<u>Supporting Information</u>

Structural Characterization and Abundance of Sialylated Milk Oligosaccharides in Holstein Cows During Early Lactation

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   E-mail address: Galuska.sebastian@fbn-dummerstorf.de

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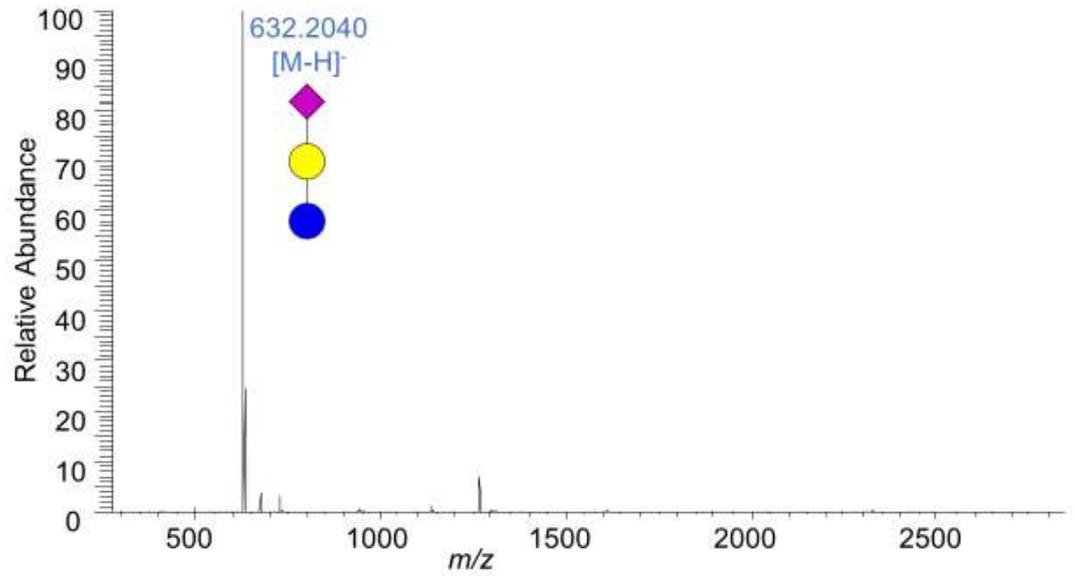
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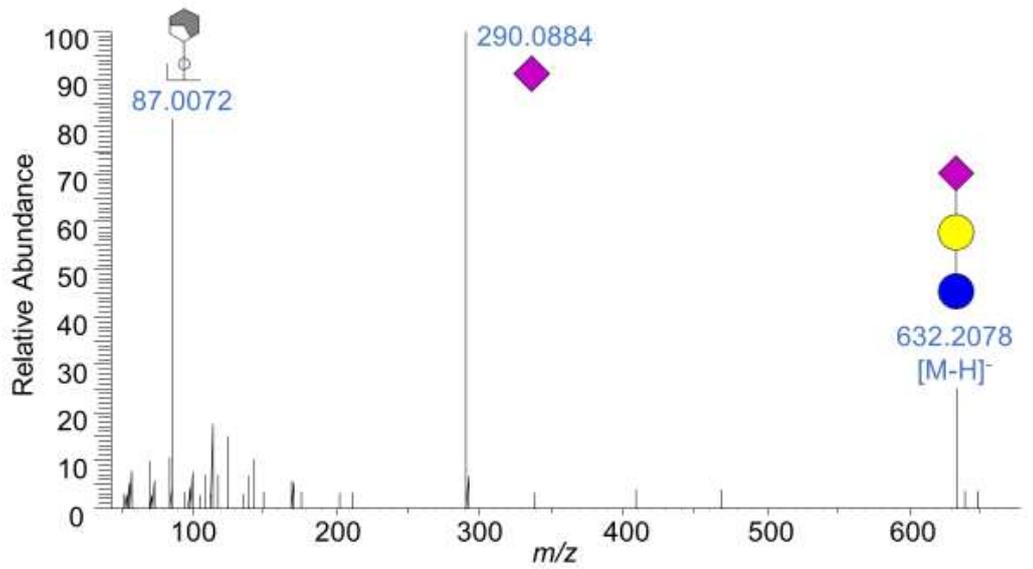
