethyl]amines. The reported fragmentation behaviours of $[M + H]^+$, $[M + Na]^+$ and $[M - H]^-$ ions differ from each other significantly; however, they can be compared to tree pruning in every case. Whereas detailed structural information on unprotected glycoclusters is obtained from all PSD experiments, only the positive-ion mode can be used to gain relevant information about the acetylated glycoclusters. © 1998 John Wiley & Sons, Ltd.

KEYWORDS: mass spectrometry; matrix-assisted laser desorption/ionization; post-source decay; positive ions; negative ions; carbohydrates; glycoclusters; glycodendrimers

INTRODUCTION

Carbohydrate–protein interactions are of great importance for biological processes such as cell–cell communication and microbial adhesion.[1–3] For the manipulation and inhibition of carbohydrate–protein interactions and for the investigation of their molecular details, monodisperse multivalent glycoconjugates are a modern and important tool.[4,5] For their preparation, specific carbohydrate derivatives have been clustered on polyvalent core molecules or hyperbranched dendritic cores, leading to so-called glycoclusters and glycodendrimers, respectively.[6,7]

Glycoclusters and glycodendrimers typically are molecules of high symmetry and molecular mass. Their structural elucidation is an important and demanding task. Owing to the special stereochemical features of glycoclusters, NMR analysis has to be supported by mass spectrometry for their characterization, to add information about purity or structural defects, which are otherwise difficult to obtain.

Mass spectrometry (MS) has been developed into an indispensable technique for the analysis of glycoconjugates in recent decades.[8–11] Especially fast atom bombardment (FAB) MS has been used to determine the monosaccharide sequence of multiantennary glycan chains of the complex type in *N*-glycoproteins.[12,13] More recently, electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) MS have become important in biochemical analysis.[14–16] MS has also been successfully applied for the determination of dendritic purity.[17] Most frequently ESI[17–20] and MALDI/MS[21–30] have been applied in dendrimer analysis and also FAB[31] and fast ion bombardment (FIB)[28,32] ionization techniques have been used.

The investigation of state-of-the-art MS is equally important for the structural analysis of monodisperse glycoclusters. To the best of our knowledge, no detailed tandem mass spectral data on glycoclusters have been reported so far. We now report on the post-source decay (PSD) fragmentation behaviour of non-glycosidic, anomerically thiourea-bridged glycoclusters which are based on tris(2-aminoethyl)amine as depicted in Fig. 1. This type of multivalent glycomimetics has recently been introduced and further developed into a useful tool in glycobiology.[33–36] Thus, thiourea-bridged glycoclusters and glycodendrimers have already shown excellent avidities in different adhesion systems.[37–39] In this paper we show that the PSD fragmentation pattern of the investigated trivalent glycoclusters (Fig. 1) is different for the corresponding protonated, sodiated and deprotonated molecules, and this is discussed in detail.

EXPERIMENTAL

Tris[2-(glycosylthiourylene)ethyl]amines

The glycoclusters studied (Fig. 1) were synthesized by the reaction of trivalent tris(2-aminoethyl)amine with

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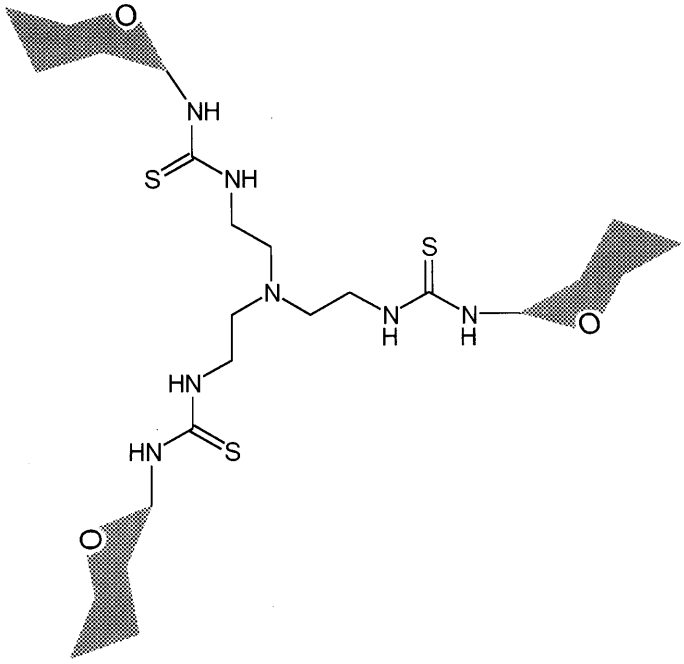


