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Use of procaine and procainamide as derivatizing co-matrices for the analysis of oligosaccharides by MALDI TOF mass spectrometry

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Abstract

RATIONALE: Analysis of oligosaccharides by matrix-assisted laser desorption/ionization (MALDI) mass spectrometry often yields only alkali metal cation adducts, which results in lower fragmentation yields and difficulty to retrieve sequence information. Derivatization by reductive amination may be used to promote Y-type glycosidic cleavages. However, this involves time-consuming preparations and purifications with sample loss. Here, procaine and procainamide were used directly as co-matrices with 2,5-dihydroxybenzoic acid (DHB).

METHODS: Acidified 10 g/L procaine hydrochloride or procainamide hydrochloride solutions in water/acetonitrile were added to the oligosaccharide solution one minute before preparing our MALDI targets using DHB with the dried-droplet method. This simple protocol resulted in deposits of very fine homogeneous crystals.

RESULTS: Positive ion mass spectra, easily acquired in an automated mode, presented a high percentage of oligosaccharides derivatized as Schiff base or glycosylamine notably detected as protonated molecules $[M + H]^+$. The high abundance of procaine or procainamide on the target did not impede the ionization process, improved the signal-to-noise ratio and eliminated the need to search for 'sweet spots'. Fragmentation of the protonated precursor ions of the derivatives largely favored Y-type glycosidic cleavages.

CONCLUSIONS: This easy and fast sample preparation, involving low toxicity and easily accessible chemicals, allowed the selection of protonated molecules as precursor ions for post-source decay analyses. This opened the possibility of simplifying sequence retrieval in routine oligosaccharide analyses

Keywords: In situ derivatization; procaine; procainamide; neutral oligosaccharides; matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

Matrix assisted laser desorption ionization (MALDI) mass spectrometry is now extensively used to analyze oligosaccharides. The application and limitations of this technique are well known and have been described in several reviews and key articles.^[1-9] One limitation is that, due to their low proton affinity, neutral oligosaccharides are often only observed as alkali metal cation adducts. This is true with all conventional matrices,^[10] among which 2,5-dihydrobenzoic acid (DHB) is the most common, but also immobilized carbon nanotubes,^[11] gold nanoparticles,^[12] ionic liquid matrices,^[13-15] or newly developed isoliquiritigenin.^[16] As a result, sensitivity is often lowered, and sequence information more difficult to obtain. In fact, coordination to alkali metal cations tends to stabilize the $[M + Na]^+$ ions and complicate the fragmentation processes.^[7] In order to improve sensitivity, alkali salts may be added as dopants^[17] or the oligosaccharides derivatized.^[8,18-25] The main target of derivatization reactions is usually the carbonyl group (reducing end) and reductive amination is by far the commonest tagging reaction. Indeed, high reaction yields, the absence of side-products and the availability of large numbers of amino compounds make it a good choice. Such procedure, however, often involves two reactive steps, sometimes with toxic chemicals, and requires purification of the excess reagents by extraction or reversed-phase HPLC. The gain in sensitivity is therefore somewhat counterbalanced by sample loss during a time-consuming preparation.^[23] Hydrazide^[26] and hydrazine derivatives^[27] have also been used with the advantage that no purification is needed. Derivatization with phenylhydrazine has recently been integrated in an automated procedure in which the reaction was carried out directly on the MALDI spot.^[28] Automation of data acquisition is now an increasing demand in particular when quantitative information is needed, but its use has been limited for oligosaccharides because the most widely used matrix, DHB, is well known for forming large

inhomogeneous crystals around the rim of the MALDI spot.^[29] Diminution of sample heterogeneity^[30] and improvement of spot to spot reproducibility^[16,31,32] is therefore a crucial goal towards a reliable use of MALDI analyses.

In addition, any valuable sample preparation must help to supply structural information^[24,33] by the fragmentation of well-chosen precursor ions. Fragmentation in MALDI is usually observed as post-source decay (PSD) or collision-induced dissociation (CID). Sodiated oligosaccharides necessitate high energy laser or collisional activation and undergo both glycosidic cleavages and cross ring fragmentations thus bringing sequential information as well as possible data on linkage and branching.^[9] Fragment ion mass spectra may be more difficult to interpret, however. Conversely, when protonated molecules of derivatized oligosaccharides are used as precursor ions, reproducible fragment ion mass spectra are obtained with mainly Y glycosidic cleavages (according to the nomenclature introduced by Domon and Costello),^[34] thus providing direct sequence information.^[19]

Recently, on-target derivatization has been proposed by several groups.^[31,35-38] Snovida *et al.*^[31] have developed a mixture of aniline with DHB involving on-target conversion of glycans to Schiff bases. It was found that, since conversion rates were low, a mixture of DHB and N,N-dimethylaniline worked as well by homogenizing sample distribution on the MALDI target.^[32] Recently, Rohmer *et al.*^[37, 39] have developed 3-aminoquinoline as matrix and derivatizing agent and Kaneshiro *et al.*^[38] used the same molecule in a mixture with α -cyano-4-hydroxycinnamic acid. Both sample preparation required long crystallization times and work remarkably well in the positive and negative ion modes.

We report here the use of two well-known derivatizing agents 4-aminobenzoic acid 2-(diethylamino) ethyl ester, also named procaine^[19,40], and N-(2-diethylamino)ethyl-4-

aminobenzamide, also named procainamide^[41], as derivatizing co-matrices. We alternatively used these compounds in an easy sample preparation to obtain Schiff bases or glycosylamine derivatives directly on the MALDI target. This sample preparation was tested on maltooligosaccharides samples and branched xyloglucan-derived oligosaccharides. In addition to its speed and simplicity for derivatization, we describe this new preparation's other advantages for automatic data acquisition and for straightforward retrieval of sequence information.

Experimental Section

Materials and reagents

Dextran (enzymatic synthesis), procainamide hydrochloride 99 % (4-amino-N-(2-diethylaminoethyl) benzamide hydrochloride), and the matrices 2,5-dihydroxybenzoic acid (98%), 2,4,6-trihydroxyacetophenone (97%), α -cyano-4-hydroxycinnamic acid (98%) were purchased from Sigma-Aldrich (Saint Quentin-Fallavier, France). Maltoheptaose, procaine hydrochloride 99 % (2-(Diethylamino)ethyl 4-aminobenzoate hydrochloride) and acetonitrile were purchased from Fisher Scientific (Illkirch, France). HPLC grade water was from a MilliQ system (Millipore, Molsheim, France), acetonitrile was HPLC grade. Acetic acid purex analytical grade was purchased from SDS (Peypin, France). Phosphoric acid for analysis, 85% solution in water was from ACROS Organics (New Jersey, USA). All chemicals and solvents were used without any further purification. The xyloglucan-derived oligosaccharides were kindly provided by F. Dardelle from the Laboratoire de Glycobiologie et Matrice extracellulaire végétale, UPRES EA 4358, Université de Rouen (Mont St Aignan, France). Briefly^[42], a few mg of tamarind xyloglucan were incubated under agitation for 16 h at 37°C with 500 μ L of endo-1,4- β -D-glucanase (12.5 units; EC 3.2.1.4; Megazyme) prepared in 10 mM ammonium acetate buffer, pH 5.0. Glucanase-

resistant material was removed by centrifugation after the addition of ethanol to reach a final volume of 2 mL. The ethanol-soluble xyloglucan oligosaccharides were concentrated by evaporation under a stream of air. The samples were diluted 10 to 100 times in ultrapure water before use.