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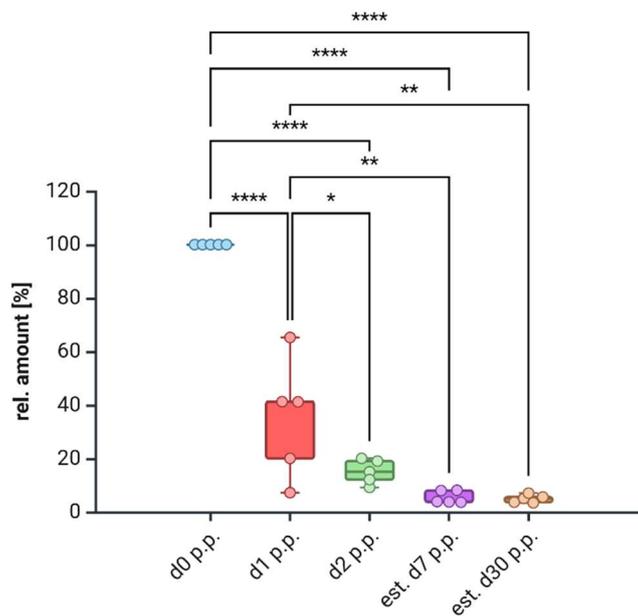
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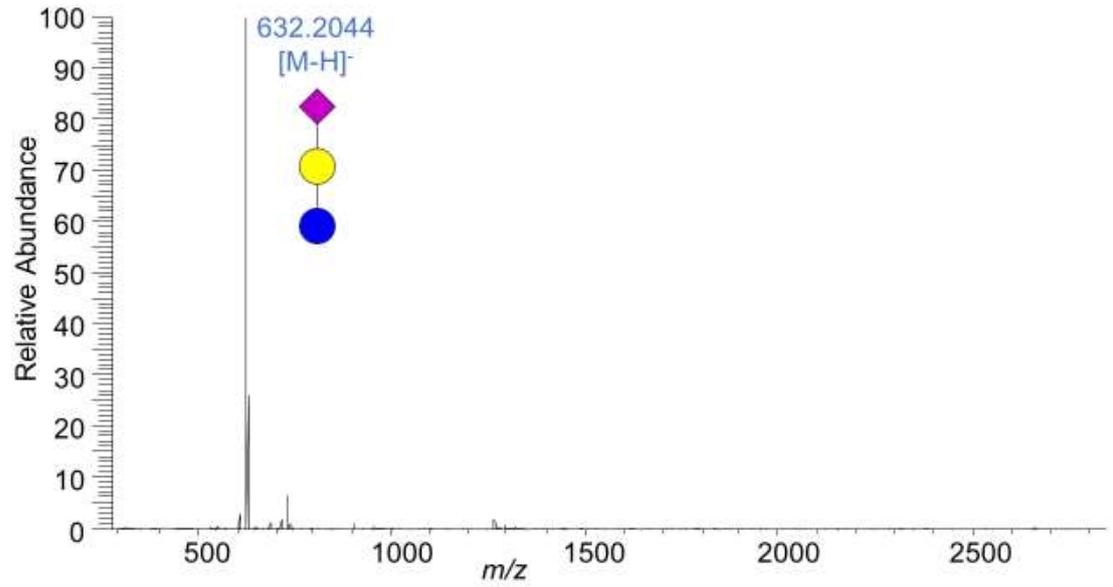
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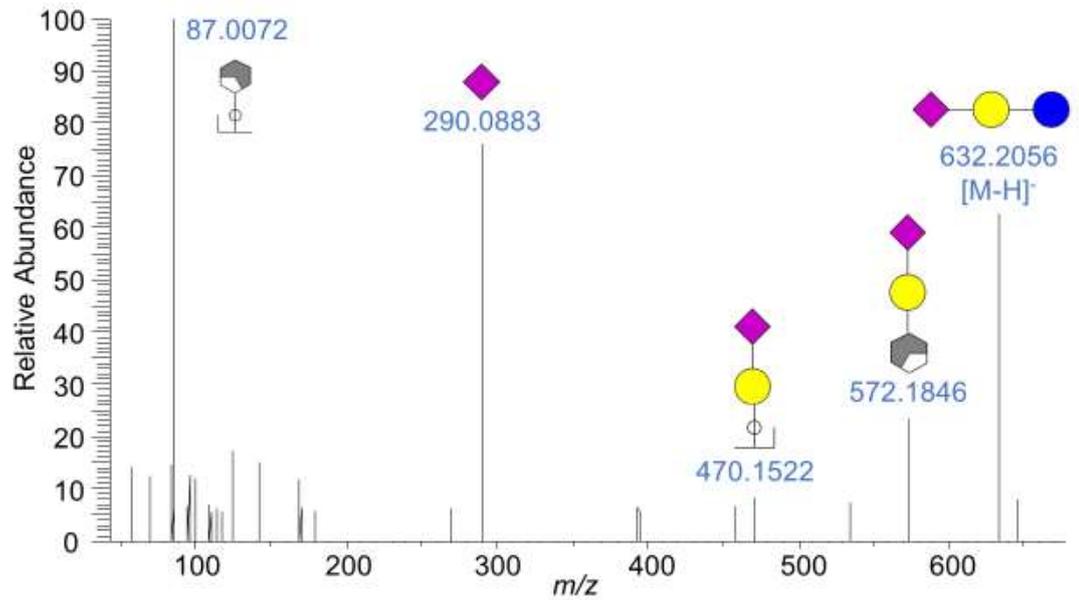
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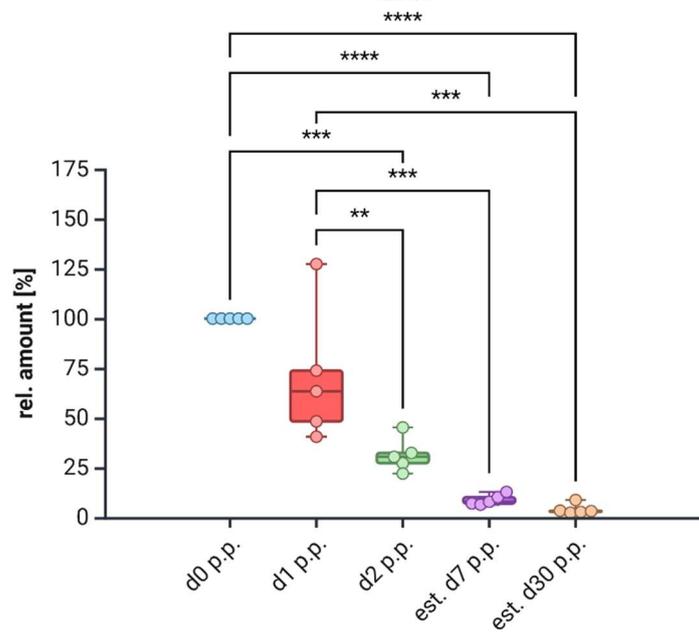
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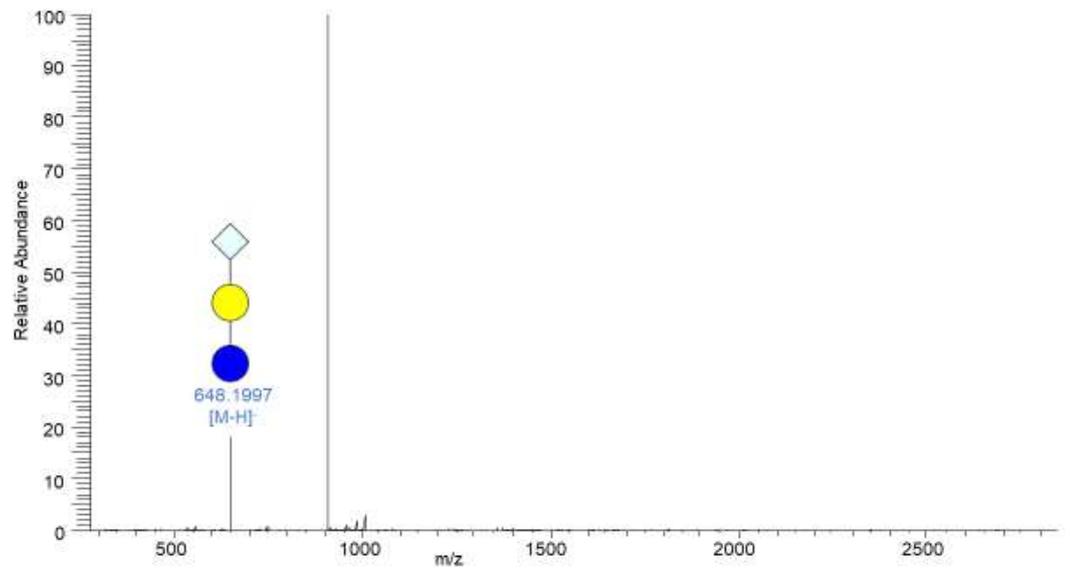