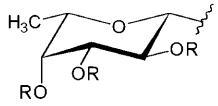
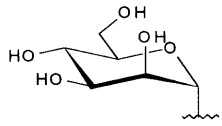
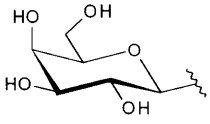
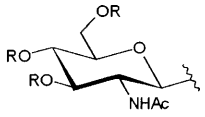
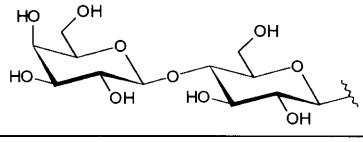
Glycocluster	Configuration		
Tris[Fuc] Tris[Fuc(OAc)$_3$]	β-L-*fuco*	R = H R = Ac	
Tris[Man]	α-D-*manno*		
Tris[Gal]	β-D-*galacto*		
Tris[GlcNAc] Tris[GlcNAc(OAc)$_3$]	2-acetamido-2-deoxy- β-D-*gluco*	R = H R = Ac	
Tris[Lac]	β-*lacto*		

Figure 1. Structures of glycoclusters.

acetylated mono- and disaccharide glycosyl isothiocyanates. The acetylated products obtained were either desalted by gel permeation chromatography (Sephadex G-10, eluted with nanopure water) to enhance the sensitivity in the negative-ion mode or deprotected by the Zemplén procedure. Finally, the deprotected compounds were desalted. Details on the synthesis and NMR characterization of the compounds are reported elsewhere.[33]

Mass spectrometry

All MALDI mass spectra were measured on a Bruker BIFLEX reflectron TOF mass spectrometer (Bruker-Franzen, Bremen, Germany) equipped with a multiprobe inlet and a gridless delayed extraction ion source. 2,5-Dihydroxybenzoic acid (DHB) (Aldrich, Steinheim, Germany) was used as a matrix in 40% acetonitrile aqueous solution at a concentration of 10 mg ml^{-1}. The

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Table 1. Principal fragments observed in the PSD mass spectra of $[M + H]^+$ ions

Ion type	Ion description[a]	m/z Tris[Fuc]	Tris[Fuc(OAc)$_3$]	Tris[Man]	Tris[Gal]	Tris[GlcNAc]	Tris[GlcNAc(OAc)$_3$]	Tris[Lac]
	$[M+H]^+$	762.3	1140.4	810.3	810.3	933.3	1311.4	1296.4
a	$MH^+ - Gly + 2H$	617.4	868.1	649.0	649.1	730.3	982.4	973.2
b	$MH^+ - (Gly–NH_2)$	599.4	850.9	632.0	631.7	713.3	965.5	956.1
c	$MH^+ - (Gly–NH_2) - H_2O$ (deprotected) or $MH^+ - (Gly–NH_2) - CH_3COOH$ (acetylated)	581.4	n.o.[b]	613.5	613.4	695.4	905.9	938.3
d	$MH^+ - (Gly–N=C=S)$	557.5	809.1	589.2	589.1	671.3	923.6	913.3
e	$MH^+ - (Gly–NHCSNHCH=CH_2)$	514.8	766.5	546.5	547.2	628.9	881.1	872.0
f	$MH^+ - 2Gly + 2H$	470.3	n.o	486.3	486.4	527.6	n.o.	n.o.
g	$MH^+ - (Gly–NH_2) - Gly + H$	453.2	578.7	469.1	469.6	510.7	635.5	631.3
h	$MH^+ - 2(Gly–NH_2)$	436.3	561.4	452.1	452.7	493.3	618.7	614.4
i	$MH^+ - (Gly–N=C=S) - Gly + H$	n.o.	n.o.	427.3	427.9	467.4	n.o.	589.7
j	$MH^+ - (Gly–N=C=S) - Gly–NH_2)$	394.3	520.2	410.2	410.3	451.1	577.2	n.o.
k	$MH^+ - (Gly–NHCSNHCH=CH_2) - Gly + H$	368.3	494.0	384.4	384.2	425.3	551.4	n.o.
l	$MH^+ - 2(Gly–N=C=S)$	351.2	476.7	367.4	367.4	408.6	533.4	529.1
m	$[Gly–NHCSNHCH_2CH_2]^+$	248.9	374.8	265.5	264.8	305.8	431.5	427.4