Sample preparation

2,5-dihydroxybenzoic acid (DHB) matrix solutions were classically prepared to a concentration of 10 g/L in a 70/30 acetonitrile/water solution containing 0,1 % trifluoroacetic acid (TFA). 2,4,6-trihydroxyacetophenone, α -cyano-4-hydroxycinnamic acid were also prepared as 10 g/L solutions in 70/30 acetonitrile/water and 50/50 methanol/acetonitrile respectively. Procaine hydrochloride and procainamide hydrochloride were also dissolved to a concentration of 10 g/L in acetonitrile containing 10% acetic acid or in a solution of water and acetonitrile with 1 % phosphoric acid. For procaine, the proportion of acetonitrile was brought up to 90% to ensure rapid drying of the spot. Procainamide was found less soluble and required a higher proportion of water (50%). The dextran standard was dissolved in ultrapure water to a concentration of 1 g/L which corresponded to approximately 10^{-3} mol/L as the estimated molecular mass was 1000 g/mol. Aqueous maltoheptaose solutions of 10^{-5} mol/L were used for the sensitivity tests. For dried droplet target preparation, 1 μ L of DHB was first deposited and allowed to dry, then one volume of the aqueous analyte solution was mixed during one minute with one volume of procaine or procainamide solutions then two volumes of DHB were added and homogenized by alternatively drawing and dispensing the solution in a micropipette tip. 1 μ L of the mixture was then deposited on the pre-spotted target and allowed to dry at room temperature and atmospheric pressure (10 to 15 minutes). For the reverse thin layer preparation^[43], one volume of analyte and one volume of derivatizing agent were mixed together

during one minute then 0.5 μL was deposited on the target and allowed to dry at room temperature and atmospheric pressure. 0.5 μL of DHB was then deposited to the target.

MALDI-MS Analysis

Mass spectrometry experiments were performed on a MALDI-TOF/TOF instrument (Autoflex III, Bruker, Bremen, Germany) equipped with a LIFT cell using FlexControl 3.3 and FlexAnalysis 3.3 software packages. They were automatically acquired using the AutoExecute program and in the positive reflectron mode. Typically 1500 scans were accumulated when low intensity signal was detected above m/z 1000. The “random walk” movement was selected for the laser. For sensitivity tests, when no signal could be detected automatically, 1500 scans were accumulated manually. Either internal or external calibration was performed from the sodiated molecules detected from our dextran (enzymatic synthesis) standard.

For fragment ion analysis in the tandem time-of-flight (TOF/TOF) mode, up to 5000 spectra were averaged. The precursor ions were accelerated to a kinetic energy of 8 kV. They were selected in a timed ion gate. Fragment ions generated by laser-induced decay (LID) of the precursor were accelerated to a kinetic energy of 19 kV in the LIFT cell and their masses were analyzed after the ion reflector passage. External calibration of MALDI-TOF mass spectra was carried out using singly charged monoisotopic peaks of angiotensin II (10 pmol/ μL), substance P (10 pmol/ μL) and ACTH 18-39 fragment (5 pmol/ μL). Fragment ions were interpreted manually with the assistance of the GlycoWorkbench software.^[44]

Results and Discussion

Figure 1 shows MALDI positive ion mass spectra obtained after the on-target derivatization of enzymatically synthesized dextran, using DHB as matrix. When procaine or procainamide with 10% acetic acid were added during the sample

preparation, derivatized oligosaccharides were detected as the most intense signals with an expected mass difference of 218.142 u for procaine derivatives (Figure 1b) and 217.158 u for procainamide derivatives (Figure 1c) compared to the native oligosaccharides (Figure 1a). Derivatized maltooligosaccharides with a degree of polymerization (DP) from 3 to 14 could be detected with good intensity and signal to noise ratio (from 7 to 46). Table 1 sums up the exact expected m/z values for the native and derivatized oligosaccharides as protonated molecules $[\text{M} + \text{H}]^+$ and sodium adducts $[\text{M} + \text{Na}]^+$. Signals from the underivatized maltooligosaccharides were still visible showing the derivatization was not complete (Figures 1b and 1c). Repeated experiments involving longer mixing times with procaine or procainamide solution during sample preparation did not significantly change the proportion of underivatized maltooligosaccharides. Similar experiments carried out with α -cyano-4-hydroxycinnamic acid showed lower conversion yields and with 2, 4, 6-trihydroxyacetophenone, no derivatization was observed. Reverse thin layer preparations gave comparable results to our dried droplet preparations, with a slightly higher proportion of underivatized maltooligosaccharides.

The yield of derivatization and the percentage of $[\text{M} + \text{H}]^+$ could be improved by changing the acetic acid (10%) to phosphoric acid (1%). The maltooligosaccharides were then observed only as protonated molecules of procaine and procainamide derivatives (Figure 1d and 1e). The presence of protonated molecules was consistent with the enhanced proton affinity of the derivatives and is one of the objectives of derivatization which is advantageous for MS/MS as we show further.

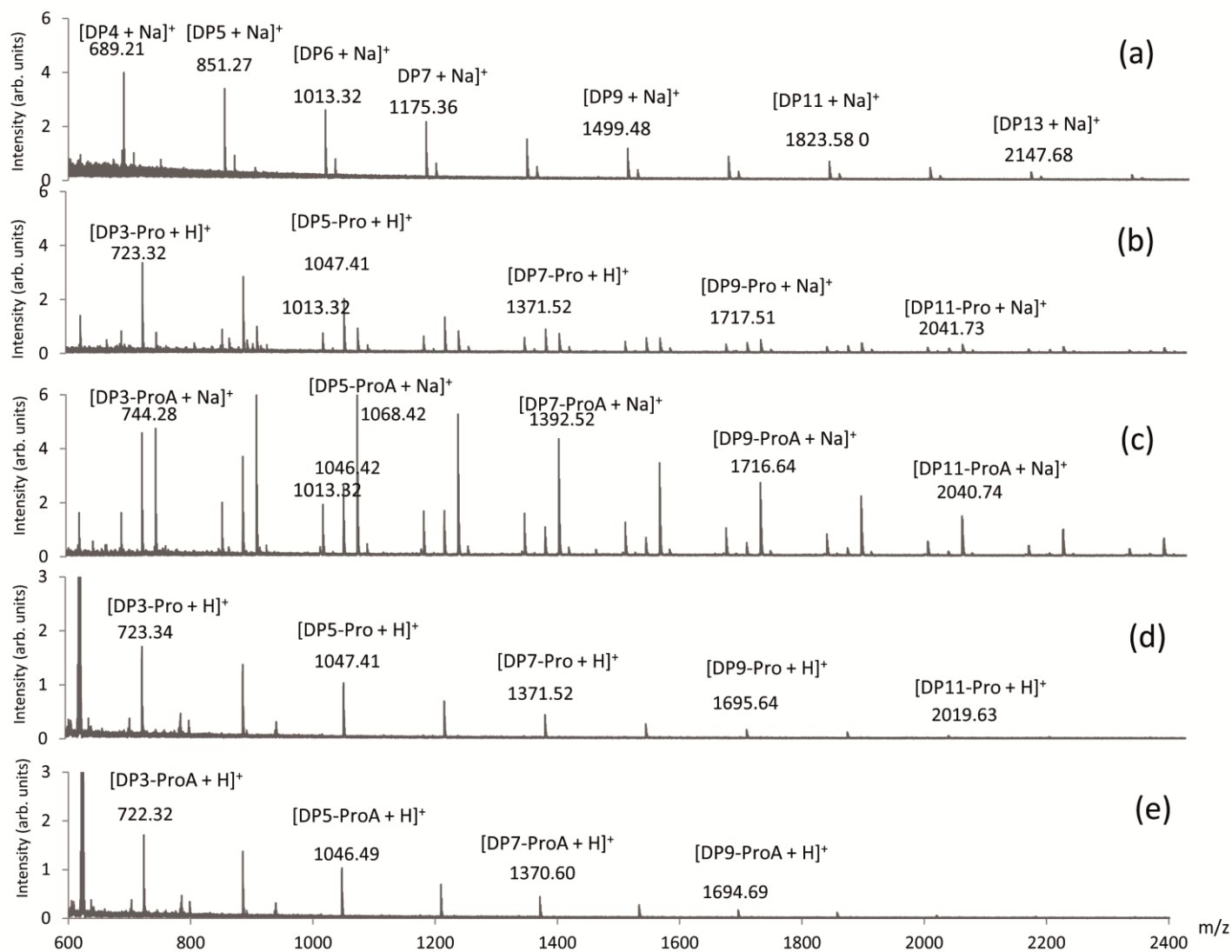


Figure 1. MALDI positive ion mass spectra (reflectron mode) with DHB matrix (a) dextran standard, (b) dextran with procaine (Pro) acidified with 10% acetic acid, (c) dextran with procainamide (ProA) acidified with 10% acetic acid, (d) dextran with procaine (Pro) acidified with 1% phosphoric acid, (e) dextran with procainamide (ProA) acidified with 1% phosphoric acid.