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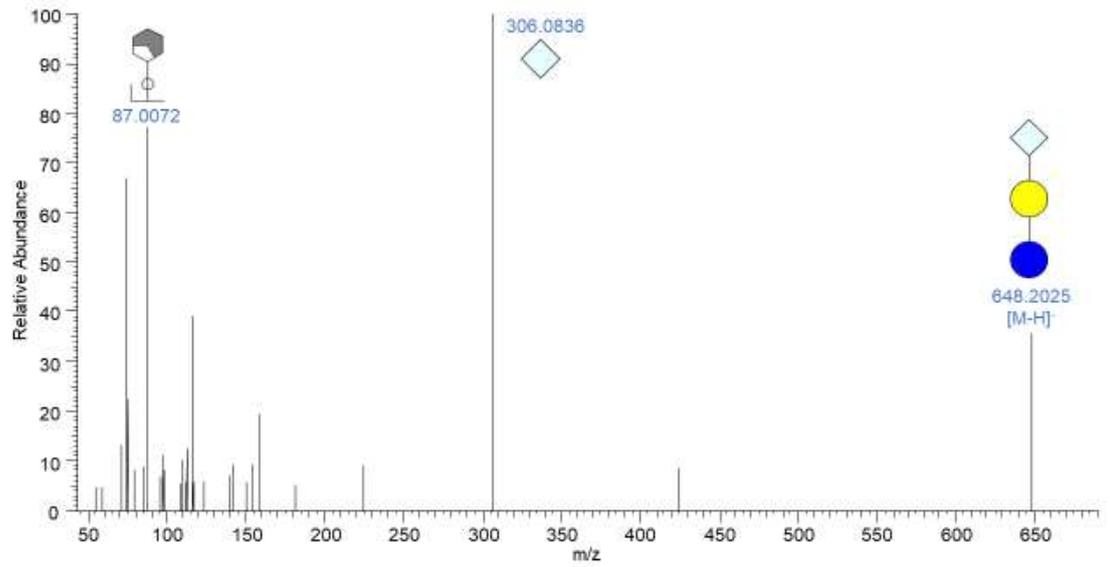
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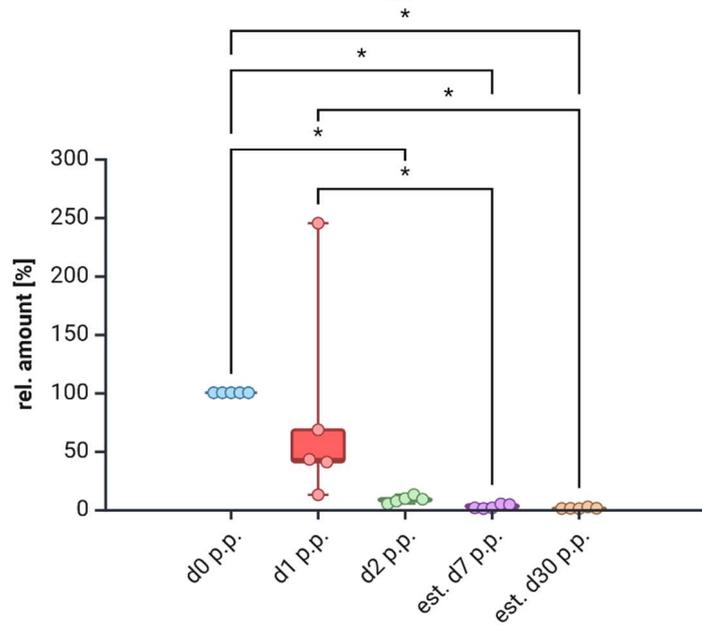
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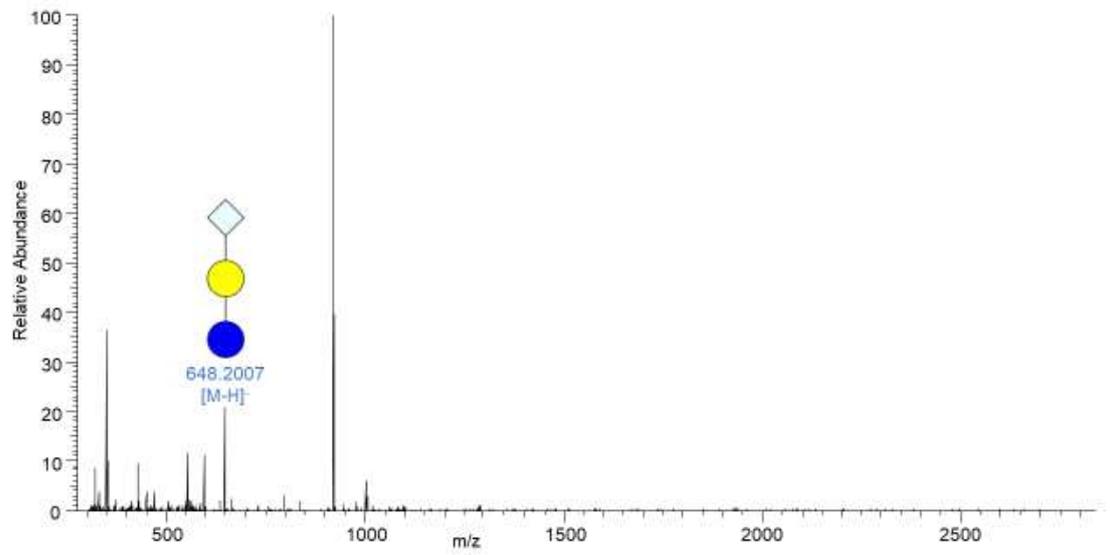
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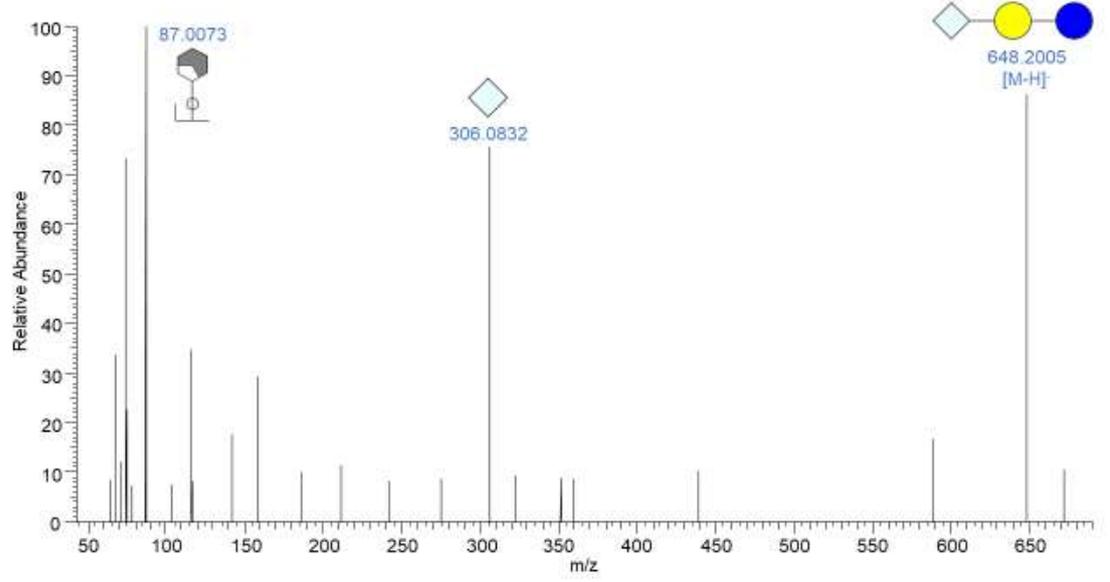
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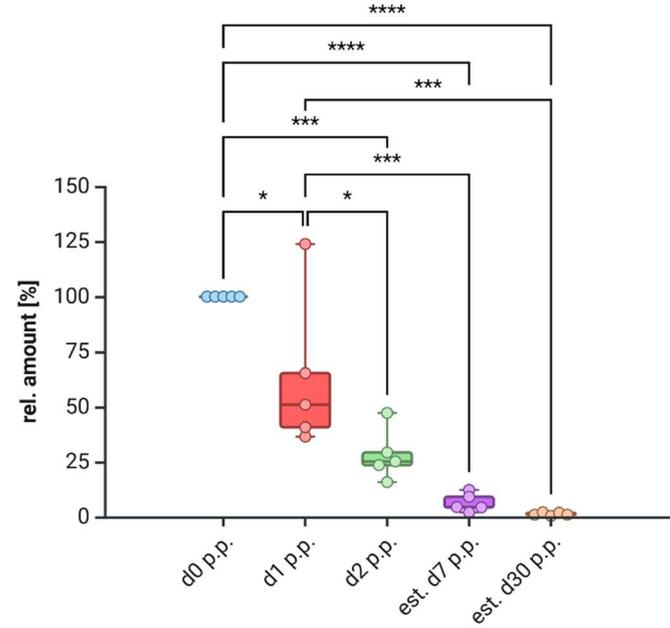