[a] Gly symbolizes the sugar moiety.
[b] Not observed.

samples were deposited on a MALDI target by the sandwich method.[40] The ion acceleration voltage was 19 kV and the reflectron (ion mirror) voltage was set at 20 kV. For gridless delayed extraction (GDE), a 5 kV potential difference between the probe and the extraction lens was applied with a time delay in the range 120–150 ns after each laser pulse. Samples were irradiated at a frequency of 5 Hz by 337 nm photons from a pulsed Laser Science (Cambridge, MA, USA) nitrogen laser. Typically 20–50 shots were summed into a single (conventional) mass spectrum. The spectra were calibrated externally using the monoisotopic $[M + H]^+$

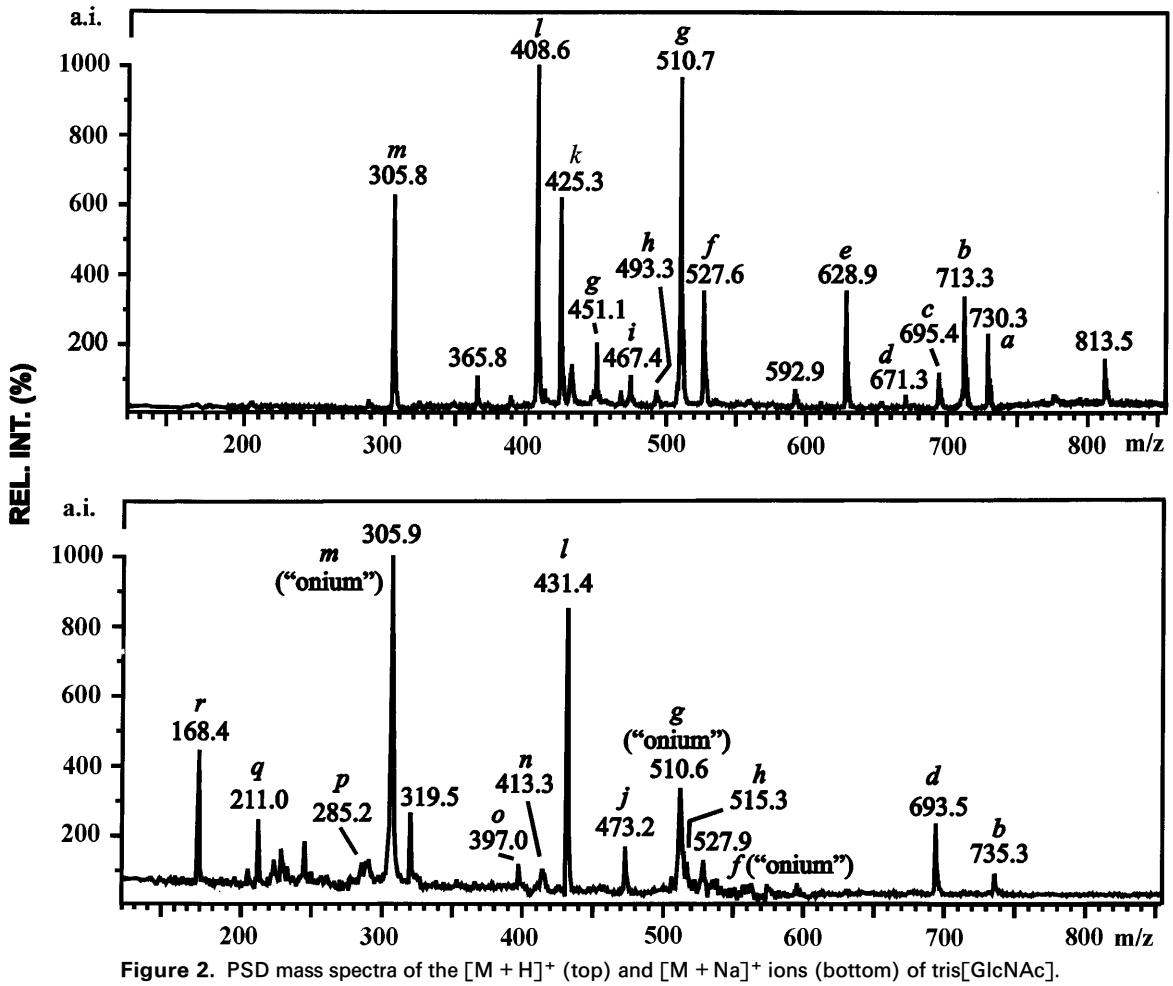

Figure 2. PSD mass spectra of the $[M + H]^+$ (top) and $[M + Na]^+$ ions (bottom) of tris[GlcNAc].

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ion of a peptide standard (bombesin; Aldrich). PSD spectra were typically recorded in 10–14 segments, each successive segment representing a 20% reduction in reflector voltage. The corresponding precursor ions were carefully selected by $FAST_{TM}$ deflecting pulses. About 200 shots were averaged per segment. The segments were pasted, calibrated and smoothed under computer control by Bruker XTOF 3.0 software. The reported fragment ion masses are monoisotopic.

RESULTS AND DISCUSSION