The signal intensities of mass spectra from samples with procaine and procainamide were comparable to that of the oligosaccharides alone with DHB matrix but the signal to noise ratio was improved. This showed that the addition of procainamide or procaine to the matrix did not impede the ionization process. Similar tests were carried out with 2-amino pyridine as derivatizing agent in which no derivatization was observed although the intensity of the sodium adducts of the oligosaccharides appeared slightly enhanced (data not shown). Enhanced signal intensities have been observed by Snovida *et al.* using DHB and aniline^[31]

or DHB and N,N-dimethylaniline^[32] as matrices and co-matrices on glycans and maltooligosaccharides. With aniline derivatives of glycans, the proportion of the Schiff base formed remains limited as the amine appears not to be a sufficiently good nucleophile. Here, the signals from the derivatized oligosaccharides were observed as quite abundant. Noticeably, when spectra were re-acquired several days after the target preparation, signals from the oligosaccharides were observed to diminish while the derivatized oligosaccharides could still be measured with an acceptable signal to noise ratio.

Table 1. Exact m/z values for derivatized and underivatized maltooligosaccharides (DP Degree of polymerization, Pro Procaine, ProA Procainamide)

n	DP _n		DP _n Pro		DP _n ProA	
	[M + H] ⁺	[M + Na] ⁺	[M + H] ⁺	[M + Na] ⁺	[M+H] ⁺	[M + Na] ⁺
3	505.176	527.158	723.318	745.300	722.334	744.316
4	667.229	689.211	885.371	907.353	884.387	906.369
5	829.282	851.264	1047.424	1069.406	1046.440	1068.422
6	991.335	1013.317	1209.477	1231.459	1208.493	1230.475
7	1153.388	1175.370	1371.530	1393.511	1370.546	1392.527
8	1315.440	1337.422	1533.582	1555.564	1532.598	1554.580
9	1477.493	1499.475	1695.635	1717.617	1694.651	1716.633
10	1639.546	1661.528	1857.688	1879.670	1856.704	1878.686
11	1801.599	1823.581	2019.741	2041.723	2018.757	2040.739
12	1963.652	1985.634	2181.794	2203.776	2180.810	2202.792
13	2125.705	2147.687	2343.846	2365.828	2342.862	2364.844
14	2287.757	2309.739	2505.899	2527.881	2504.915	2526.897
15	2449.810	2471.792	2667.952	2689.934	2666.968	2688.950

This is illustrated on figure 2 which shows mass spectra of xyloglucan-derived oligosaccharides. Although the derivatization was not complete immediately after sample drying when the sample was prepared with a 1% phosphoric acid solution of the derivation reactant (Figure 2b), the spectra acquired 24h hours after sample spot preparation showed the $[M + H]^+$ of the derivatives as the most abundant ion (Figure 2c). Peak intensities showed degradation of the native oligosaccharides was likely to account for the diminution of their signals, but the persistence of the signals from the procaine and procainamide labeled molecules confirmed the stability of the derivatives, which may be either a Schiff base or a glycosylamine. It has been shown indeed that unreduced alkylamine derivatives of monosaccharides exist as closed-ring glycosylamines.^[45]

Reductive amination being the usual derivatization performed on oligosaccharides, an attempt at in situ reduction by adding reducing agents borane dimethylamine complex or sodium triacetoxyborohydride was carried out which

resulted in a complete disappearance of all signals from the oligosaccharides.

Maltoheptaose was used to assess the potential benefit of the in-situ derivatization for sensitivity. Figure 3 shows the results obtained with deposits of 2.5 pmol maltoheptaose on the target. The spectra were acquired in the reflectron positive ion mode using an automatic acquisition that involved 50 laser shots on 30 random spots of the target. A total of 1500 spectra were thus accumulated on the condition that a low intensity signal is detected above m/z 1150. In the spectra acquired a few minutes after drying, only modest benefit of the addition of procaine or procainamide could be inferred. Indeed, the intensity of the signal from the sodium adduct of underivatized maltoheptaose when only DHB matrix was used was comparable or slightly higher, but the noise level was lower and consequently the signal to noise ratio was improved when acidified procaine was used with the DHB matrix. The gain in sensitivity, however, could not be considered as significant and attempts at detection of lower amounts remained unsuccessful.

