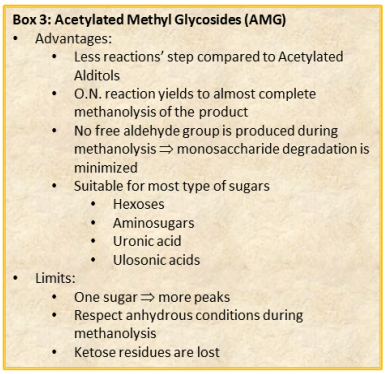

Acetylated Methyl Glycosides: Advantages & Limitations

Description

Box 3: Acetylated Methyl Glycosides (AMG)

- Advantages:
  - Less reactions' step compared to Acetylated Alditols
  - O.N. reaction yields to almost complete methanolysis of the product
  - No free aldehyde group is produced during methanolysis ⇒ monosaccharide degradation is minimized
  - Suitable for most type of sugars
    - Hexoses
    - Aminosugars
    - Uronic acid
    - Ulosonic acids
- Limits:
  - One sugar ⇒ more peaks
  - Respect anhydrous conditions during methanolysis
  - Ketose residues are lost

The chemical approach to produce these derivatives follows the scheme shown below shown, and experimental details can be found elsewhere [link to “Further Readings”].

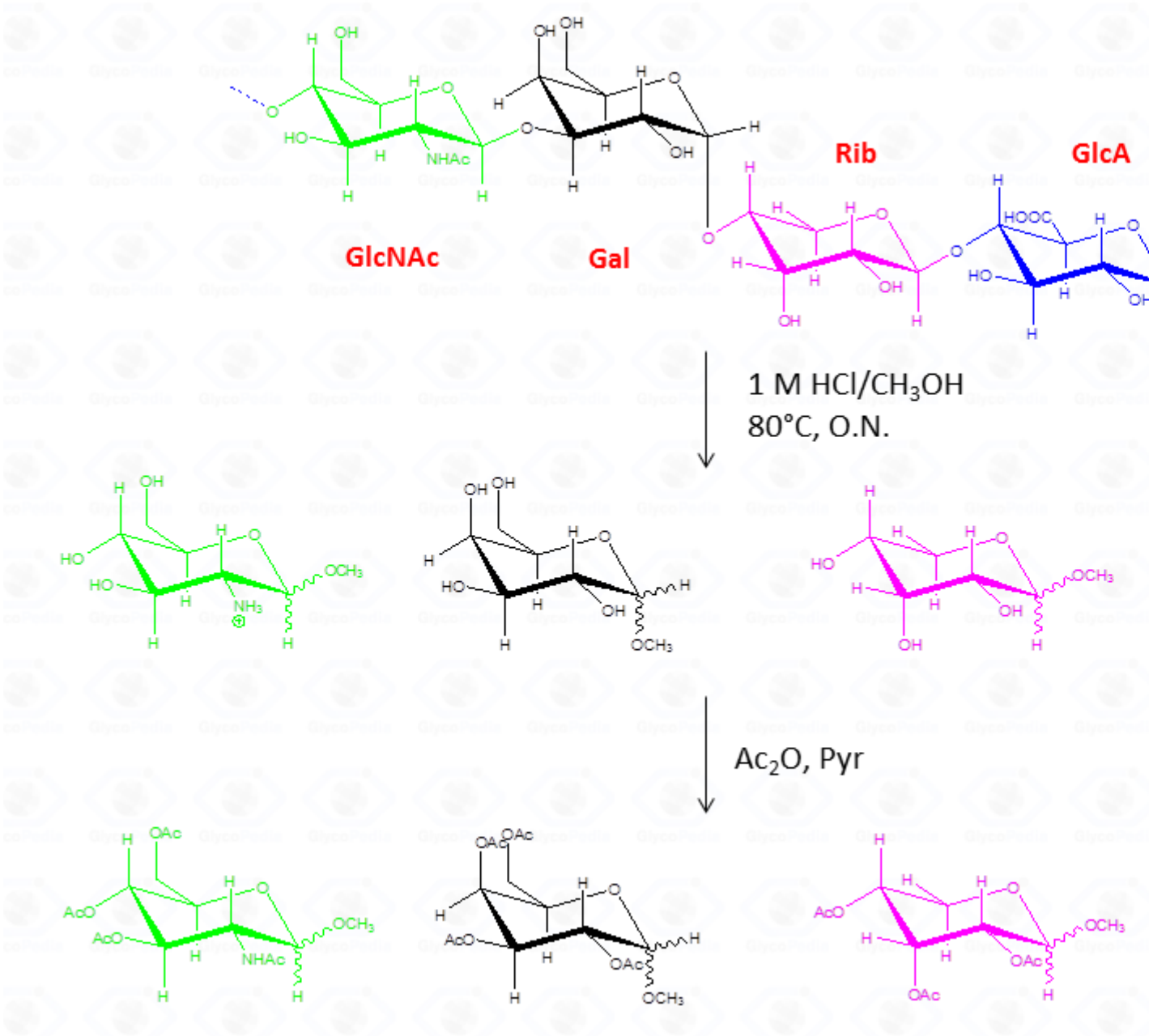

Figure 10: Scheme of the reactions occurring during transformation of a glycan in the corresponding Acetylated Methyl Glycosides (AMG)

Whereas this procedure is suitable for the detection of almost all type of monosaccharides (neutral or basic aldoses, uronic and ulosonic acids), it fails in the detection of ketoses, as fructose. This approach differs from that of the acetylated alditols for many aspects because monosaccharides never expose their reducing end so that the parasite reactions of the aldehyde group do not occur. All this translates in an increase in the recovery yield of each component.

By this reaction mechanism each sugar produces a mixture of different glycosides, which differ for the configuration at the anomeric center (? and ?) as well as for the ring size (pyranic or furanic).