Conventional (reflectron) positive-ion MALDI mass spectra of the trivalent carbohydrate derivatives exhibit the corresponding abundant protonated and sodiated molecules. The majority of negative-ion mode spectra display a strong $[MNa - 2H]^-$ species and almost no $[M - H]^-$ peak. Consequently, the formation of the deprotonated molecules is enhanced by desalting of the samples prior to the measurements (see Experimental).

The fragmentation behaviour of all types of molecular ions studied can be compared to tree pruning: the symmetrical molecular ions either lose the sugar moiety from the branches, or portions thereof, or lose even larger parts of the carbohydrate-carrying antennae. In the following text the detailed fragmentation patterns observed in the different PSD spectra are discussed.

PSD mass spectra of $[M + H]^+$ ions

Generally, the ions *a*, *b*, *e*, *g*, *l* and *m* (see Table 1 for structure assignments) are the most prominent fragments observed in the spectra of all compounds (Figs 2 and 3). The charge is mostly localized on the aglycone part of a given fragment. The only complementary ion pair is represented by species *e* and *m*, i.e. $MH^+ - (Gly–NHCSNHCH{=}CH_2)$ and $[Gly–NHCSNHCH_2CH_2]^+$, respectively, where the abbreviation Gly symbolizes the sugar unit. The elemental compositions for hexose moieties (α-D-Man, β-D-Gal), 6-deoxyhexose (β-L-Fuc), peracetylated 6-deoxyhexose (β-L-Fuc$(OAc)_3$), 2-acetamido-2-deoxy-β-D-hexose (β-D-GlcNAc), peracetylated 2-acetamido-2-deoxy-β-D-hexose (β-D-GlcNAc$(OAc)_3$) and lactose (β-Lac) are $C_6H_{11}O_5$, $C_6H_{11}O_4$, $C_{12}H_{17}O_7$, $C_8H_{14}NO_5$, $C_{14}H_{20}NO_8$ and $C_{12}H_{21}O_{10}$, respectively.