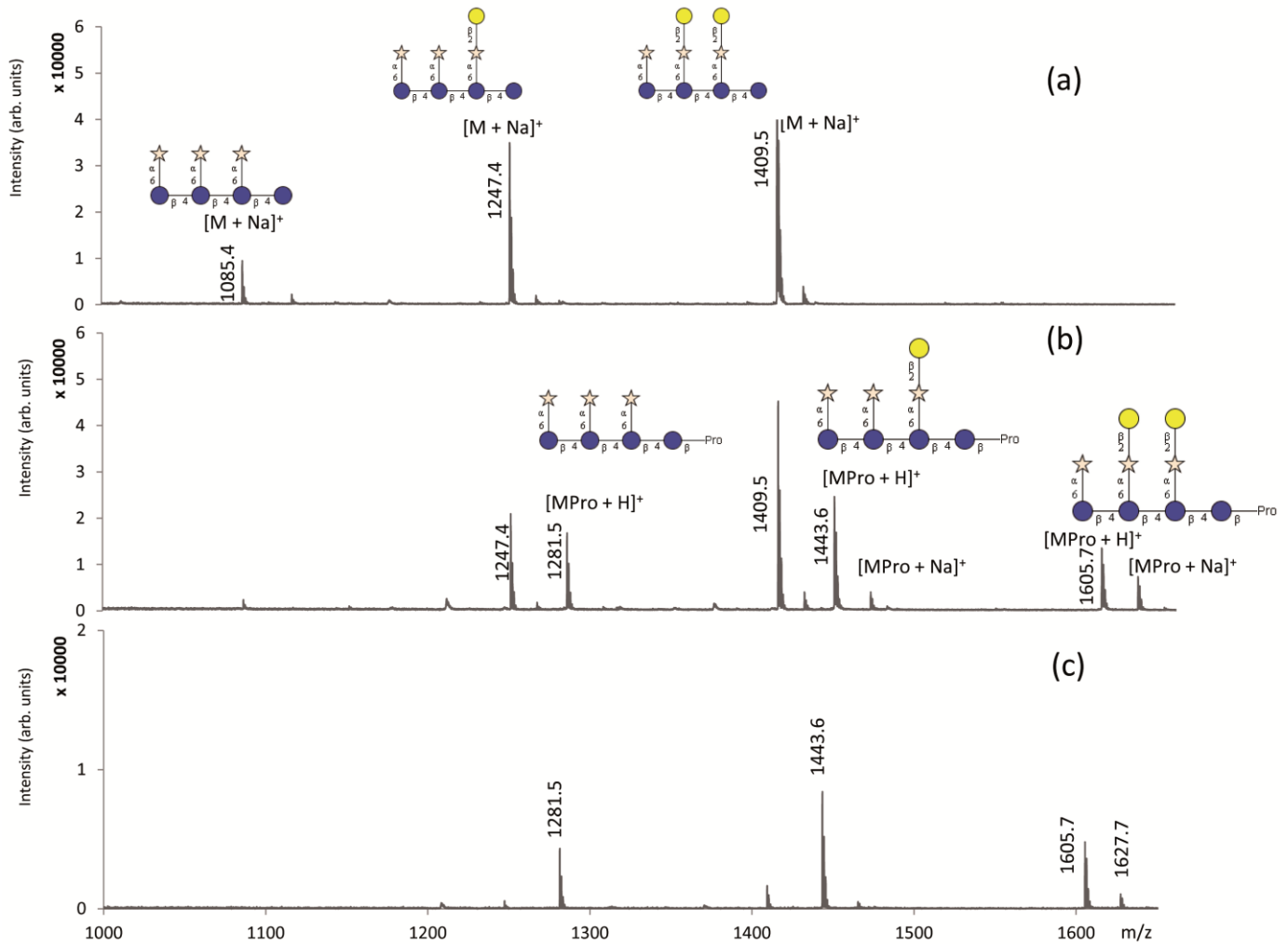


Figure 2. MALDI positive ion mass spectra (reflectron mode) of xyloglucan-derived oligosaccharides (a) with DHB matrix alone, (b) with DHB matrix and acidified procaine (Pro), 1% phosphoric acid immediately after sample drying, (c) with DHB matrix and acidified procaine (Pro), 1% phosphoric acid 24 h after sample drying. (Blue circles ● : glucose, residue mass 162.1; Yellow circles ○ : galactose, residue mass 162.1; stars ☆ : xylose, residue mass : 132.0)

One additional advantage of the procaine and procainamide sample preparation with acetic acid, however, was that, after drying, the deposits were composed of very fine homogeneous crystals contrary to DHB alone which tends to form large inhomogeneous crystals around the rim of the MALDI spot. The acquisition of the mass spectra was therefore facilitated by making unnecessary the search for “sweet spots”: the automated acquisition was much faster and successful than for targets with DHB alone. This was less true when phosphoric acid was used.

In order to determine the structural information

these derivatives could impart, MALDI TOF/TOF experiments, using laser induced dissociation (LID) and the LIFT MS/MS facility, were carried out with precursor ions $[M + H]^+$ and $[M + Na]^+$ of maltooligosaccharides of various degree of polymerization. Figures 4 and 5 show the fragment ion mass spectra for dextran DP7 derivatives with procaine and procainamide. Consistently with what has been observed before in earlier PSD analyses,^[19] and in contrast to results with high energy electrospray MS/MS,^[40] protonated molecules $[M + H]^+$ displayed very easily identifiable fragmentation: the Y type glycosidic cleavages largely predominated (Figure 4a and 5a). These cleavages

whether induced by the charge or resulting from a remote charge mechanism proceed to Y-type fragment ions as the proton is expected to remain on the modified reducing end. Rapid and unambiguous sequence information may be thus deduced from the fragment ion mass spectra. Here, several B ion series were also distinguishable for the procainamide derivative and in the very low m/z region (B_1 m/z 163, B_2 m/z 325 and B_3 m/z 649) for the procaine derivative as well.

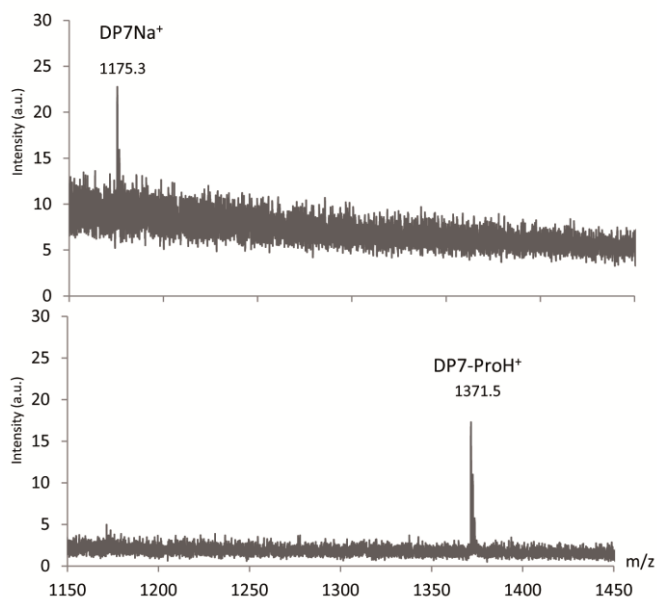


Figure 3. MALDI-TOF mass spectra of 2.5 pmol deposits of maltoheptaose. (a) DHB alone (b) DHB and procaine acidified with 1% phosphoric acid.

For $[M + Na]^+$ precursor ions, sodium ions are not expected to be localized on the derivatized end and logically, both Y and B fragment ion series were observed (Figures 4b and 5b). Sequence ions were much less visible with precursor ions $[M + Na]^+$ than for protonated precursor ions as fragmentation was largely dominated by the loss of 277 u or 278 u which correspond to the loss of the procaine or procainamide label with an addition of two carbon, three hydrogen and one oxygen atoms from the reducing end hexose residue in a $^{0,2}A_7$ type fragmentation (Scheme 1). This very abundant fragment ion at m/z 1115 came together with the corresponding complementary ion observed at m/z 279 for procaine. This suggested that the label may

not remain bonded as a Schiff base (open-chain imino compound) but rather as a glycosylamine. Indeed, in the case of an open chain structure, fragmentation towards ions at m/z 279 would necessitate the unlikely migration of two hydrogen atoms. Furthermore, protonated procaine and procainamide appeared in the fragment ion spectra of all precursor ions at m/z 237 and m/z 236 respectively. These fragment ions concurred to show that a cyclisation probably occurred, the alcohol function reacting with the carbon involved in the imine bond, as is observed on derivatives of D-mannose.^[45] Such cyclisation could account for the stability of these unreduced derivatives. Other sequence ions, here referred to as Y_n^* , were interpreted as Y_n ions minus 277 u (for procainamide) and 278 u (for procaine). In procaine derivatives, an abundant ion could be observed at m/z 100 (Scheme 1). This ion is a characteristic fragment from the procaine tag and has been previously described.^[19] Finally, the ion at m/z 399.12 was thought to derive from residual metastable fragmentation of $[M + H]^+$. Indeed, protonated molecules could not completely be eliminated as shown by the wide peak at m/z 1370.6 (Figure 5b). As expected, the resolving power of ion selection was observed to lower as m/z increased. Therefore, exclusive selection of $[M + H]^+$ or $[M + Na]^+$ was more difficult to achieve for maltooligosaccharides of higher degree of polymerization. Low resolution in the precursor ion selection of $[M + H]^+$ precursor ions did not compromise sequence information, however, as the fragmentation yield was much higher for protonated molecules.

Figure 6 shows the fragment ion mass spectra obtained for one of the xyloglucan-derived oligosaccharides. The monosaccharide residue content could be read directly from the Y ion series fragment observed for the $[M + H]^+$ precursor of the oligosaccharide derivatized by procaine (Figure 6b) or procainamide (Figure 6c), although mass accuracy was lower than for DP7.