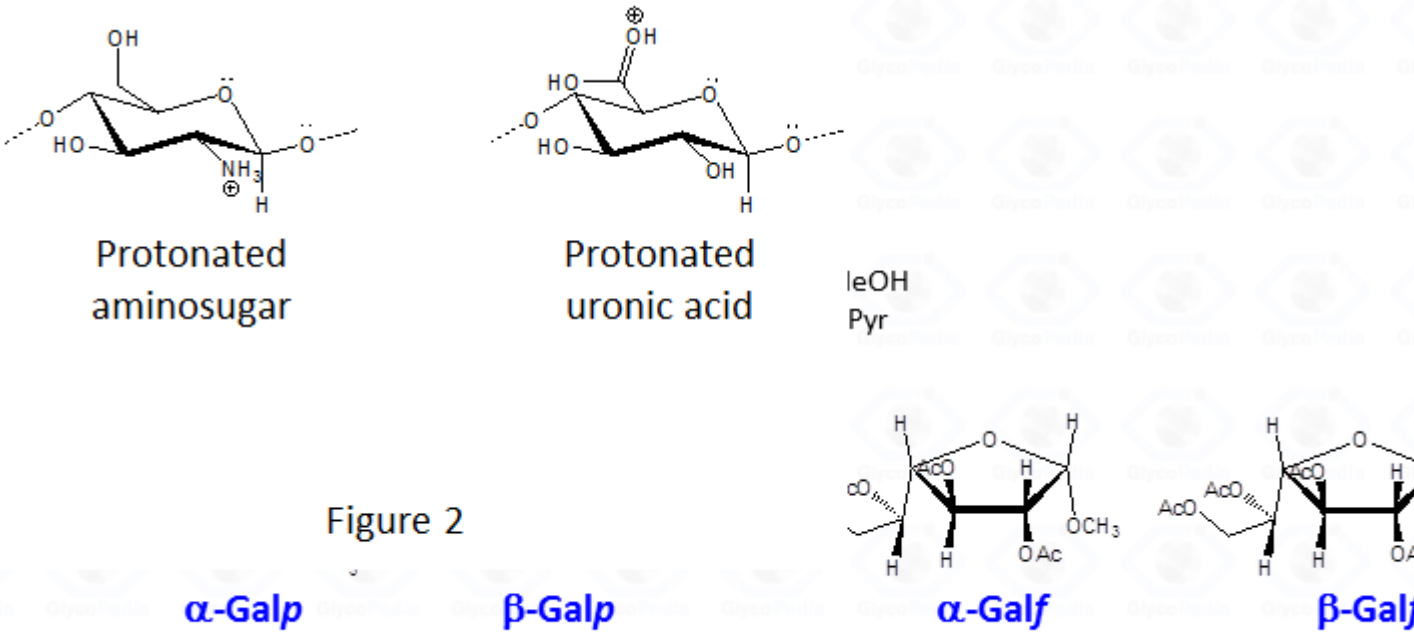

Figure 2

Figure 11: Possible isomers formed by galactose upon methanolysis treatment.

In the case of uronic acids intramolecular lactones may be produced.

With regard to the analysis of EI-MS spectra, is that, in common to acetylated alditols, different isomers have the same fragmentation pattern and it is not possible to discriminate among them on the basis of the spectrum.

Box 4: AMG fragmentation rules

- The most stable ions will be observed in the EI-MS spectrum
- Isomeric sugars (as Glc and Gal) give the same EI-MS spectrum
- The radical cation of the methylglycosides undergoes several pathways:
  - A, B, C, D, E, F, H, J, and K (example given for an hexose)
  - Fragments gives a series of daughter ions by loss of neutral molecules (AcOH, Ac_2O, $AcO^{\bullet}$, $Ac^{\bullet}$, $CH_2=C=O$)
- Occurrence of acetamido, deoxy, methylester groups, may direct the fragmentation pathway to a preferential route.
- Along with the ions from the fragmentation pathways, triacetoxonium (*m/z* 145) and diacetoxonium (*m/z* 103) ions maybe observed.

Different isomers must be discriminated by their retention time, which in turn depends on the type of GC-column used as well as on the experimental set up of the chromatographic apparatus of the instrument.

In general, the spectra from peracetylated methylglycosides do not contain the signal of the molecular ion. It can be indirectly deduced either by the occurrence of the primary ion formed (loss of acetoxyl radical (m/z 59)) or by the weak signals of two different oxonium ions which occur throughout the A and E pathways.