Additional fragments formed by the elimination of acetic acid can be found in the case of the paracetylated glycoclusters. One or two molecules of acetic acid are released from the $[M + H]^+$ ion producing ions at *m/z* 1251 and 1191 in the case of the GlcNAc$(OAc)_3$ cluster (Fig. 3). A similar pattern rationalizes the nature of species at *m/z* 905 (ion *c*) and 845, which are generated from ion *b* (*m/z* 965). Whereas for the acetylated glycoclusters the ion *c* can be described as $MH^+ - (Gly–NH_2) - CH_3COOH$, in the case of the unprotected analogues it should be labeled as MH^+

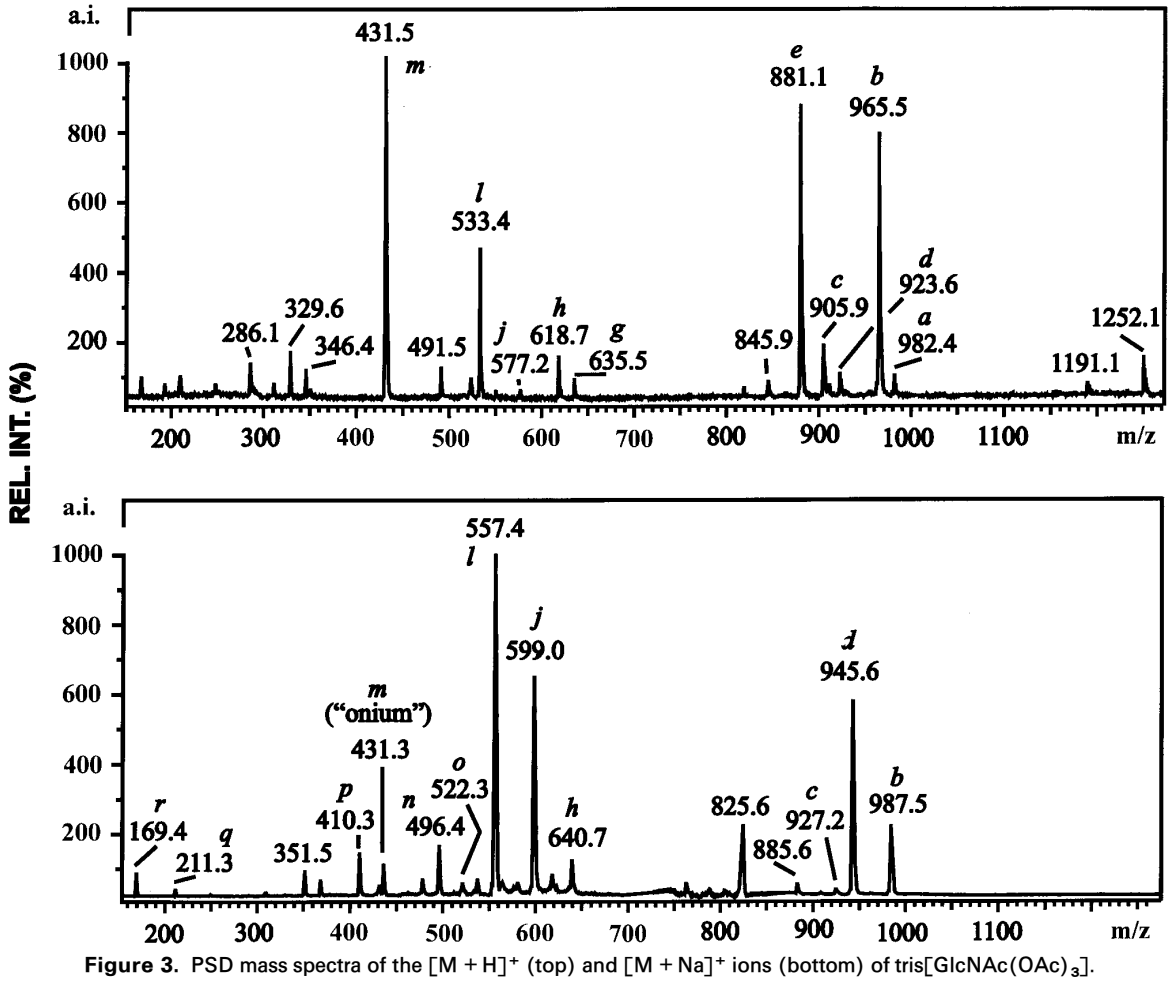

Figure 3. PSD mass spectra of the $[M + H]^+$ (top) and $[M + Na]^+$ ions (bottom) of tris[GlcNAc$(OAc)_3$].