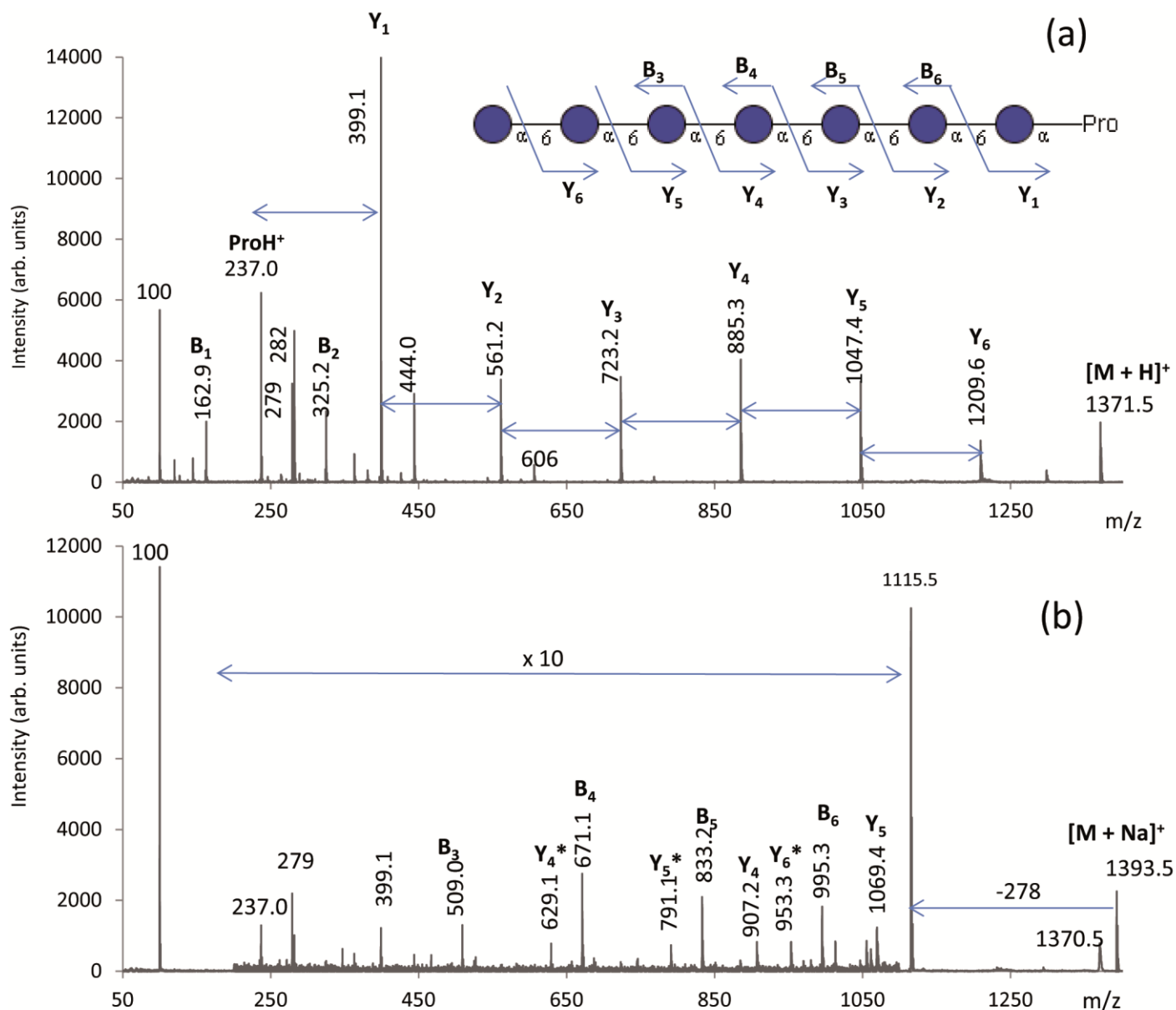


Figure 4. MALDI TOF/TOF mass spectra of procaine derivatized maltoheptaose (a) $[M+H]^+$ precursor ion (m/z 1371.5) (b) $[M+Na]^+$ precursor ion (m/z 1393.5). Y^* ions are Y glycosidic cleavages minus 278 u.

Such interpretation would be more difficult from the $[M+Na]^+$ of the native oligosaccharide (Figure 6a). Indeed, in the derivatized branched oligosaccharide only one Y -type fragment ion was observed for each residue with no overlap from the different branches. Assignment of the fragment ions signals revealed that multiple Y -glycosidic cleavages took place. Ambiguities remained in the attributions due to the similarity of the middle branches (α and β') and isomeric glucose and galactose residues. Most ions could be interpreted with one or two cleavages, the interpretation involving three cleavages are given although

considered as less probable.

Information as to linkage and branching cannot be derived from the labeled molecules alone, complementary data would need to be retrieved from fragment ion mass spectra of other precursor ions. An incomplete derivatization reaction may then be an advantage as then structural information may be further confirmed and completed by the compared analysis of the fragment ions from the sodium adducts of the derivatized and underivatized oligosaccharides also present in the mass spectra.