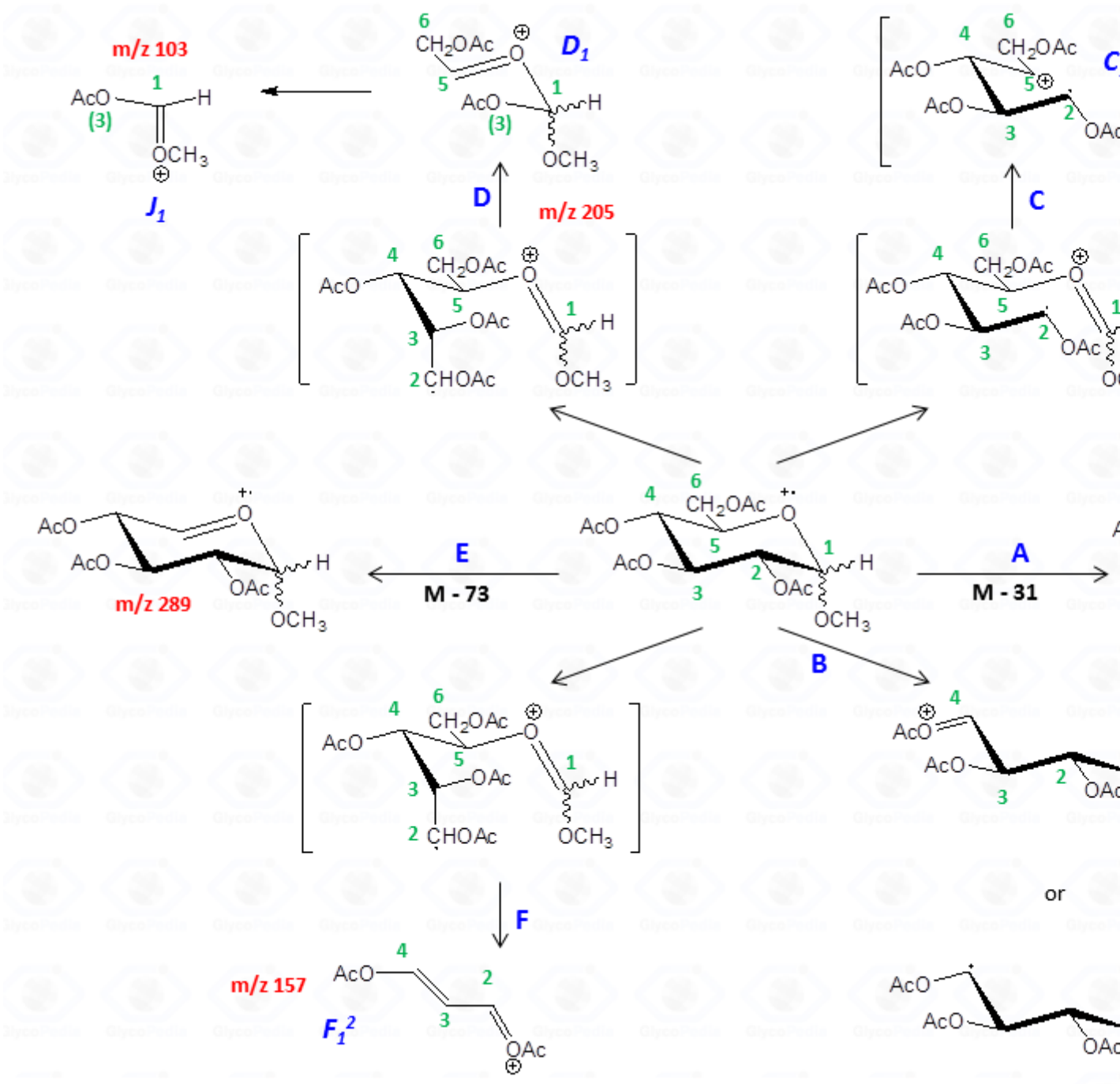

Figure 12a: Fragmentation pathway of a fully acetylated methylhexopyranose

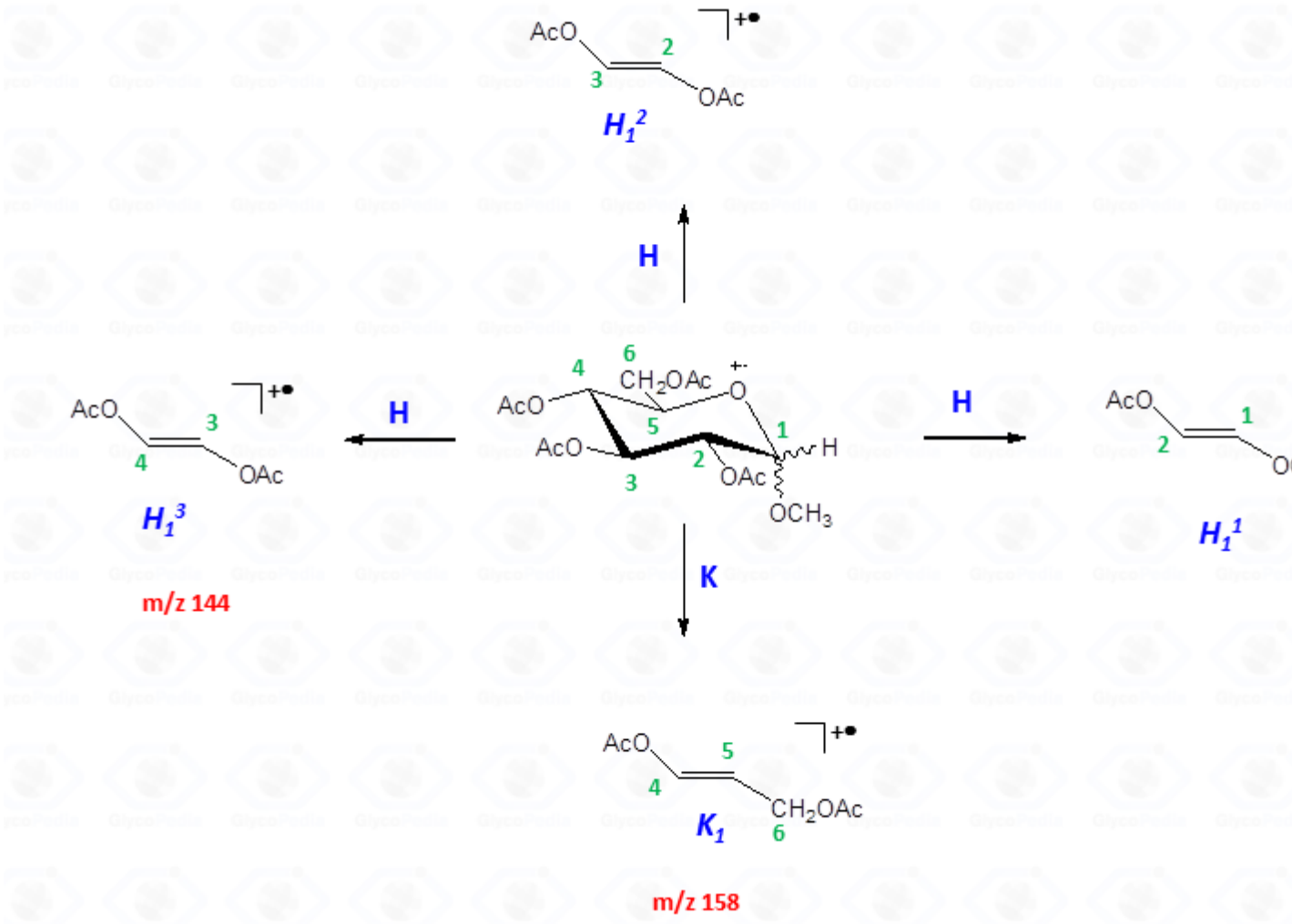

Figure 12b: Fragmentation pathway of a fully acetylated methylhexopyranose

These fragments can further loose neutral moiecules, such as acetic acid (CH_3COOH, *m/z* 60), acetic anhydride (Ac_2O, *m/z* 102) or chetene (CH_2CO, *m/z* 42)

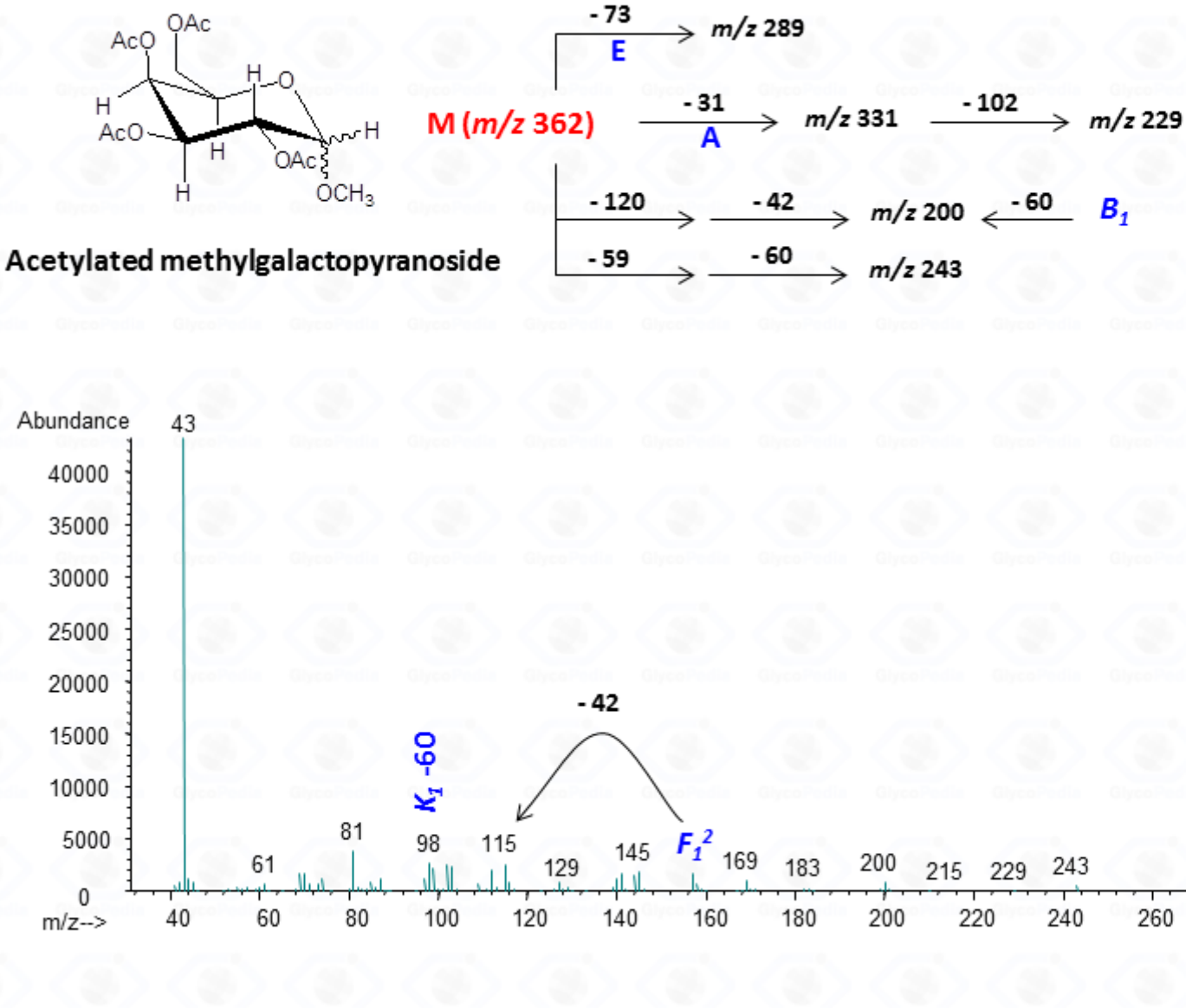

Figure 13: EI-MS spectrum of a fully acetylated methylhexopyranose.

The other fragmentation pathways (see Figs 12a and 12b) yield signals in the low mass range of the spectrum that are less useful to deduce the structure of the residue.