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Table 2. Principal fragments observed in the PSD mass spectra of $[M + Na]^+$ ions

Ion type	Ion description	m/z Tris[Fuc]	Tris[Fuc(OAc)$_3$]	Tris[Man]	Tris[Gal]	Tris[GlcNAc]	Tris[GlcNAc(OAc)$_3$]	Tris[Lac]
	$[M + Na]^+$	784.3	1162.4	832.3	832.3	955.3	1333.4	1318.4
b	$MNa^+ - (Gly–NH_2)$	621.3	872.8	654.0	653.1	735.3	987.5	977.8
d	$MNa^+ - (Gly–N{=}C{=}S)$	579.8	830.9	611.2	611.0	693.5	945.6	936.4
h	$MNa^+ - 2(Gly–NH_2)$	458.6	583.4	474.6	474.7	515.3	640.7	636.5
j	$MNa^+ - Gly–N{=}C{=}S - (Gly–NH_2)$	417.1	541.3	432.4	432.3	473.2	599.0	594.0
l	$MNa^+ - 2(Gly–N{=}C{=}S)$	374.3	500.1	390.5	390.3	431.4	557.4	552.3
n	$MNa^+ - 2(Gly–N{=}C{=}S) - H_2O$ (deprotected) or $MNa^+ - 2(Gly–N{=}C{=}S) - CH_3COOH$ (acetylated)	n.o.[a]	439.3	372.3	372.6	413.3	496.4	534.2
o	$MNa^+ - 2(Gly–NHCSNH_2)$	340.8	465.3	356.2	356.8	397.0	522.3	517.2
p	$[Gly–N{=}C{=}S + Na]^+$	228.2	353.5	244.2	244.1	285.2	410.3	405.6
q	$[S{=}C{=}N–CH_2CH_2N(CH_2CH_2NH_2)_2 + Na]^+$	211.0	211.9	211.4	211.0	211.0	211.3	210.7
r	$[N(CH_2CH_2NH_2)_3 + Na]^+$	169.0	168.7	169.2	168.7	168.4	169.4	168.5

[a] Not observed.

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Table 3. Principal fragments observed in the PSD mass spectra of $[M - H]^-$ ions

Ion description	*m/z* Tris[Fuc]	Tris[Man]	Tris[Gal]	Tris[GlcNAc]
$[M - H]^-$	760.3	808.3	808.3	931.3
$[M - H]^- - HS^{\cdot}$	727.2	774.9	775.1	898.6
$[M - H - C_3H_6O_3]^-$ (Hex, HexNAc) or $[M - H - C_3H_6O_2]^-$ (Fuc)	686.3	718.0	718.8	n.o.
$s = [M - H - C_4H_8O_4]^-$ (Hex, HexNAc) or $[M - H - C_4H_8O_3]^-$ (Fuc)	656.8	688.3	689.4	811.7
$s - HS^{\cdot}$	623.4	654.7	655.8	778.3
$s - C_2H_4O_2$	n.o.[a]	628.8	628.8	n.o.
$s - C_3H_6O_3$ (Hex, HexNAc) or $s - C_3H_6O_2$ (Fuc)	582.7	598.1	598.7	n.o.
$t = [M - H - 2(C_4H_8O_4)]^-$ (Hex, HexNAc) or $t = [M - H - 2(C_4H_8O_3)]^-$ (Fuc)	552.6	568.2	568.9	691.5
$t - HS^{\cdot}$	519.0	534.5	535.3	657.9
$t - C_2H_4O_2$	n.o.	508.5	508.4	n.o.
$t - C_3H_6O_3$ (Hex, HexNAc) or $t - C_3H_6O_2$ (Fuc)	478.0	477.9	478.5	n.o.
$u = [M - H - 3(C_4H_8O_4)]^-$ (Hex, HexNAc) or $u = [M - H - 3(C_4H_8O_3)]^-$ (Fuc)	448.3	448.1	448.8	571.2

[a] Not observed.

$- (Gly–NH_2) - H_2O$.

Low-mass ions such as those at *m/z* 169 and 211 are observed in the majority of the PSD spectra of $[M + H]^+$ ions and they most probably are related to the tris(2-aminoethyl)amine core.