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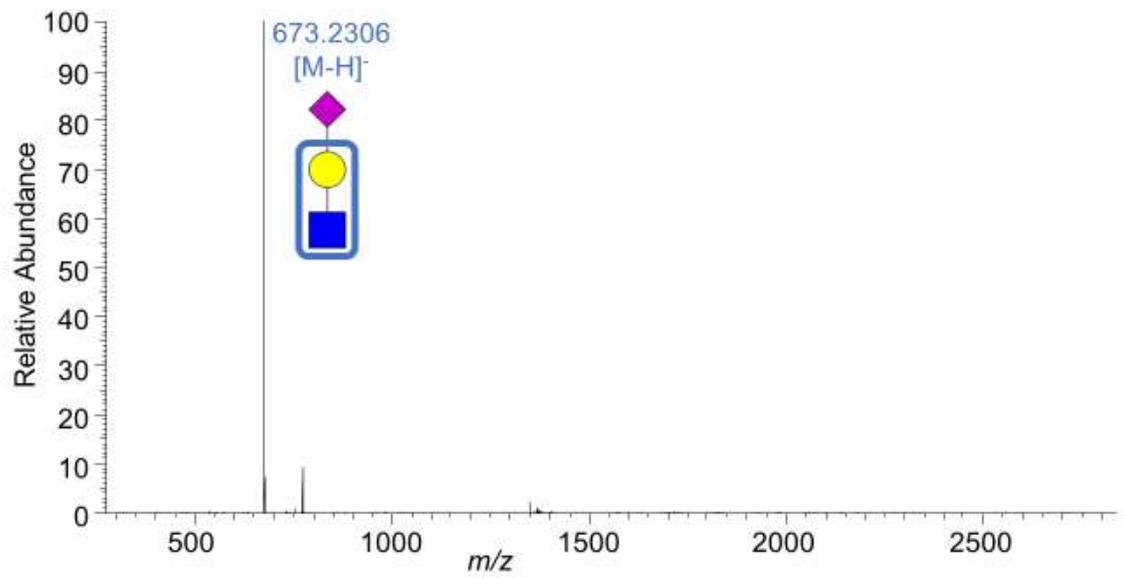
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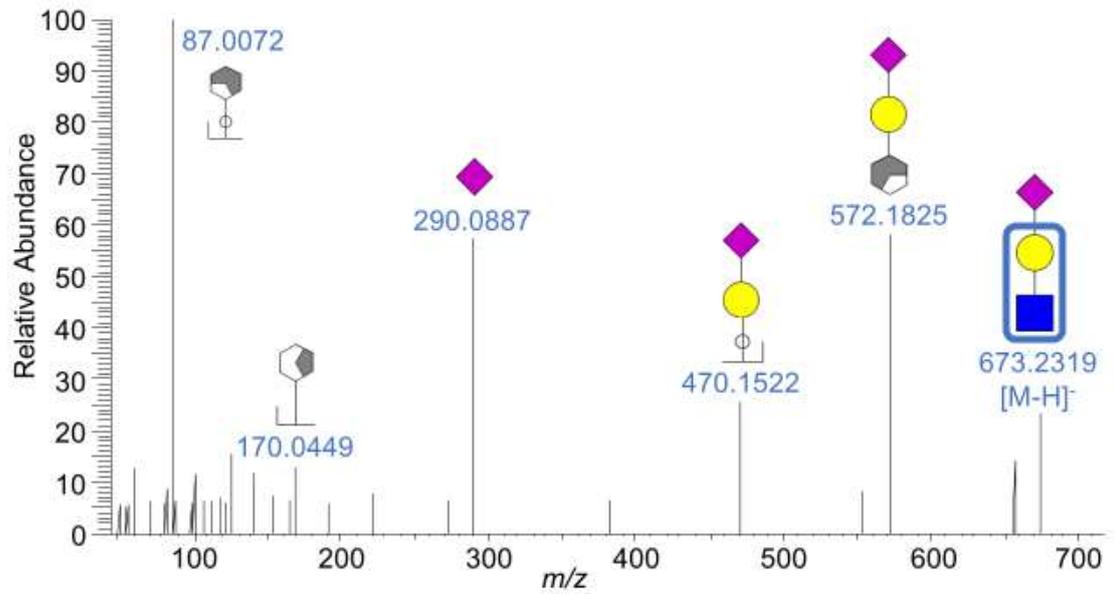
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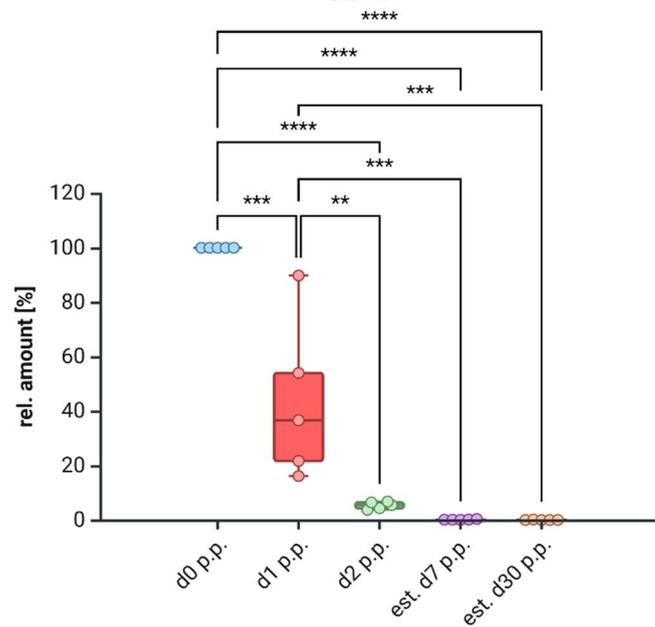
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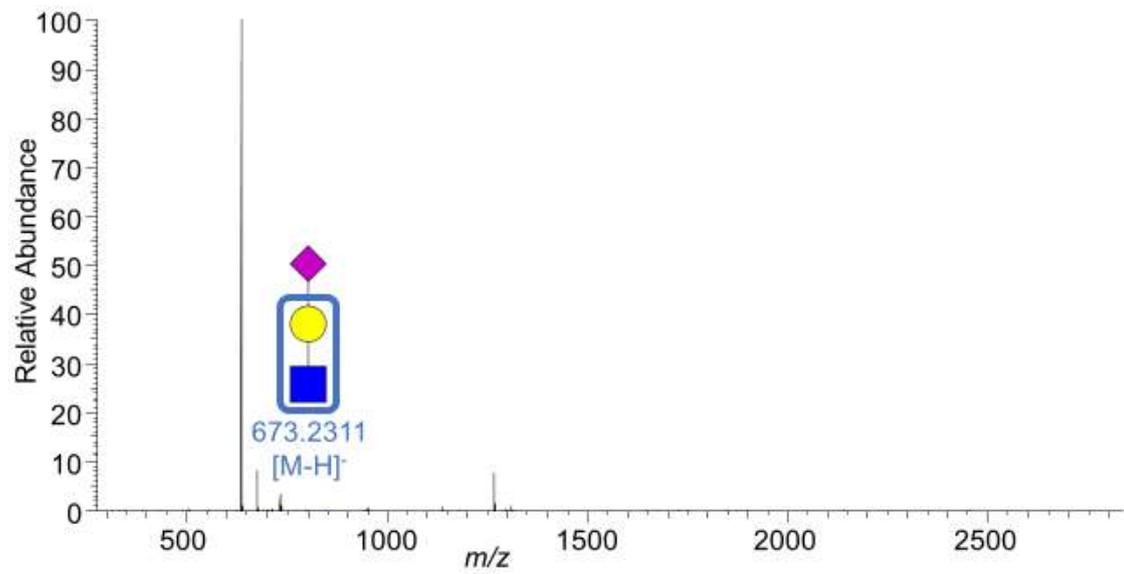
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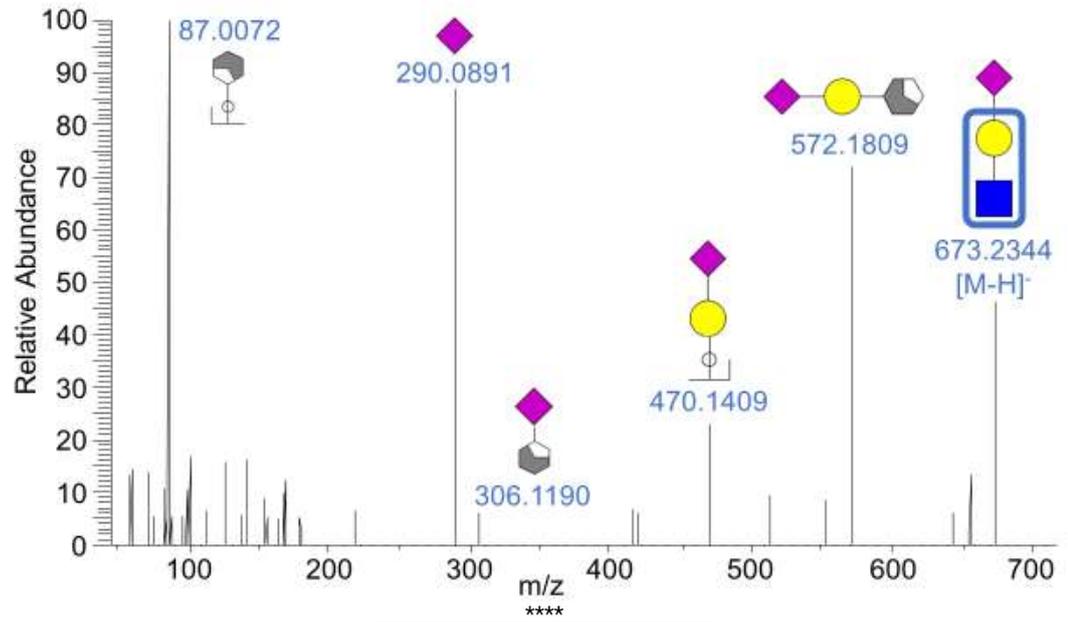