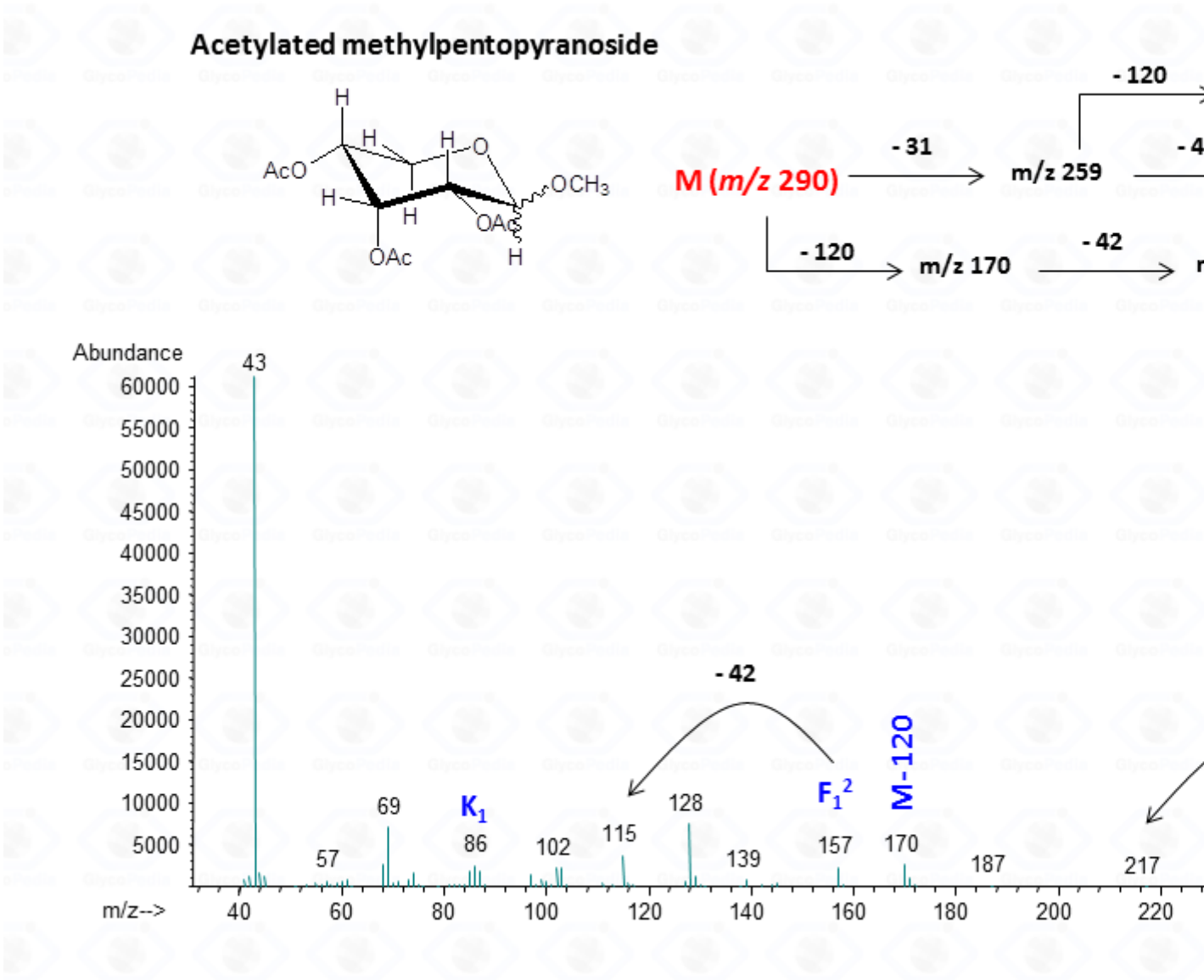

Figure 14: EI-MS spectrum of a fully acetylated methylpentopyranose

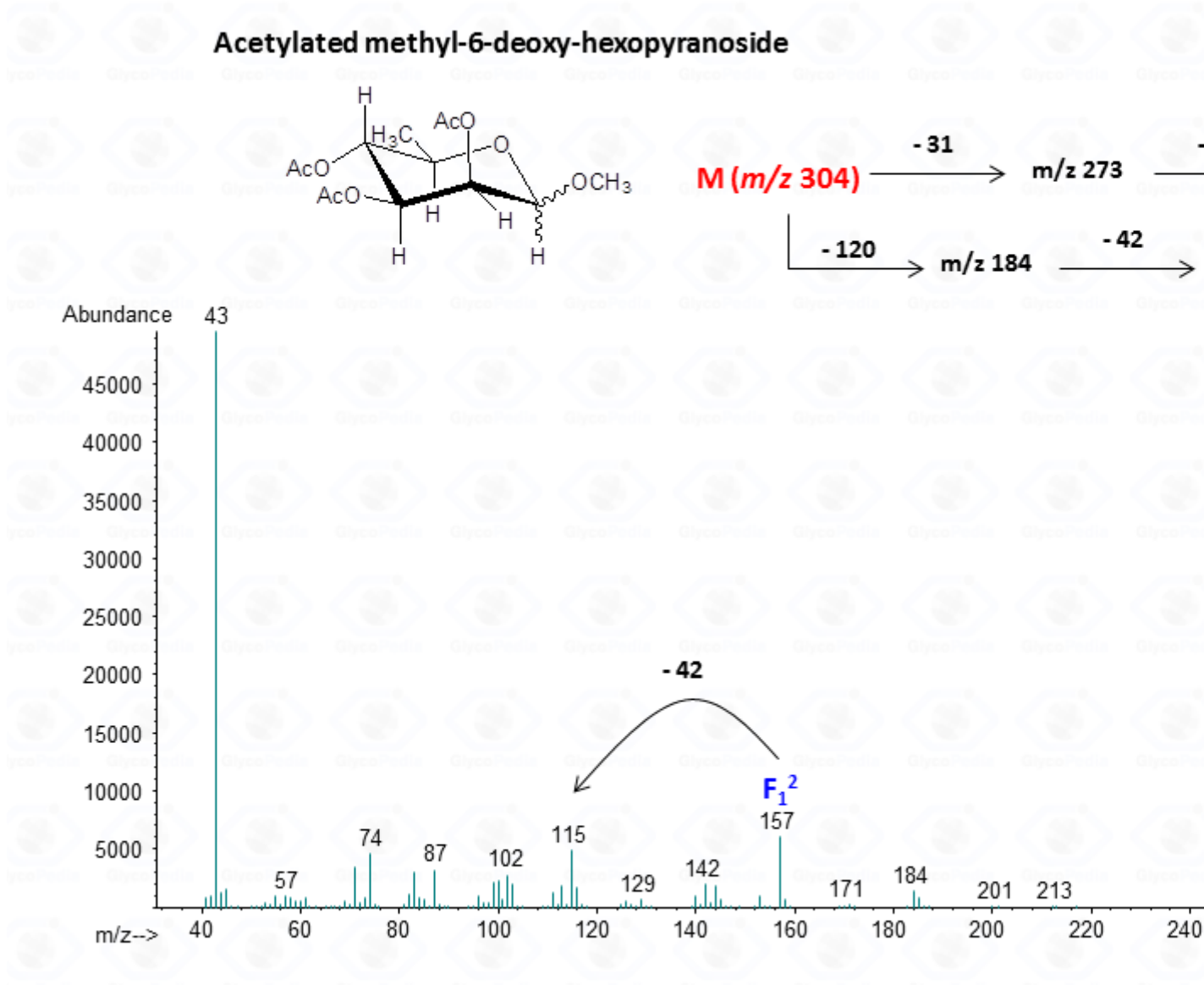

Figure 15: EI-MS spectrum of a fully acetylated methyl-6-deoxy-hexopyranose

The acetylated methyl glycosides method allows detection of acidid monosaccharides. Indeed, uronic acids can be identified from their distinctive fragmentation pattern.
For the pyranose form