PSD mass spectra of $[M + Na]^+$ ions

The majority of ions observed in the PSD spectra of sodiated molecules are sodiated fragments (Table 2) and only a few of them are of the 'onium' type (ions *f*, *g*, *l* and *m*). One portion of the cationized fragment ions represents the sodiated analogues of those also observed in the PSD spectra of the $[M + H]^+$ ions (species *b*, *d*, *h*, *j* and *l*). The second series represents entirely different fragments such as *n*, *p* and *o* (Figs 2 and 3).

The most abundant fragments are the ions *l*, *j*, *d* and *b*. Similarly to the PSD spectra of $[M + H]^+$ ions, the elimination of water and/or acetic acid from some species is also observed. In the case of the protected compounds acetic acid is released from ions *d* and *l*,

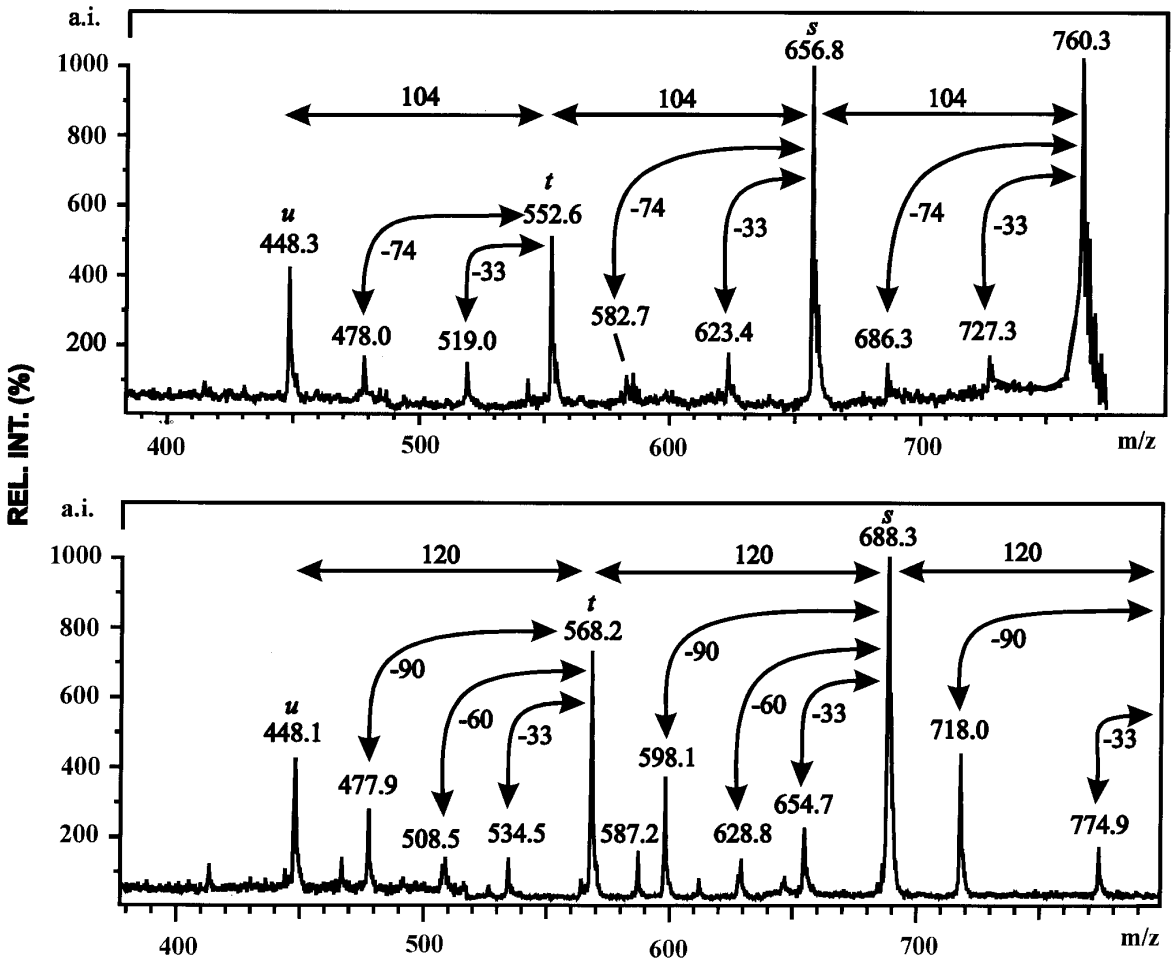

Figure 4. PSD mass spectra of the $[M - H]^-$ ions of tris[Fuc] (top) and tris[Man] (bottom).