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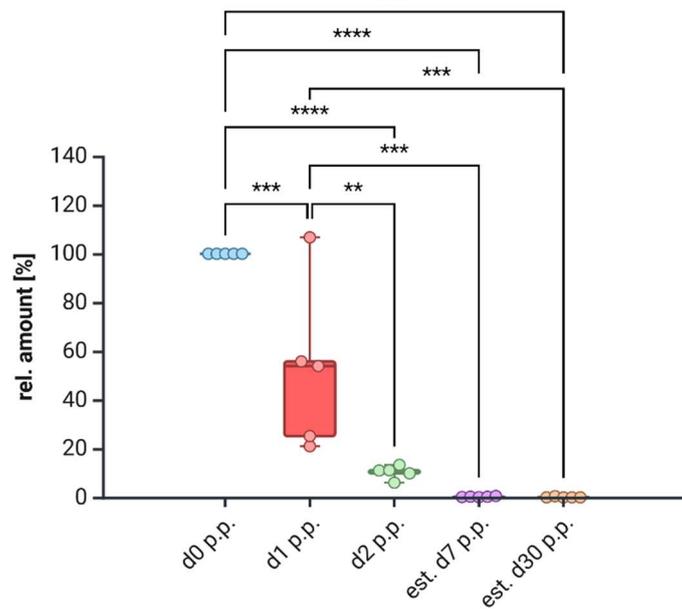
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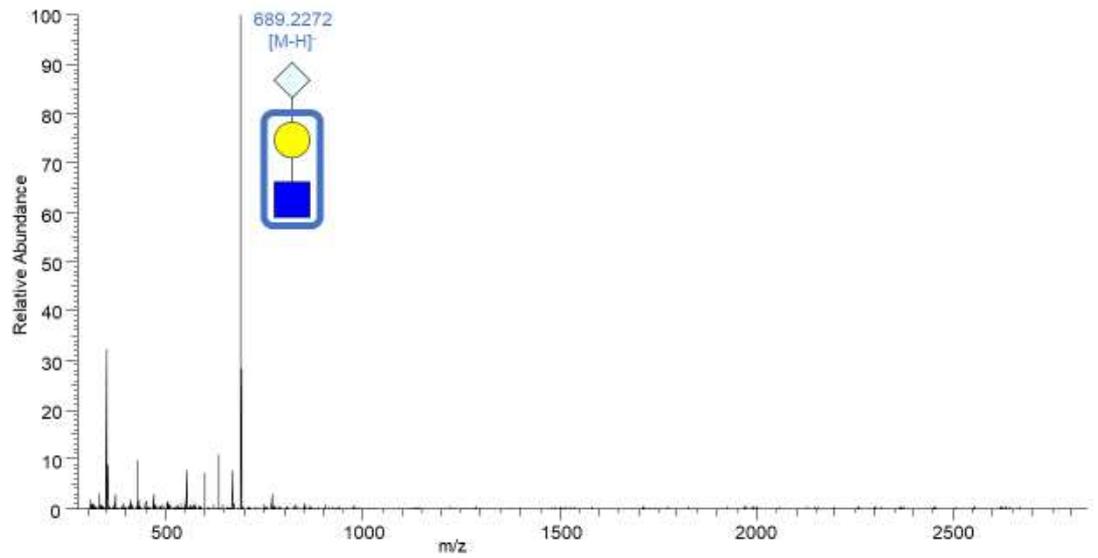
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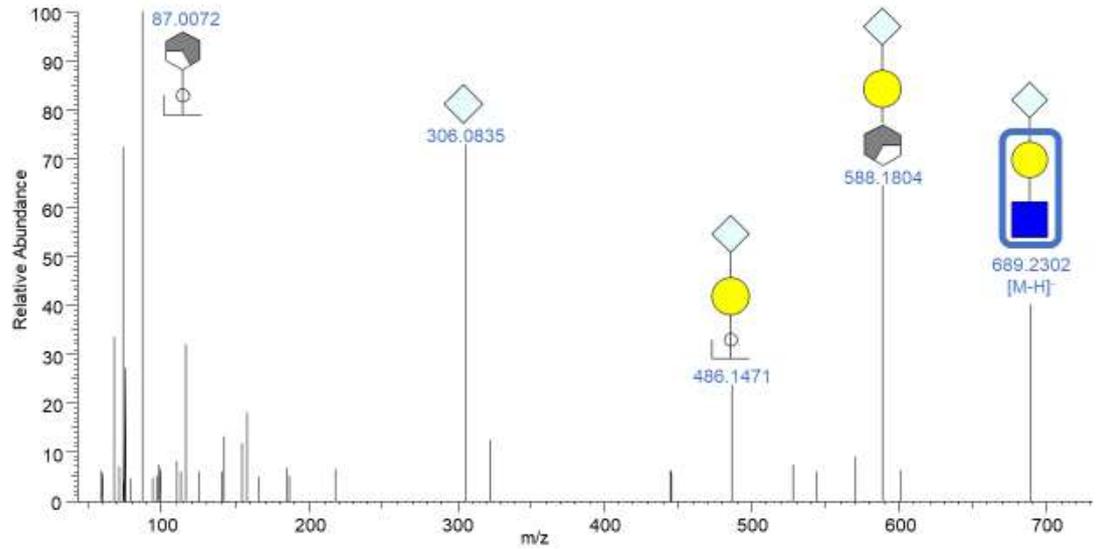
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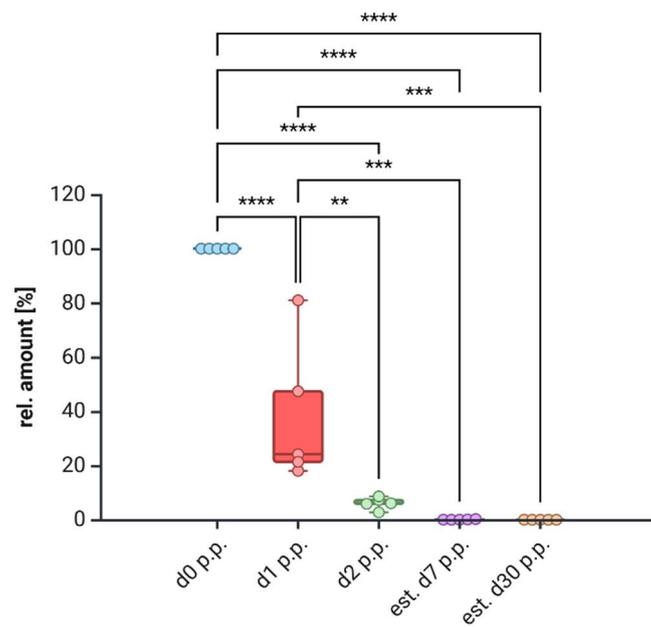
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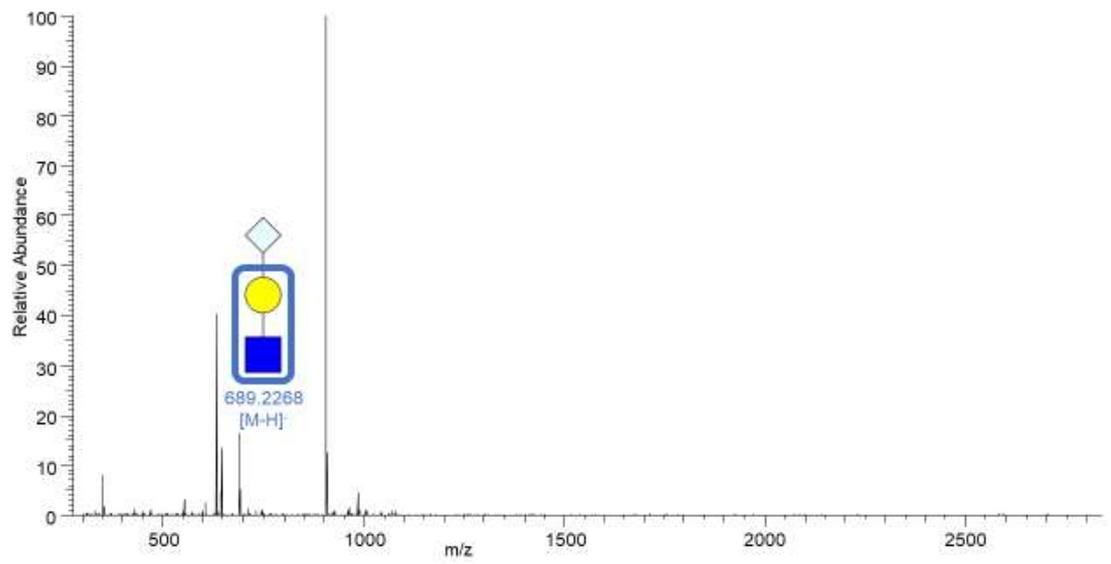