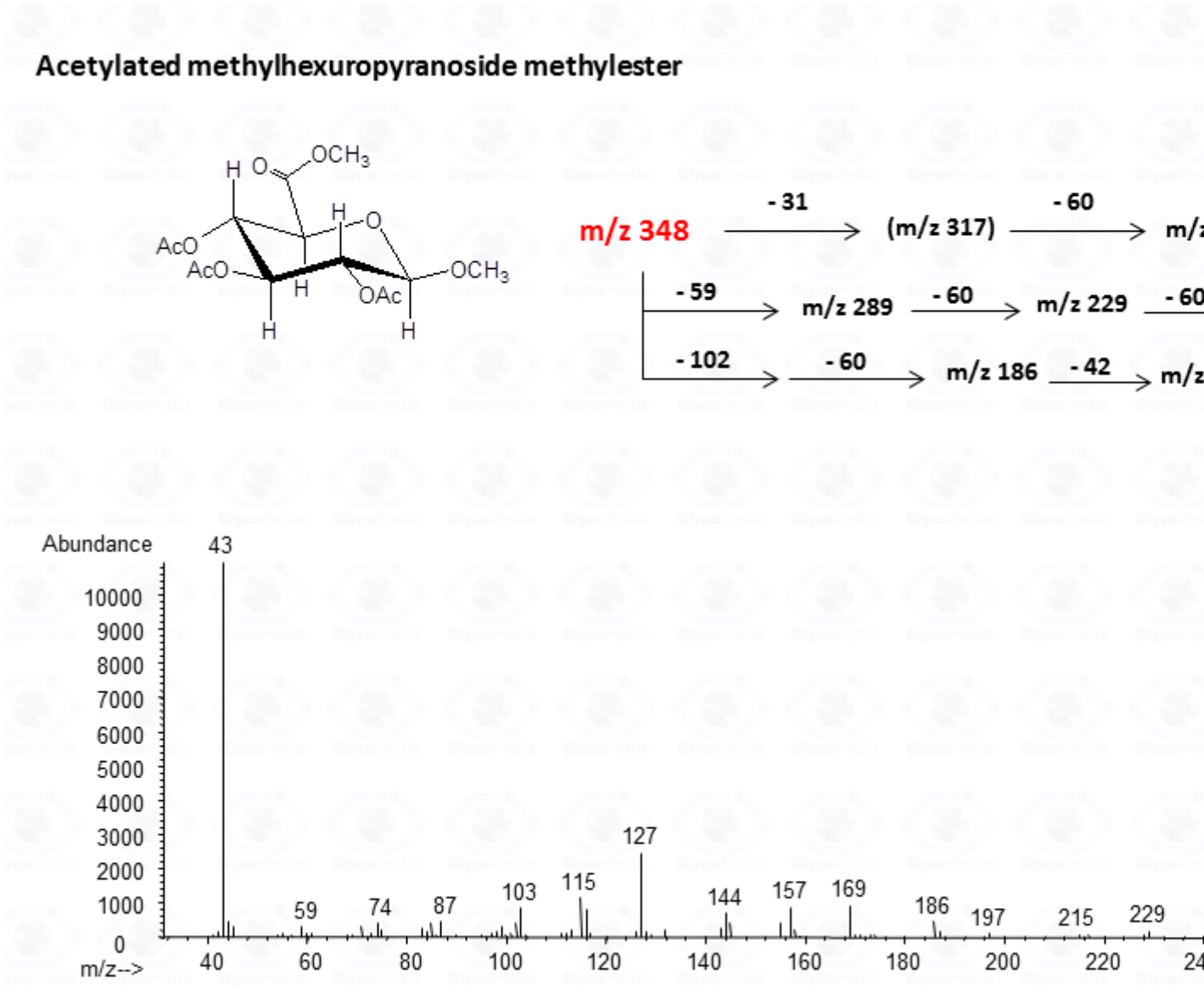

Figure 16: EI-MS spectrum of a fully acetylated methylglycoside from glucuronic acid

For the intramolecular lactone

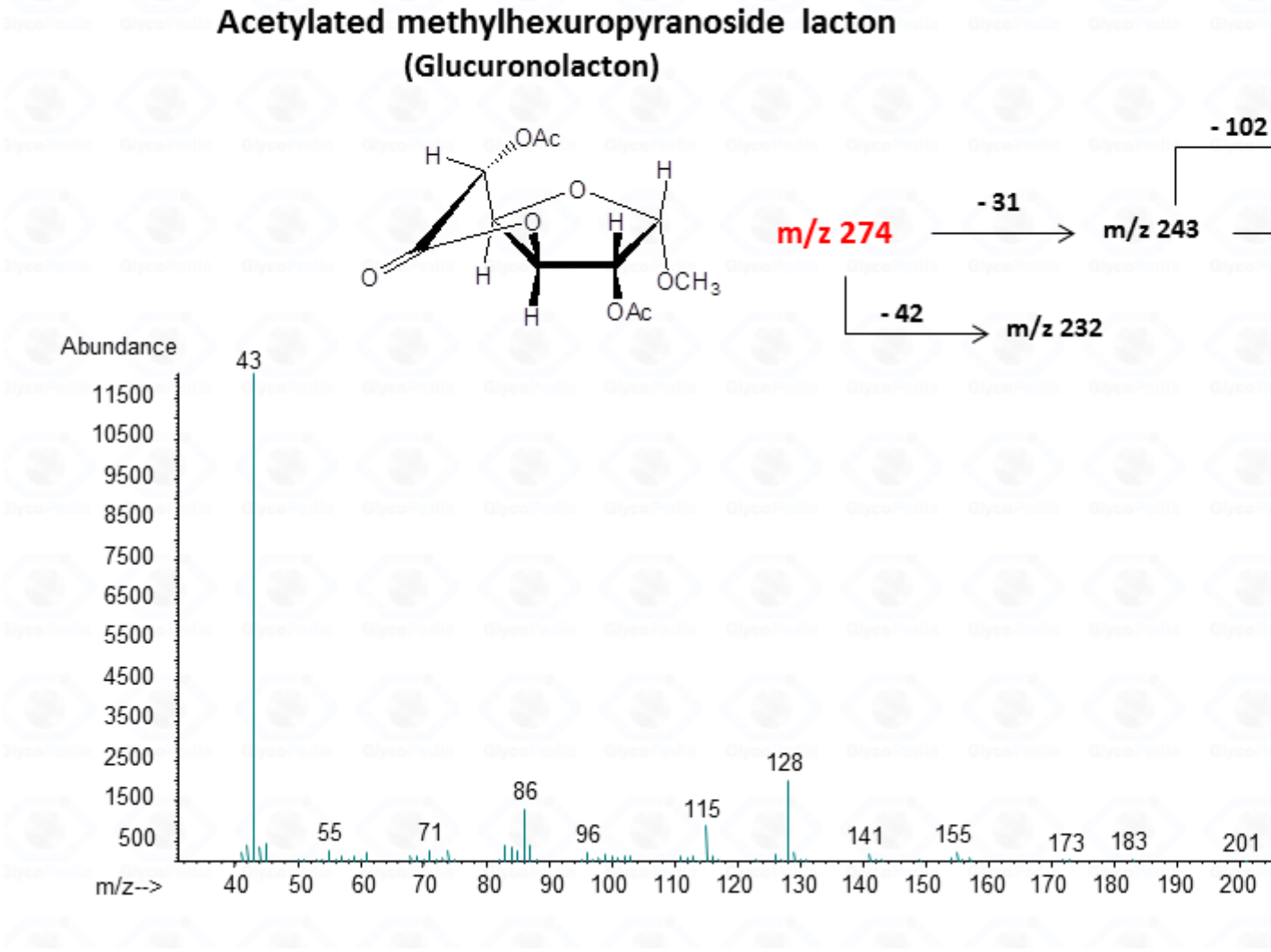

Figure 17: EI-MS spectrum of a fully acetylated methylglycoside of the internal lacton from glucuronic acid.