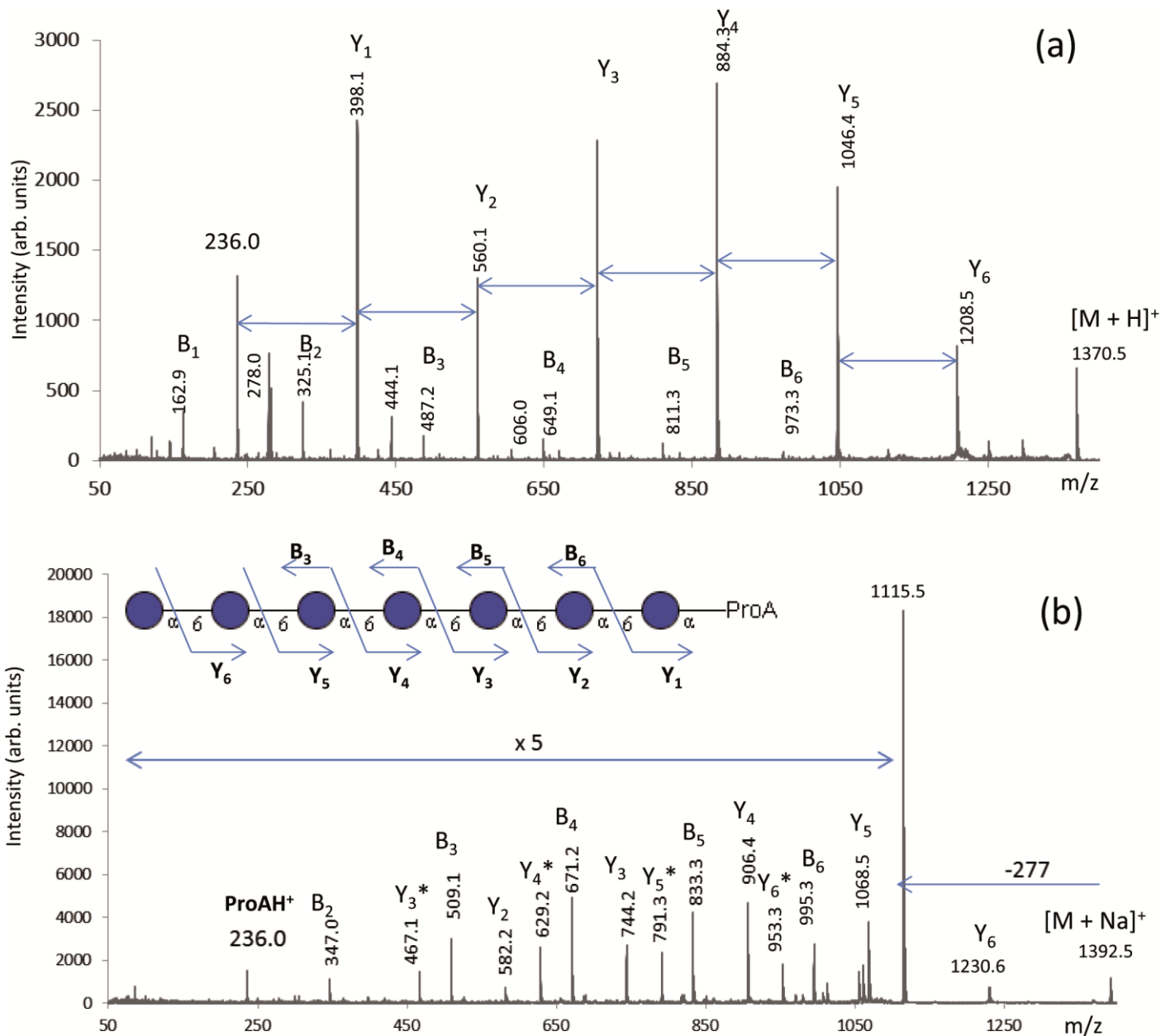
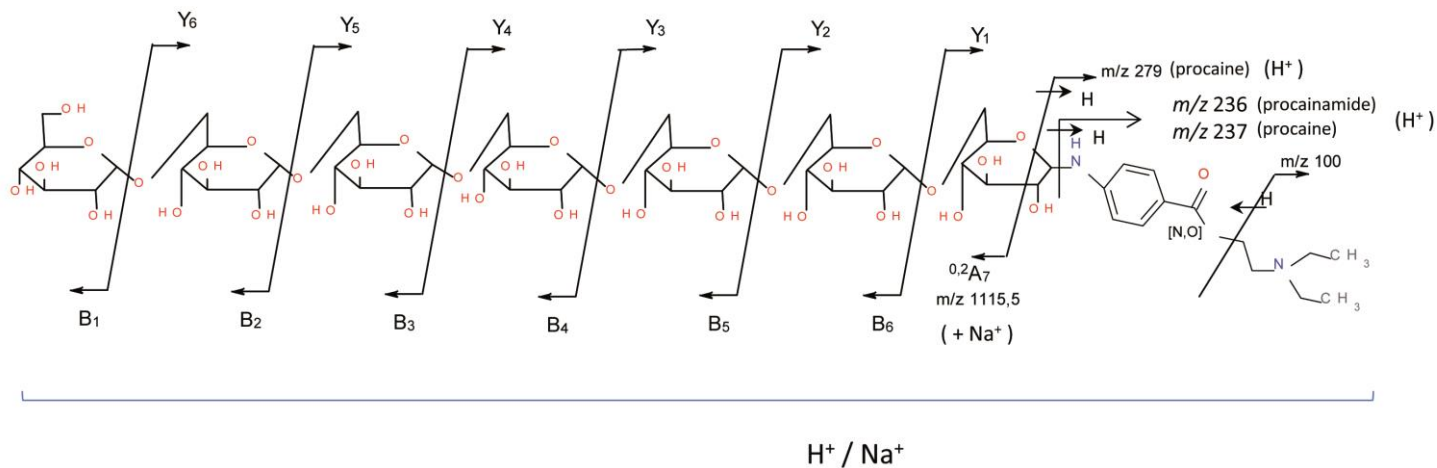


Figure 5. MALDI TOF/TOF mass spectra of procainamide derivatized maltoheptaose (a) $[M+H]^+$ precursor ion (m/z 1370.5) (b) $[M+Na]^+$ precursor ion (m/z 1392.5). Y^* ions are Y glycosidic cleavages minus 277 u.



Scheme 1. Fragmentation of maltoheptaose derivatives.

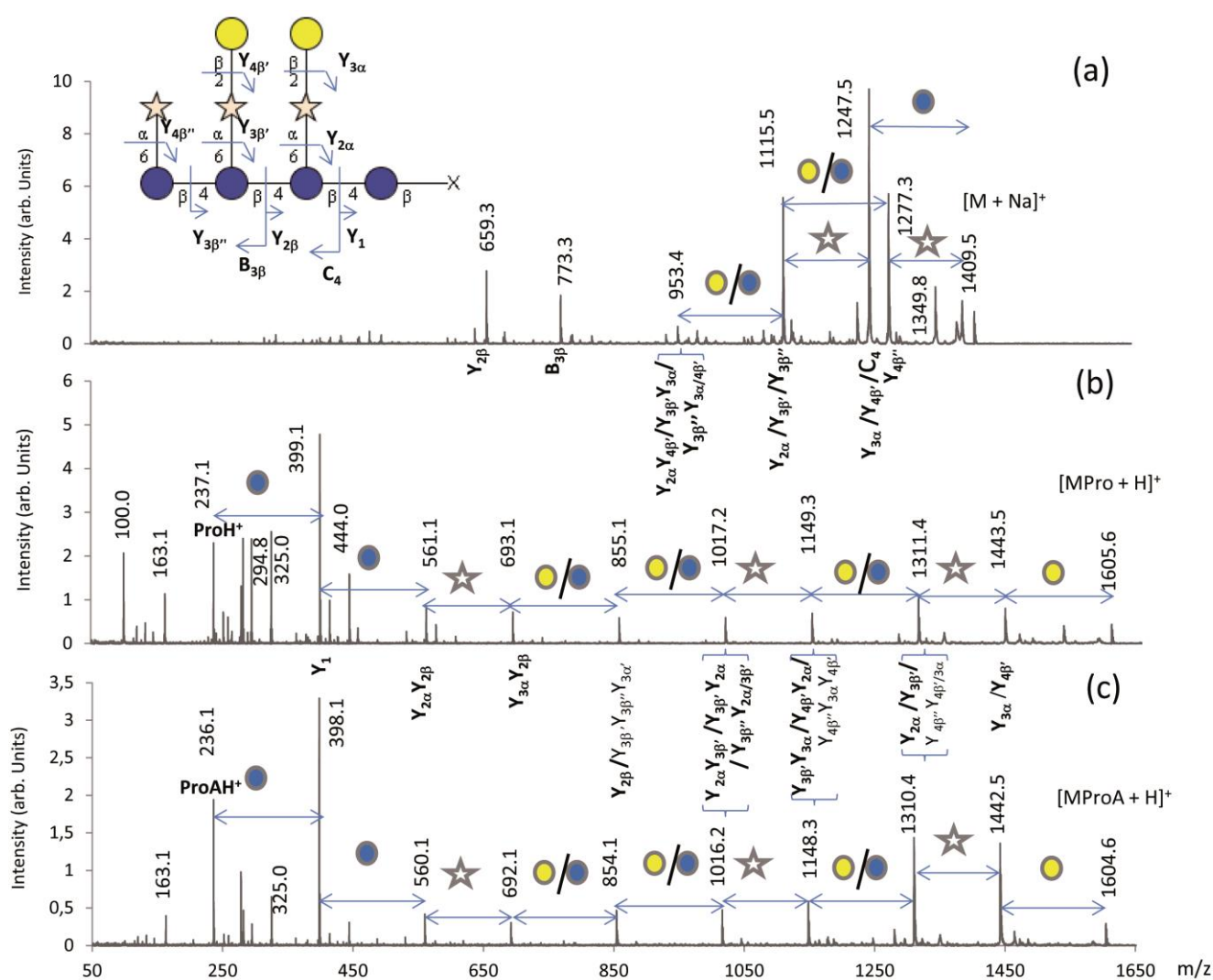


Figure 6. MALDI TOF/TOF mass spectra of $[M+H]^+$ of procaine derivatized xyloglucan-derived oligosaccharide. Most probable assignments of fragment ions signals are given in bold characters. (Blue circles ● : glucose, residue mass 162.1; Yellow circles ○ : galactose, residue mass 162.1; stars ☆ : xylose, residue mass : 132.0).