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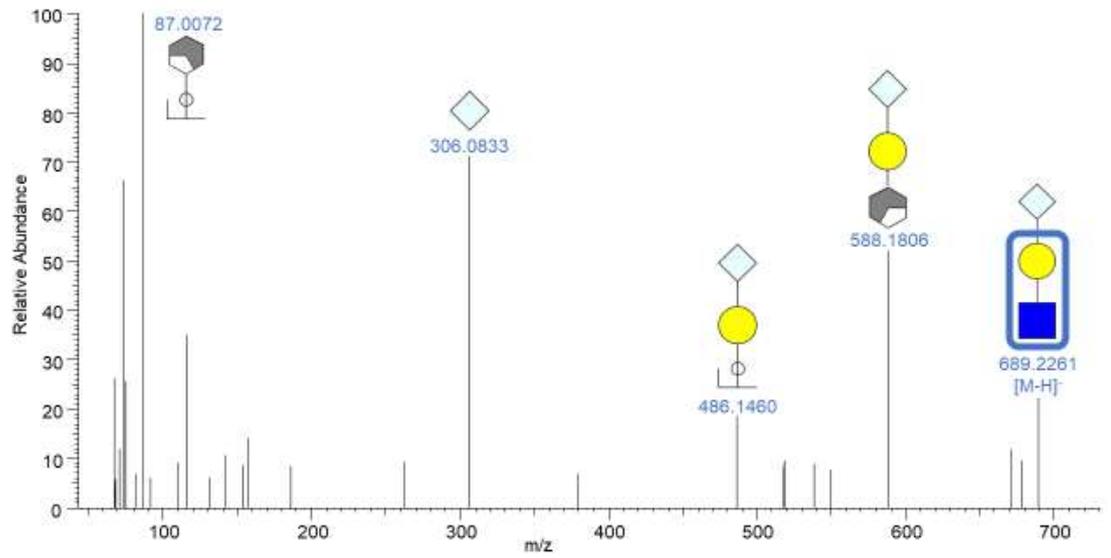
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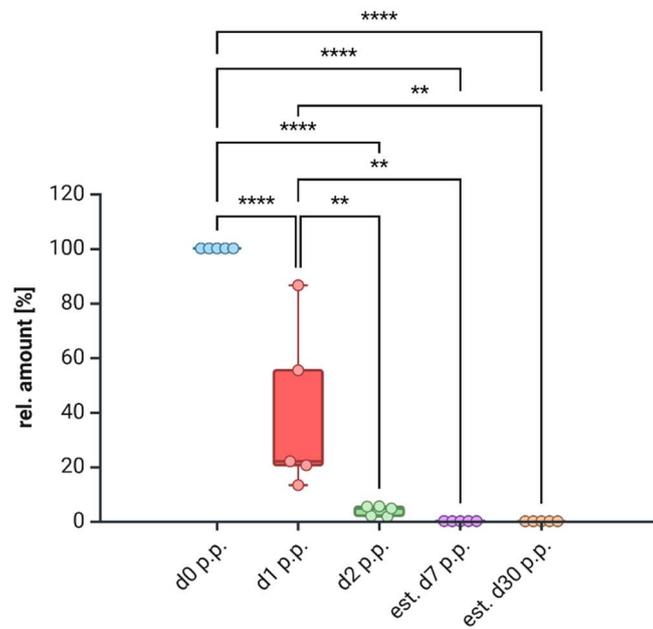
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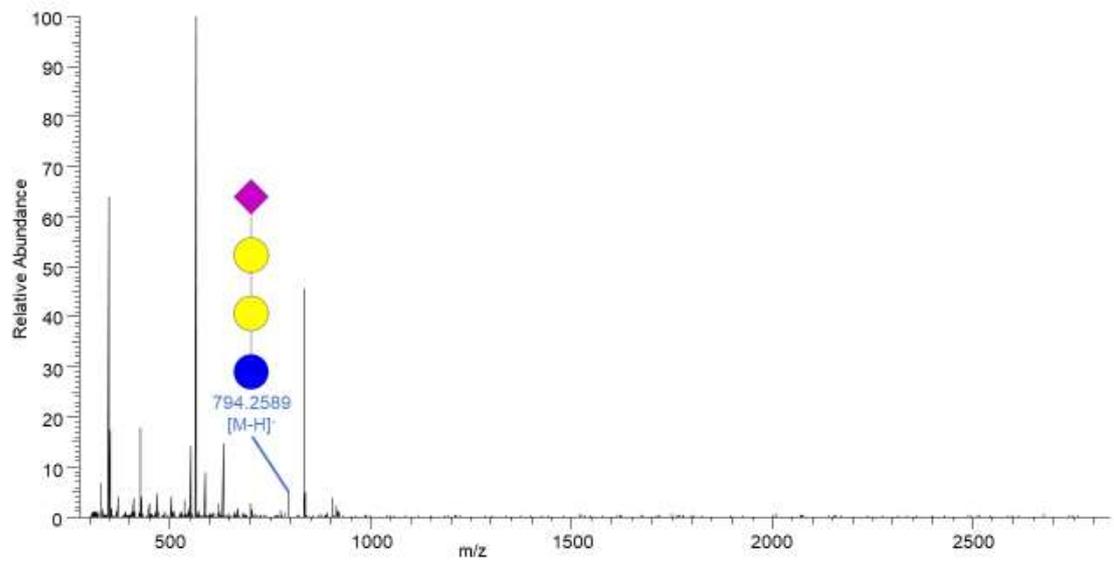
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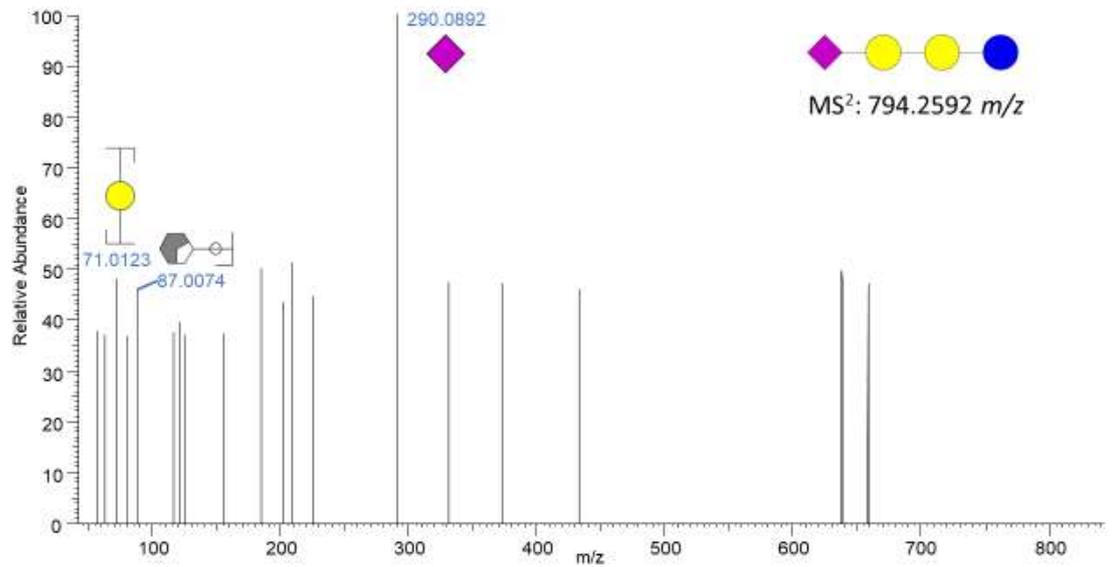
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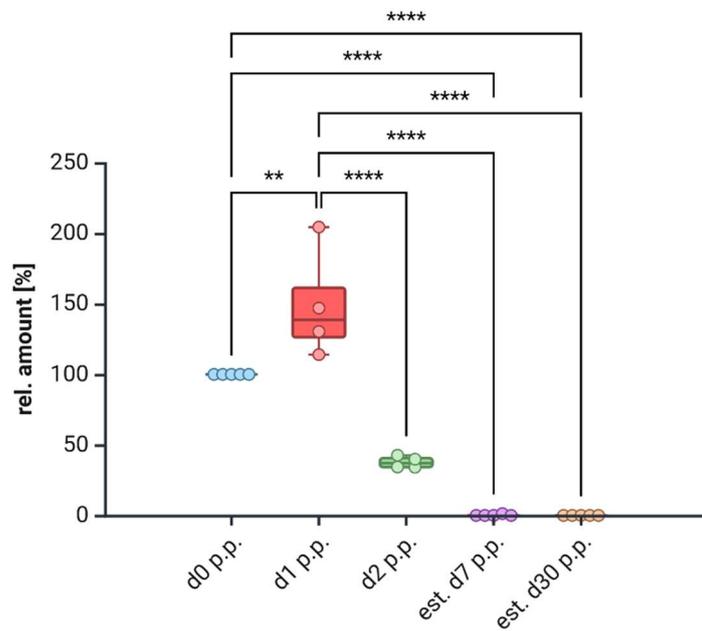