For ulosonic acids, as Kdo

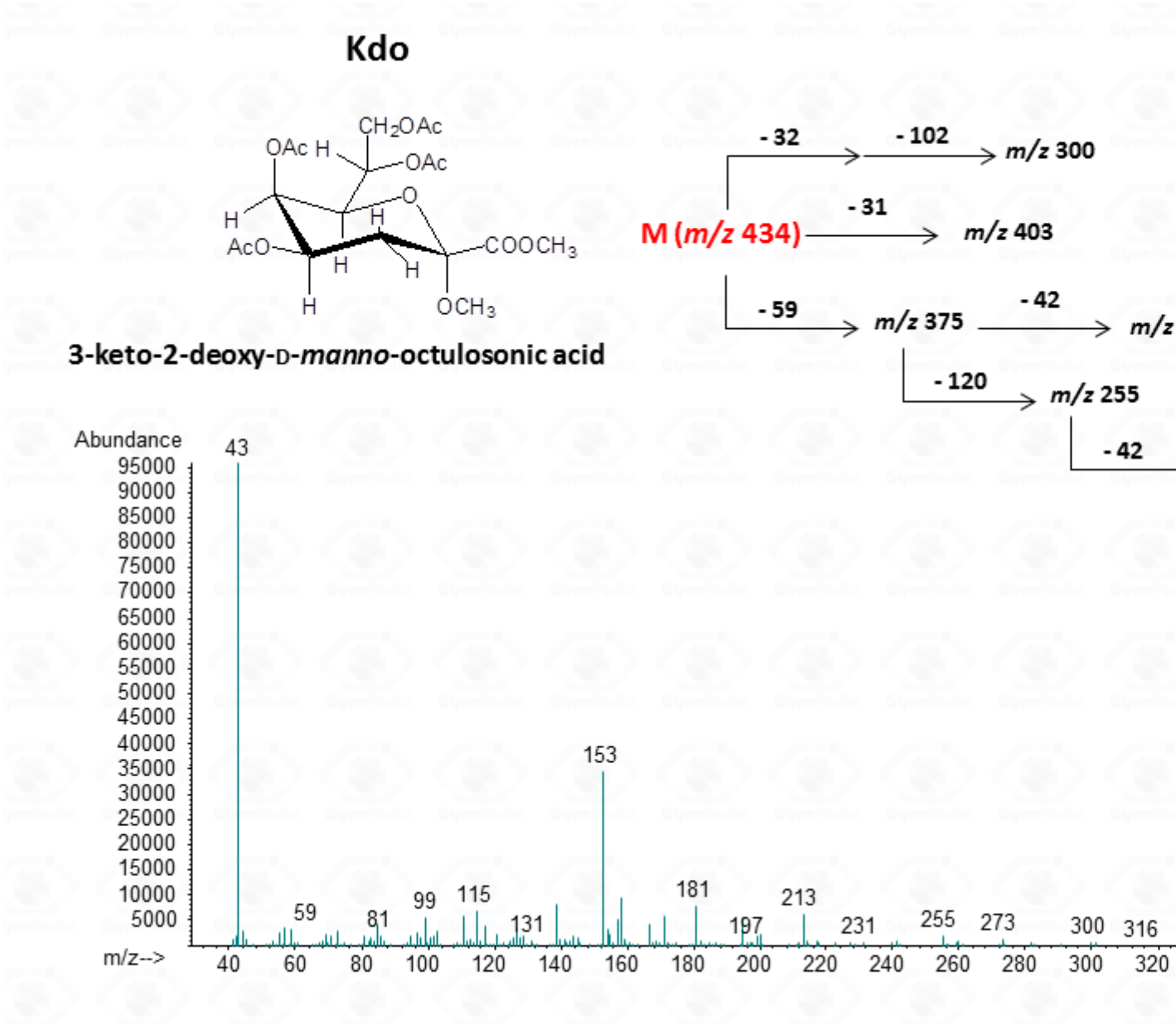

Figure 18: EI-MS spectrum of a fully acetylated methylglycoside methylester of Kdo.