Here, the presence of $Y_{4\beta''}$ (m/z 1277.3) in the fragment ion mass spectrum of the $[M + Na]^+$ precursor of the native oligosaccharide (Figure 6a) confirmed the presence of a pentose terminal residue, and the complementary $Y_{2\beta}$ (m/z 659.3) and $B_{3\beta}$ (m/z 773.3) attested to the position of an hexose on the α branch. Permethylation,^[46-48] which gives rise to cross ring fragmentations also remains for an in-depth characterization of oligosaccharides.

Conclusions

The addition of acidified procaine or procainamide to solutions of oligosaccharides was found to be advantageous for MALDI analyses as it allowed high

yield conversion of the analyzed carbohydrates to stable derivatives while homogenizing the crystallization on the target. As a result, protonated molecules of procaine-labeled oligosaccharides could be rapidly detected using automatic data acquisition. Although the ease and speed of this sample preparation was one of its benefits, another important one was that it involved easily available chemicals of low toxicity. And while no significant gain in sensitivity could be obtained, the addition of the derivatizing agents allowed the selection of protonated molecules as precursor ions for post source decay analysis using the LIFT MS/MS facility. This should open the possibility of simplifying the

sequence retrieval of complex oligosaccharides, by the predominance of Y type glycosidic cleavages in $[M + H]^+$. Structural information may be further confirmed and completed by the compared analysis of the fragment ions from the sodium adducts of derivatized and underivatized oligosaccharides also present in the mass spectra. In this study, protonated molecules were detected more abundantly from procaine derivatives than with procainamide. The sample preparation was faster with procaine as it was more soluble in water and acetonitrile mixtures. The fragmentation pattern of procainamide-labeled maltooligosaccharides provided higher quality structural information. Indeed, when the protonated molecule of procainamide-labeled maltooligosaccharides were chosen as precursor ions, the fragment ions were more evenly distributed between high and low mass Y series fragments, and when sodium adducts were fragmented, the glycosidic cleavage ions were observed with a better S/N ratio because less dominated by the ions at m/z 1115 and m/z 100. Nevertheless, this sample preparation with either derivatizing agent showed good potential for routine analyses in carbohydrate analysis.

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Abbreviations: Arb. units or a. u., arbitrary units; DHB, 2,5 dihydrobenzoic acid; DP, degree of polymerization; LID, laser induced dissociation; Pro, procaine (4-aminobenzoic acid 2-(diethylamino)ethyl ester); ProA, procainamide (N-(2-diethylamino)ethyl-4-aminobenzamide); TFA,

trifluoroacetic acid.

References

- [1] D. J. Harvey. Analysis of carbohydrates and glycoconjugates by matrix-assisted laser desorption/ionization mass spectrometry: An update covering the period 2001–2002. *Mass Spectrom. Rev.* **2008**, *27*, 125.
- [2] D. J. Harvey. Analysis of carbohydrates and glycoconjugates by matrix-assisted laser desorption/ionization mass spectrometry: An update for 2003-2004. *Mass Spectrom. Rev.* **2009**, *28*, 273.
- [3] D. J. Harvey. Analysis of carbohydrates and glycoconjugates by matrix-assisted laser desorption/ionization mass spectrometry: An update for the period 2005-2006. *Mass Spectrom. Rev.* **2011**, *30*, 1.
- [4] D. J. Harvey. Matrix-assisted laser desorption/ionization mass spectrometry of carbohydrates and glycoconjugates. *Int. J. Mass Spectrom.* **2003**, *226*, 1.
- [5] J. Zaia. Mass spectrometry of oligosaccharides. *Mass Spectrom. Rev.* **2004**, *23*, 161.
- [6] B. Stahl, M. Steup, M. Karas, F. Hillenkamp. Analysis of neutral oligosaccharides by matrix-assisted laser desorption ionization mass spectrometry. *Anal. Chem.* **1991**, *63*, 1463.
- [7] M. T. Cancilla, S. G. Penn, J. A. Carroll, C. B. Lebrilla. Coordination of alkali metals to oligosaccharides dictates fragmentation behavior in matrix assisted laser desorption ionization/fourier transform mass spectrometry. *J. Am. Chem. Soc.* **1996**, *118*, 6736.
- [8] M. Okamoto, K.-I. Takahashi, T. Doi, Y. Takimoto. High-sensitivity detection and postsource decay of 2-aminopyridine-derivatized oligosaccharides with matrix-assisted laser desorption/ionization mass spectrometry. *Anal. Chem.*, **1997**, *69*, 2919.
- [9] Y. Mechref, M. V. Novotny, C. Krishnan. Structural characterization of oligosaccharides using MALDI-TOF/TOF tandem mass spectrometry. *Anal. Chem.* **2003**, *75*, 4895.
- [10] M. Tikarovska. Determination of neutral oligosaccharides in vegetables by matrix-assisted laser desorption/ionization mass spectrometry. *Anal. Chim. Acta*, **2004**, *520*, 47.