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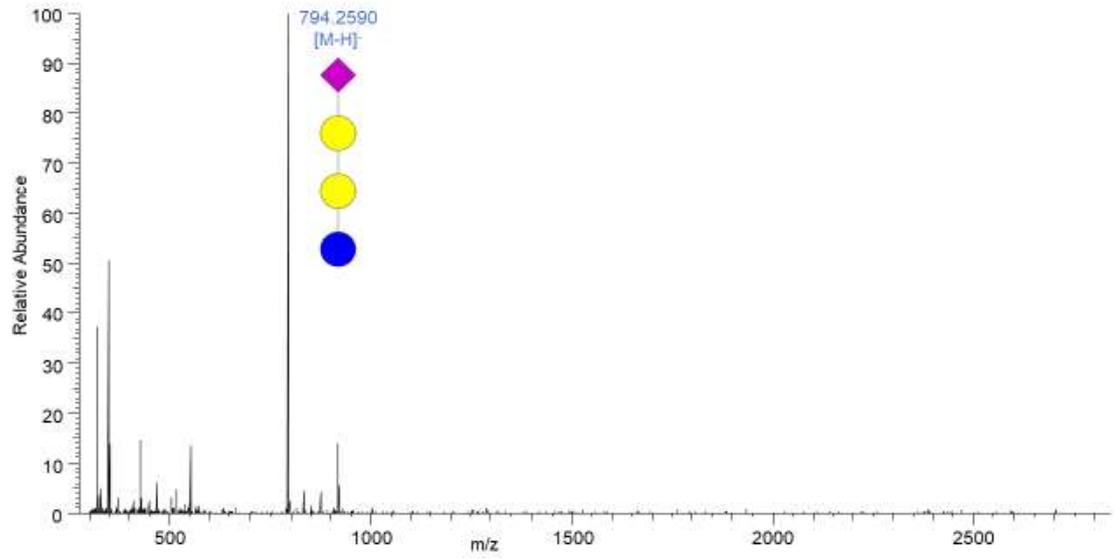
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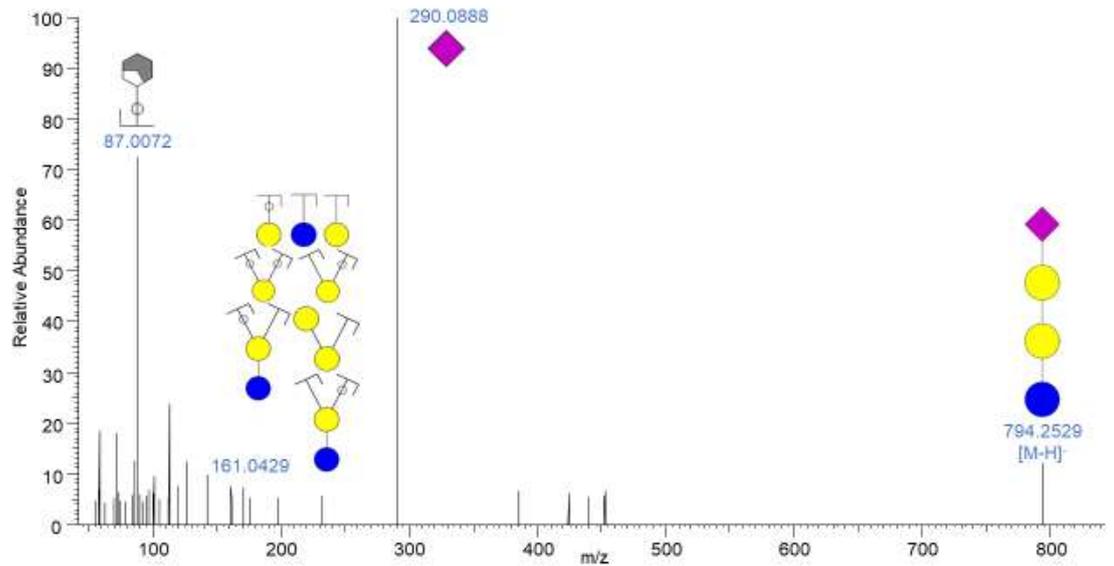
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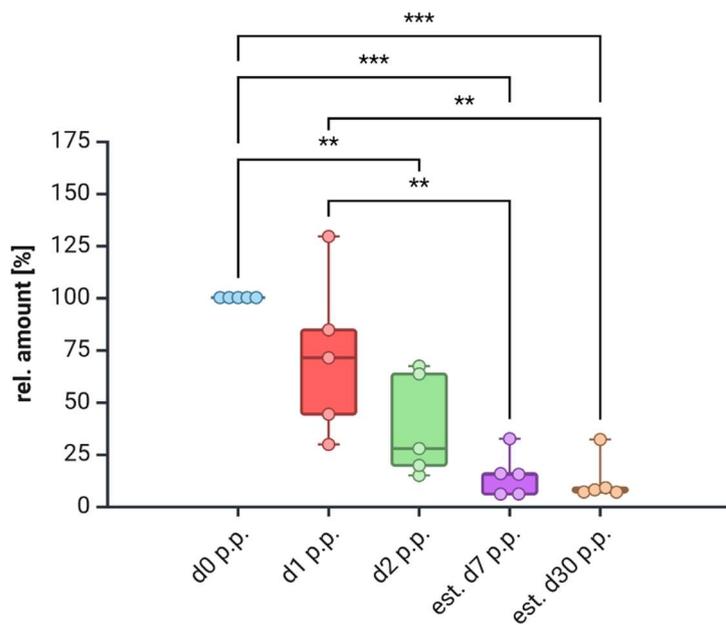
#12
(RT 8.9 min)

A
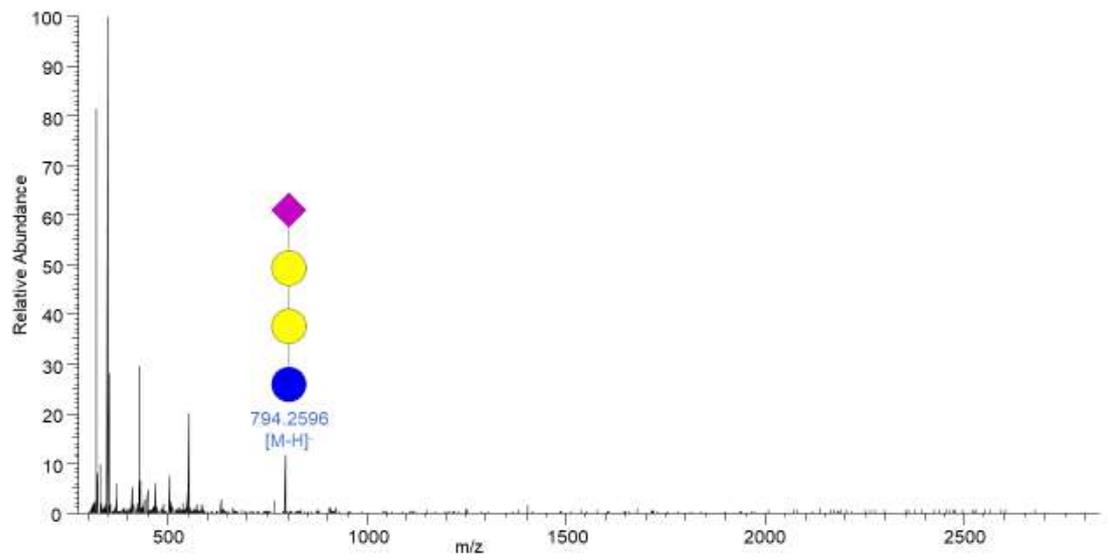
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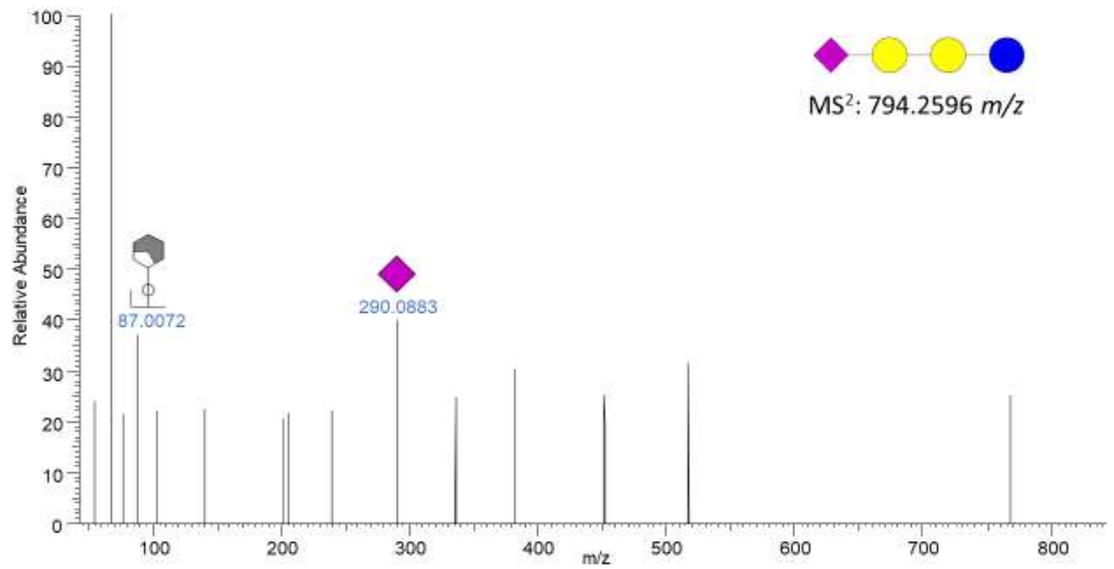
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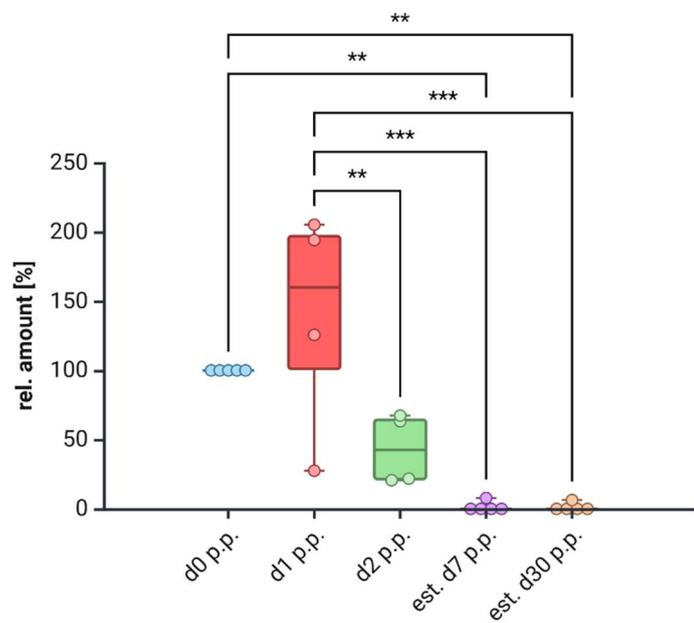