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producing the species *c* and *n*, respectively (Table 2). Ion *c* can further lose an additional acetic acid molecule. For tris[GlcNAc(OAc)$_3$] (Fig. 3, bottom) the corresponding ion is observed at *m/z* 825.6.

The low-mass ions at *m/z* 169 and 211, which were already observed in the spectra of $[M + H]^+$ ions, are also observed in the PSD spectra of sodiated molecules; however, in the latter case they could also correspond to the ions $[N(CH_2CH_2NH_2)_3 + Na]^+$ and $[S{=}C{=}NCH_2CH_2N(CH_2CH_2NH_2)_2 + Na]^+$, respectively (Table 2).

With the tris[Lac] derivative no significant cleavages of the glycosidic bonds were observed both in the PSD mass spectra of $[M + Na]^+$ and $[M + H]^+$ ions.

PSD mass spectra of $[M - H]^-$ ions

The fragmentation behaviour of the trivalent clusters in the negative ion mode (Table 3) is completely different when compared with the PSD spectra of the $[M + H]^+$ and/or $[M + Na]^+$ ions.

The selective fragmentation in the negative-ion mode has already been used for the characterization of monosaccharide linkages and the identification of the reducing end in linear oligosaccharides.[41] This information is gained from the occurrence of a combination of diagnostic ions, such as $[M - H - 30]^-$, $[M - H - 60]^-$, $[M - H - 90]^-$ and $[M - H - 120]^-$. Analogous ions are observed in the PSD spectra of the investigated glycoclusters. For the hexose-coated clusters (Fig. 4, bottom) the most abundant product ions are separated by 120 mass units. This difference is attributed to the loss of $C_4H_8O_4$ and is also found in the analogous spectrum of tris[GlcNAc]. This indicates an exclusive removal of sugar ring carbons C-3, C-4, C-5 and C-6 (including their attached hydroxy groups) from the sugar moiety during the fragmentation. The lowest mass anion *u* still contains the C-1 and C-2 atoms including the functional group of the latter. With tris[deoxyHex] and tris[Hex] this ion is displayed at *m/z* 448 (all compounds carry equal functionalities at the corresponding sugar hydroxy groups, when protected); in the case of tris[GlcNAc] the ion *u* shifts to *m/z* 571 (Table 3).

The basic $[M - H]^-$ ion series, *–s–t–u–*, overlaps with further fragments (Fig. 4), where the losses of 33 and 60 mass units should be ascribed to HS$^\cdot$ and $C_2H_4O_2$ eliminations, respectively. The loss of 90 u ($C_3H_6O_3$) from $[M - H]^-$, *s* and *t* ions is diagnostic for a hexose, whereas the presence of a deoxyhexose is indicated by the losses of 74 u.

The mass spectra of the acetylated trivalent glycoclusters taken in the negative-ion mode (not reported here) cannot be interpreted unambiguously, owing to nonselective release of acetic acid molecules (multiples of 60 u are observed) from the $[M - H]^-$ ion. On the other hand, the intense losses of 33 u from the $[M - H]^-$, *s* and *t* ions observed in the spectra of all protected compounds enable us to describe this mechanism as the elimination of HS$^\cdot$ and not, e.g., of CH_5O.

The deprotonated molecule of tris[Lac] was not detected at all. Since the sample was desalted, this may be rationalized by a fast fragmentation of this ion in the ion source.

This study has shown that PSD MALDI/MS significantly facilitates the structural elucidation of multivalent glycomimetics and that it will be of importance for the detailed characterization of larger analogous glycoclusters, such as glycodendrimers.

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