- [11] S.-F. Ren, L. Zhang, Z.-H. Cheng, Y.-L. Guo. Immobilized carbon nanotubes as matrix for MALDI-TOF-MS analysis: applications to neutral small carbohydrates. *J. Am. Soc. Mass Spectrom.* **2005**, *16*, 333.
- [12] C.-L. Su, W.-L. Tseng. Gold nanoparticles as assisted matrix for determining neutral small carbohydrates through laser desorption/ionization time-of-flight mass spectrometry. *Anal. Chem.* **2007**, *79*, 1626.
- [13] T. N. Laremore, F. Zhang, R. J. Linhardt. Ionic liquid matrix for direct UV-MALDI-TOF-MS analysis of dermatan sulfate and chondroitin sulfate oligosaccharides. *Anal. Chem.* **2007**, *79*, 1604.
- [14] Y. Fukuyama, S. Nakaya, Y. Yamazaki, K. Tanaka. Ionic liquid matrixes optimized for MALDI-MS of sulfated/sialylated/neutral oligosaccharides and glycopeptides. *Anal. Chem.* **2008**, *80*, 2171.
- [15] J. A. Crank, D. W. Armstrong. Towards a second generation of ionic liquid matrices (ILMs) for MALDI-MS of Peptides, proteins, and carbohydrates. *J Am Soc Mass Spectrom* **2009**, *20*, 1790.
- [16] H. Yang, J. Wang, F. Song, S. Liu, Y. Zhou. Isoliquiritigenin (4, 2', 4'-trihydroxychalcone): a new MALDI matrix with outstanding properties for the analysis of neutral oligosaccharides. *Anal. Chim. Acta* **2010**, *701*, 45.
- [17] Y.-K. Tzeng, Z. Zhu, H.-C. Chang. Alkali-hydroxide-doped matrices for structural characterization of neutral underivatized oligosaccharides by MALDI time-of-flight mass spectrometry. *J. Mass Spectrom.* **2009**, *44*, 375.
- [18] F. Lamari, R. Kuhn, N. Karamanos. Derivatization of carbohydrates for chromatographic, electrophoretic and mass spectrometric structure analysis. *J. of Chrom. B* **2003**, *793*, 15.
- [19] W. Mo, T. Takao, H. Sakamoto, Y. Shimonishi. Structural analysis of oligosaccharides derivatized with 4-aminobenzoic acid 2-(diethylamino) ethyl ester by matrix-assisted laser desorption/ionization mass spectrometry. *Anal. Chem.* **1998**, *70*, 4520.
- [20] X. Liu, G. Zhang, K. Chan, J. Li. Microwave-assisted Kochetkov amination followed by permanent charge derivatization: a facile strategy for glycomics. *Chem. Commun.* **2010**, *46*, 7424.
- [21] S. Broberg, A. Broberg, J. Ø. Duus. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry of oligosaccharides derivatized by reductive amination and N,N-dimethylation. *Rapid Commun. Mass Spectrom.* **2000**, *14*, 1801.
- [22] J. Lemoine, M. Cabanes-Macheteau, M. Bardor, J.-C. Michalski, L. Faye, P. Lerouge. Analysis of 8-aminonaphthalene-1,3,6-trisulfonic acid labelled N-glycans by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry. *Rapid Commun. Mass Spectrom.* **2000**, *14*, 100.
- [23] M. Pabst, D. Kolarich, G. Pörtl, T. Dalik, G. Lubec, A. Hofinger, F. Altmann. Comparison of fluorescent labels for oligosaccharides and introduction of a new postlabeling purification method. *Anal. Biochem.* **2009**, *384*, 263.
- [24] A. H. Franz, T. F. Molinski, C. B. Lebrilla. MALDI-FTMS characterization of oligosaccharides labeled with 9-aminofluorene. *J Am Soc Mass Spectrom* **2001**, *12*, 1254.
- [25] J. Hsu, S. J. Chang, A. H. Franz. MALDI-TOF and ESI-MS analysis of oligosaccharides labeled with a new multifunctional oligosaccharide tag. *J Am Soc Mass Spectrom* **2006**, *17*, 194.
- [26] A. Kameyama, Y. Kaneda, H. Yamanaka, H. Yoshimine, H. Narimatsu, Shinohara, Y. Detection of Oligosaccharides Labeled with Cyanine Dyes Using Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry. *Anal. Chem.* **2004**, *76*, 4537.
- [27] T. J. P. Naven, D. J. Harvey. Cationic Derivatization of Oligosaccharides with Girard's T Reagent for Improved Performance in Matrix-assisted Laser Desorption/Ionization and Electrospray Mass Spectrometry. *Rapid Commun. Mass Spectrom.* **1996**, *10*, 829.
- [28] K. Yamada, S. Hyodo, M. Kinoshita, T. Hayakawa, K. Takechi. Hyphenated Technique for Releasing and MALDI MS Analysis of O-Glycans in Mucin-Type Glycoprotein Samples. *Anal. Chem.* **2010**, *82*, 7436.
- [29] Y. Kim, G. B. Hurst, M. J. Doktycz, M. V. Buchanan. Improving Spot Homogeneity by Using Polymer Substrates in Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry of Oligonucleotides. *Anal. Chem.* **2001**, *73*, 2617.
- [30] R. W. Garden, J. V. Sweedler. Heterogeneity within MALDI Samples As Revealed by Mass Spectrometric Imaging. *Anal. Chem.* **2000**, *72*, 30.
- [31] S. I. Snovidá, V. C. Chen, H. Perreault. Use of a 2,5-Dihydroxybenzoic Acid/Aniline MALDI Matrix for

- Improved Detection and On-Target Derivatization of Glycans: A Preliminary Report. *Anal. Chem.* **2006**, *78*, 8561.
- [32] S. I. Snovida, H. Perreault. A 2,5-dihydroxybenzoic acid/N,N-dimethylaniline matrix for the analysis of oligosaccharides by matrix-assisted laser desorption/ionization mass spectrometry. *Rapid Commun. Mass Spectrom.* **2007**, *21*, 3711.
- [33] E. Lattová, S. Snovida, H. Perreault, O. Krokhin. Influence of the labeling group on ionization and fragmentation of carbohydrates in mass spectrometry. *J Am Soc Mass Spectrom* **2005**, *16*, 683.
- [34] B. Domon, C. E. Costello. A systematic nomenclature for carbohydrate fragmentations in FAB-MS/MS spectra of glycoconjugates. *Glycoconjugate J* **1988**, *5*, 397.
- [35] E. Lattová, V. C. Chen, S. Varma, T. Bezabeh, H. Perreault. Matrix-assisted laser desorption/ionization on-target method for the investigation of oligosaccharides and glycosylation sites in glycopeptides and glycoproteins. *Rapid Commun. Mass Spectrom.* **2007**, *21*, 1644.
- [36] Y. Zhang, T. Iwamoto, G. Radke, Y. Kariya, K. Suzuki, A. H. Conrad, J. M. Tomich, G. W. Conrad. On-target derivatization of keratan sulfate oligosaccharides with pyrenebutyric acid hydrazide for MALDI-TOF/TOF-MS. *J. Mass Spectrom.* **2008**, *43*, 765.
- [37] M. Rohmer, B. Meyer, M. Mank, B. Stahl, U. Bahr, M. Karas. 3-Aminoquinoline Acting as Matrix and Derivatizing Agent for MALDI MS Analysis of Oligosaccharides. *Anal. Chem.* **2010**, *82*, 3719.
- [38] K. Kaneshiro, Y. Fukuyama, S. Iwamoto, S. Sekiya, K. Tanaka. Highly Sensitive MALDI Analyses of Glycans by a New Aminoquinoline-Labeling Method Using 3-Aminoquinoline/ α -Cyano-4-hydroxycinnamic Acid Liquid Matrix. *Anal. Chem.* **2011**, *83*, 3663.
- [39] M. Rohmer, D. Baeumlisberger, B. Stahl, U. Bahr, M. Karas. Fragmentation of neutral oligosaccharides using the MALDI LTQ Orbitrap. *Int. J. of Mass Spectrom.* **2011**, *305*, 199.
- [40] K.-I. Yoshino, T. Takao, H. Murata, Y. Shimonishi. Use of the derivatizing agent, 4-aminobenzoic acid 2-(diethylamino)ethyl ester, for high-sensitivity detection of oligosaccharides by electrospray ionization mass spectrometry. *Anal. Chem.* **1995**, *67*, 4028.
- [41] D. J. Harvey. N-(2-Diethylamino)ethyl-4-aminobenzamide derivative for high sensitivity mass spectrometric detection and structure determination of N-linked carbohydrates. *Rapid Commun. Mass Spectrom.* **2000**, *14*, 862.
- [42] F. Dardelle, A. Lehner, Y. Ramdani, M. Bardor, P. Lerouge, A. Driouich, J.-C. Mollet. Biochemical and Immunocytological Characterizations of Arabidopsis Pollen Tube Cell Wall. *Plant Physiol.* **2010**, *153*, 1563.
- [43] T. Nishikaze, J. Amano. Reverse thin layer method for enhanced ion yield of oligosaccharides in matrix-assisted laser desorption/ionization. *Rapid Commun. Mass Spectrom.* **2009**, *23*, 3787.
- [44] A. Ceroni, K. Maass, H. Geyer, R. Geyer, A. Dell, S.M. Haslam. *J. Proteome Res.* **2008**, *7*, 1650.
- [45] W. H. Ojala, J. M. Ostman, C. R. Ojala. Schiff bases or glycosylamines: crystal and molecular structures of four derivatives of D-mannose. *Carbohydr. Res.* **2000**, *326*, 104.
- [46] C. Zhao, B. Xie, S.-Y. Chan, C. E. Costello, P. B. O'Connor. Collisionally activated dissociation and electron capture dissociation provide complementary structural information for branched permethylated oligosaccharides. *J Am Soc Mass Spectrom* **2008**, *19*, 138.
- [47] I. Ciucanu, F. Kerek. A simple and rapid method for the permethylation of carbohydrates. *Carbohydr. Res.* **1984**, *131*, 209.
- [48] M. Wührer, A. M. Deelder. Matrix-assisted laser desorption/ionization in-source decay combined with tandem time-of-flight mass spectrometry of permethylated oligosaccharides: targeted characterization of specific parts of the glycan structure. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 943.