For sialic acid,

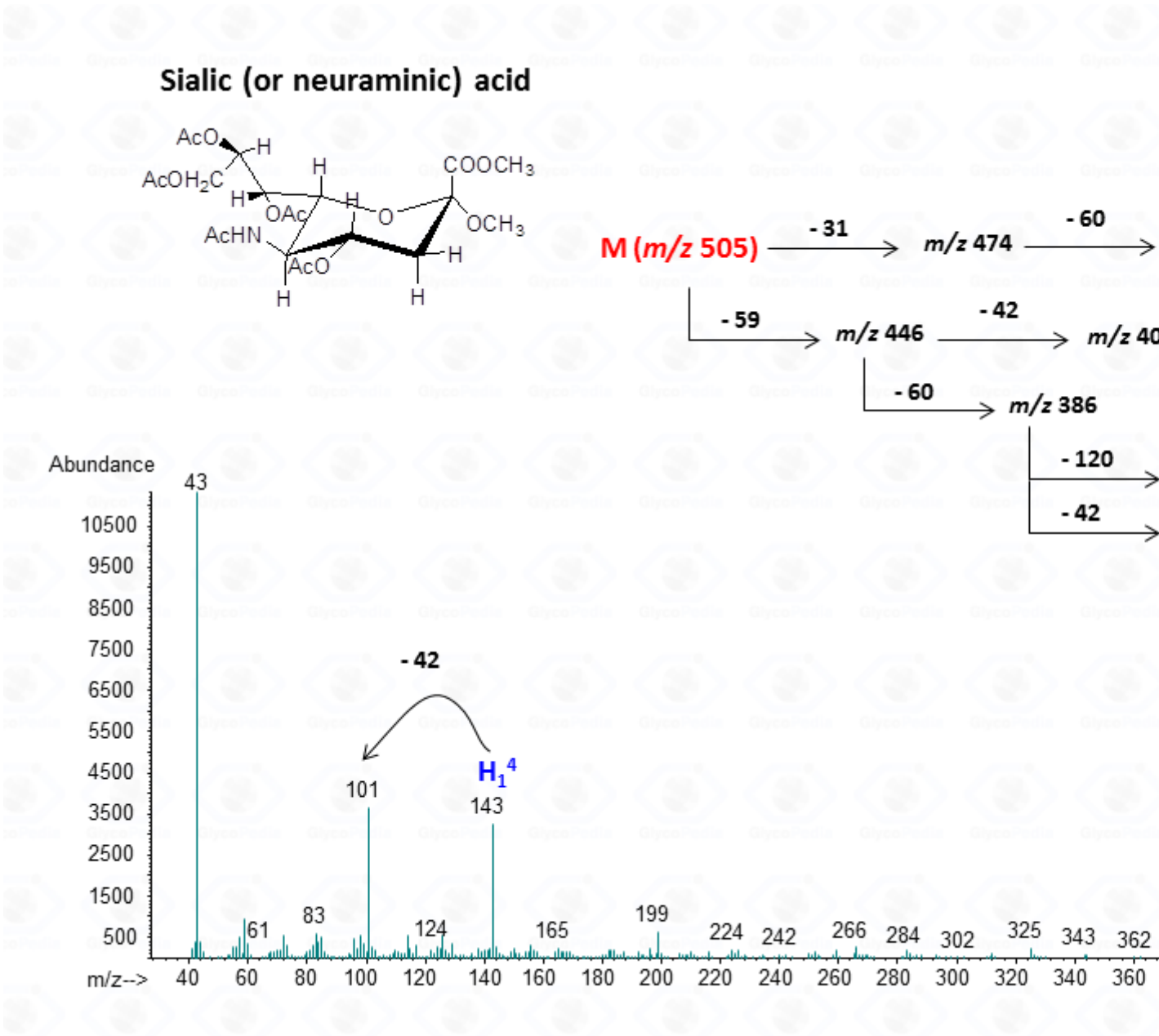

Figure 19: EI-MS spectrum of a fully acetylated methylglycoside methylester of Sialic acid

For legionaminic acid)

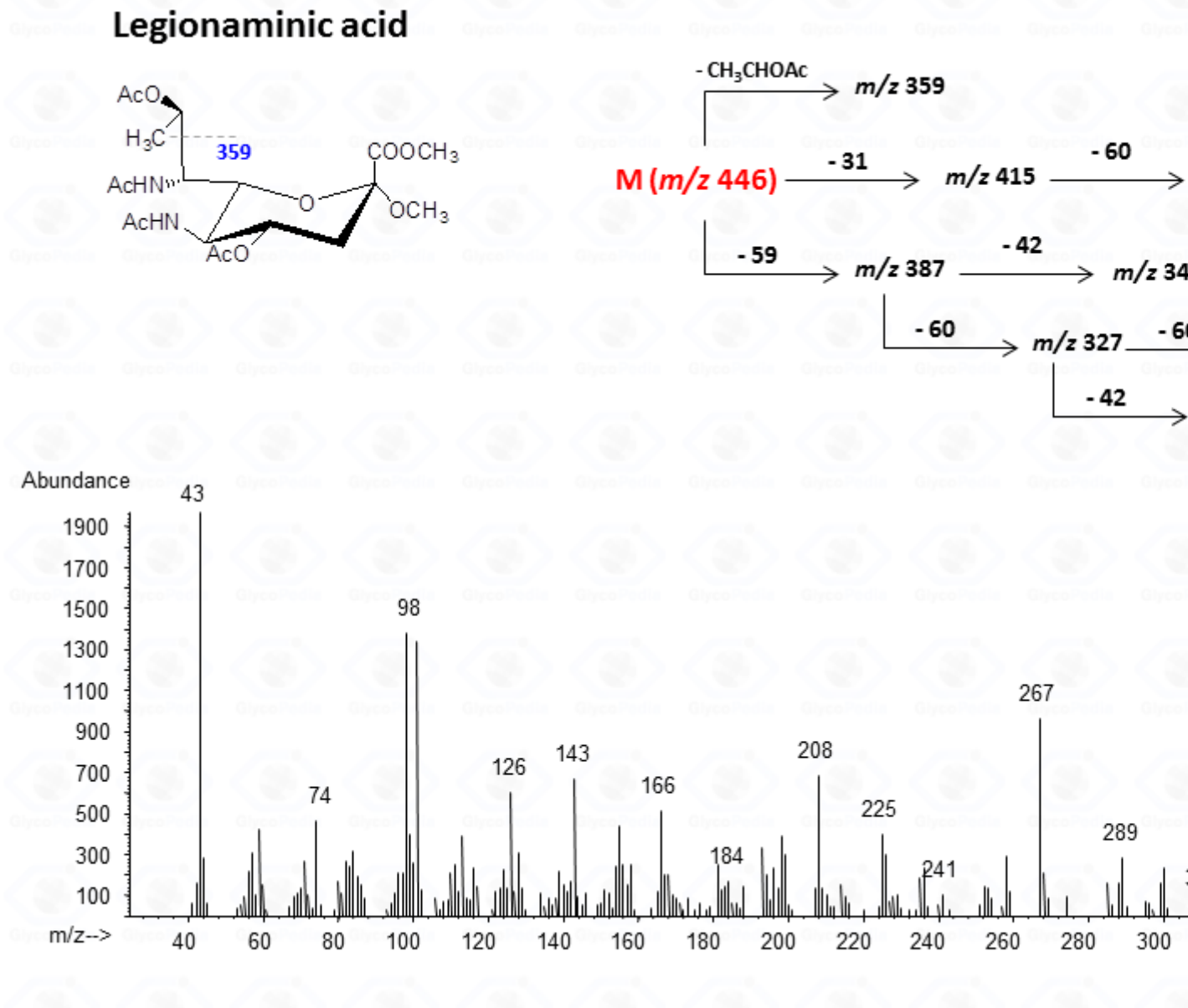

Figure 20: EI-MS spectrum of a fully acetylated methylglycoside methylester of Legionaminic acid.

For aminouronic acids

diapositive22.png

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Figure 21: EI-MS spectrum of a fully acetylated methylglycoside methylester of mannosaminuronic acid.

The lost from the molecular ion of the carboxymethyl group (derived from the acidic function) gives always a rather intense signal at high molecular mass range and this peak is highly informative of the composition of the compound. Clearly, the Acetylated Methyl Glycosides method finds its application also to aminosugars, as simple hexosamines (glucosamine in Fig. 22)

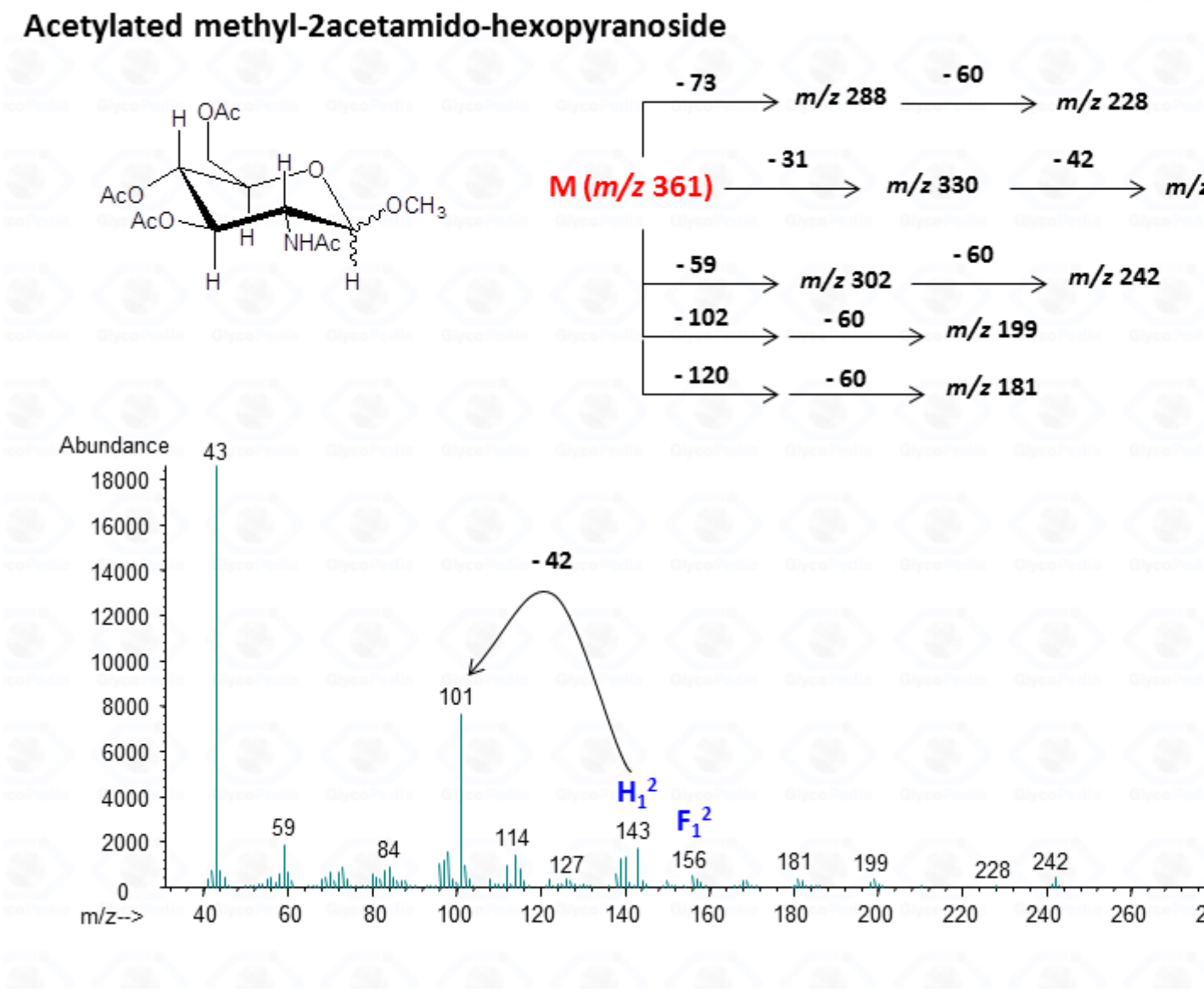

Figure 22: EI-MS spectrum of a fully acetylated methylglycoside of glucosamine.

or to more complex residues, as the above mentioned aminuronic acids (Fig. 21), or muramic acid.

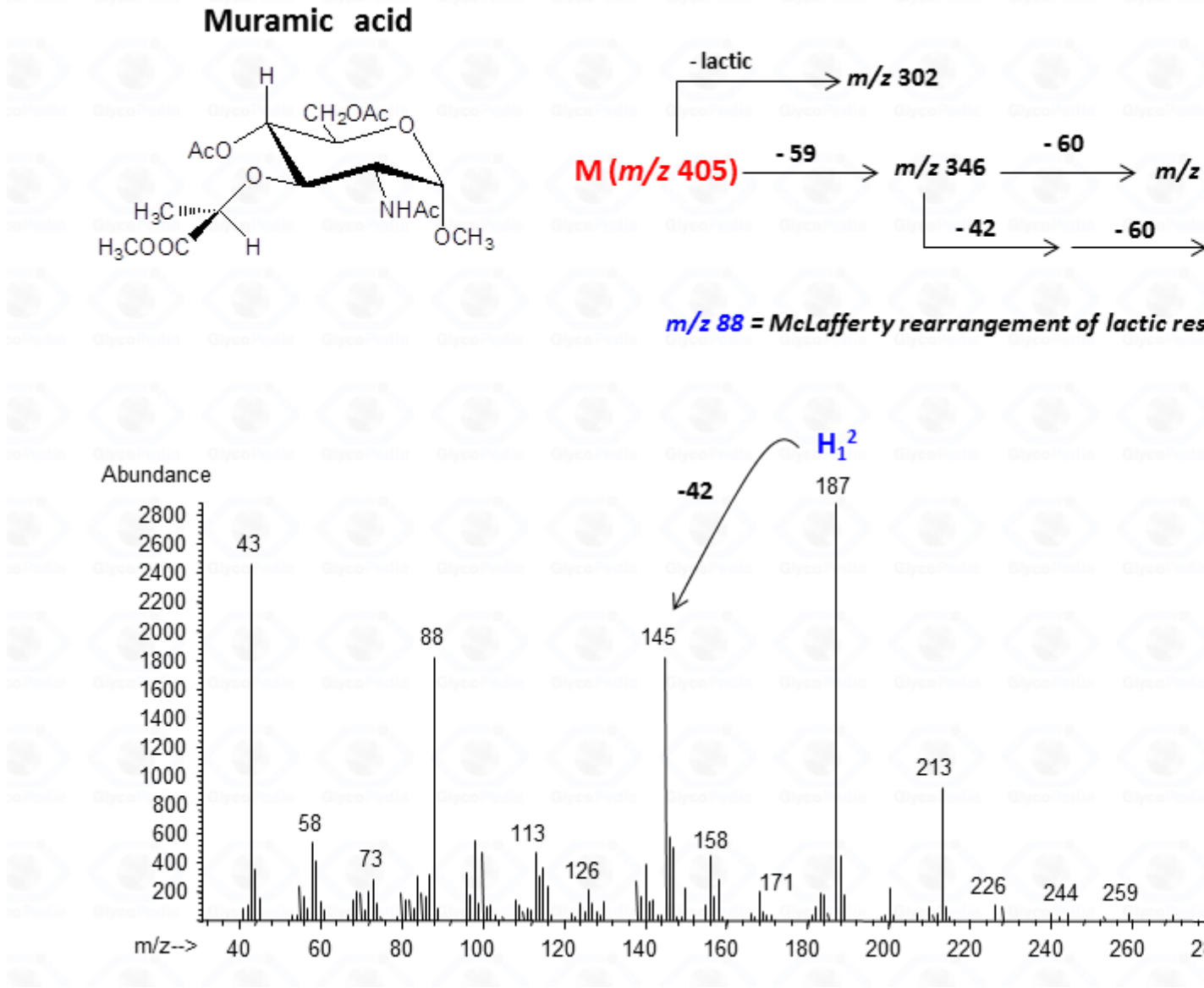

Figure 23: EI-MS spectra of the acetylated methylglycoside methylester of muramic acid

In few cases, this methodology can be used to discriminate among different sugar isomers, as the three isomeric 6-deoxy-N-Acetyl-hexosamines for which the spectra display small but nevertheless marked differences.

diapositive25.png

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Figure 24: EI-MS spectra of three different isomeric 6-deoxy-hexosamines (A1 fragment at m/z 272)

Such a differentiation is possible at the condition that the spectra of all the possible isomers are present in the library.
EI-MS fragmentation spectra contain all the information necessary for the identification of the monosaccharide components. Usually, the most informative peaks are those occurring at high mass ranges. In some cases, few diagnostic ions can be found in the low molecular range of the spectrum. This is exemplified by the comparison of the spectra obtained for a hexose (Fig. 13), a pentose (Fig. 14) and a 6-deoxyhexose (Fig. 15). The acetylated methyl glycosides of the hexose presents *m/z* 200 as diagnostic peak, whereas *m/z* 128 and 170 occur for the pentose and *m/z* 142 and 184 for 6-deoxyhexose, respectively. Clearly, during the process of monosaccharide identification, it is always safe to cross-check the results gathered from the fragmentation pattern data with other information, as those derived from the retention time of the suspected monosaccharide unit.
Acetylated methyl glycosides derivatives are stable standards. Once prepared, they can be stored and used for several years.

Category

1. News