#12
(RT 9.2 min)

A

B

C

**#13
(RT 10.3 min)
A**

B

MS^2: 810.2541 m/z
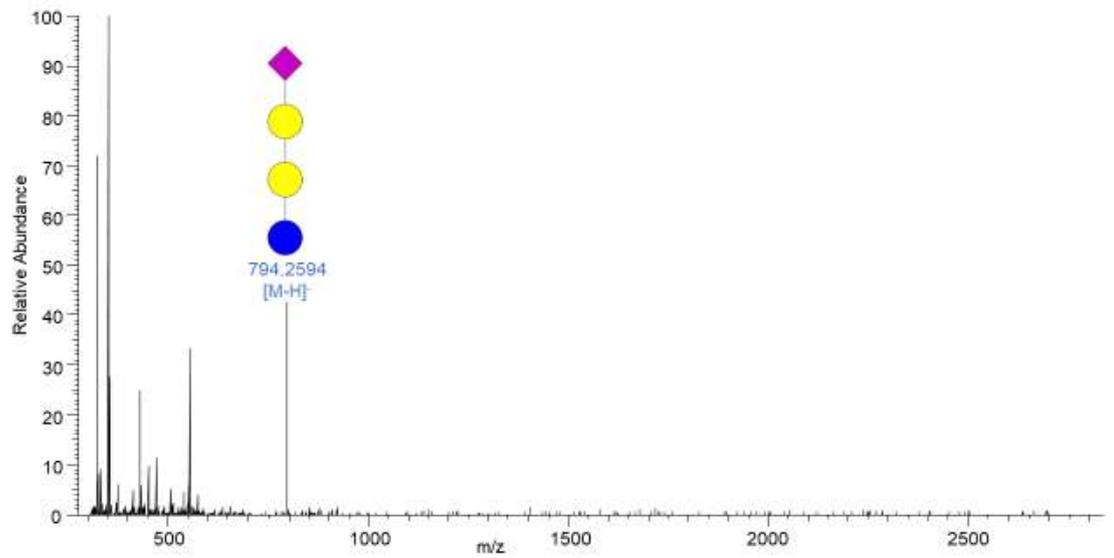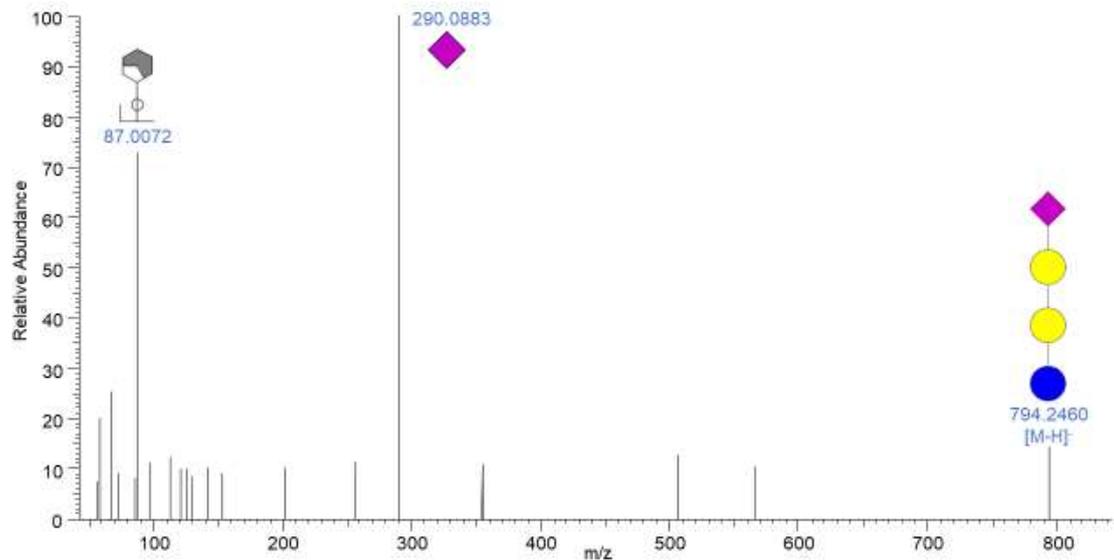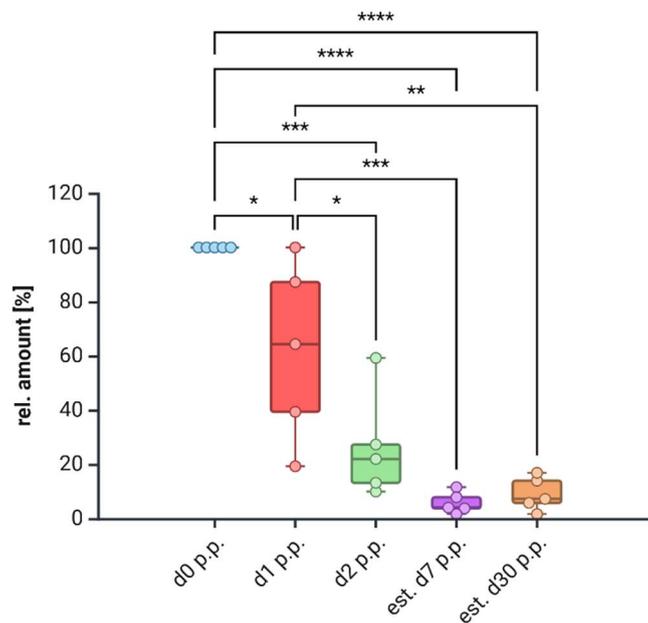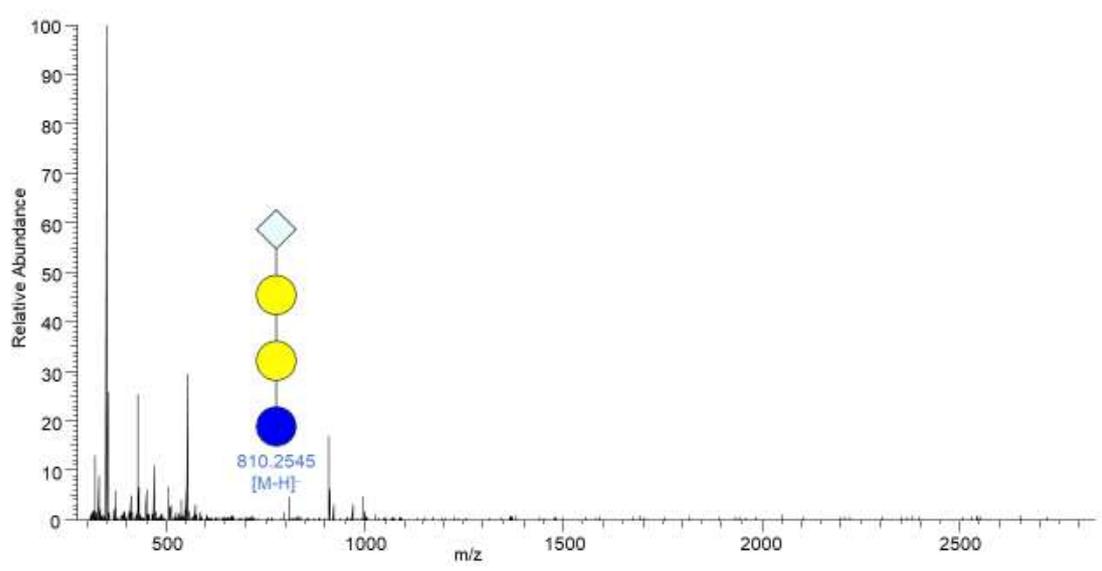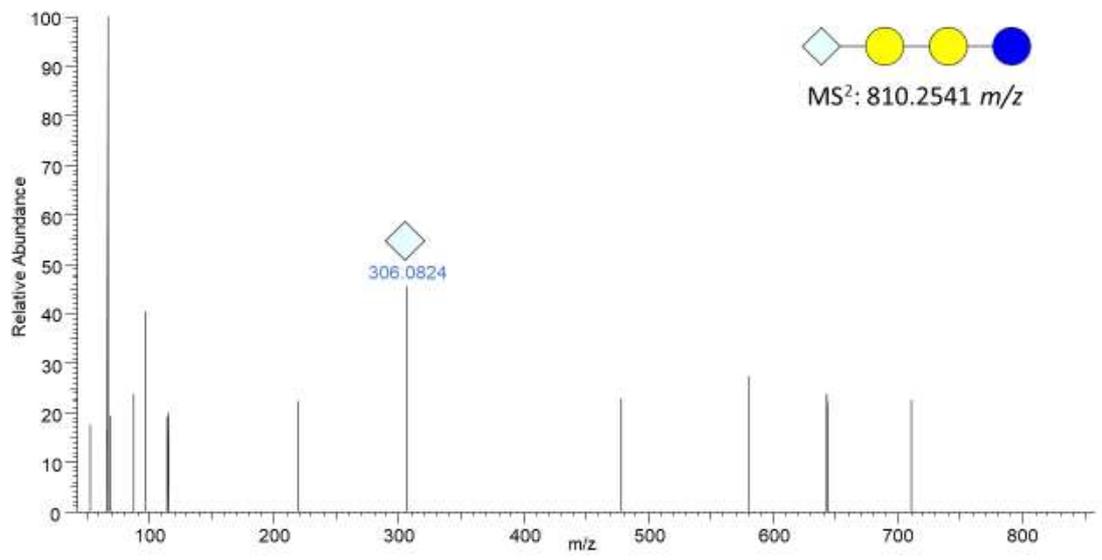
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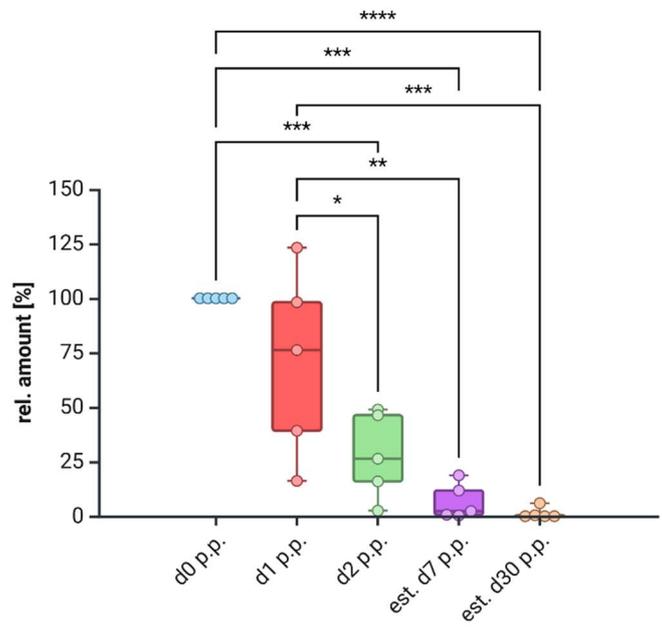
**#14
(RT 7.9 min)**

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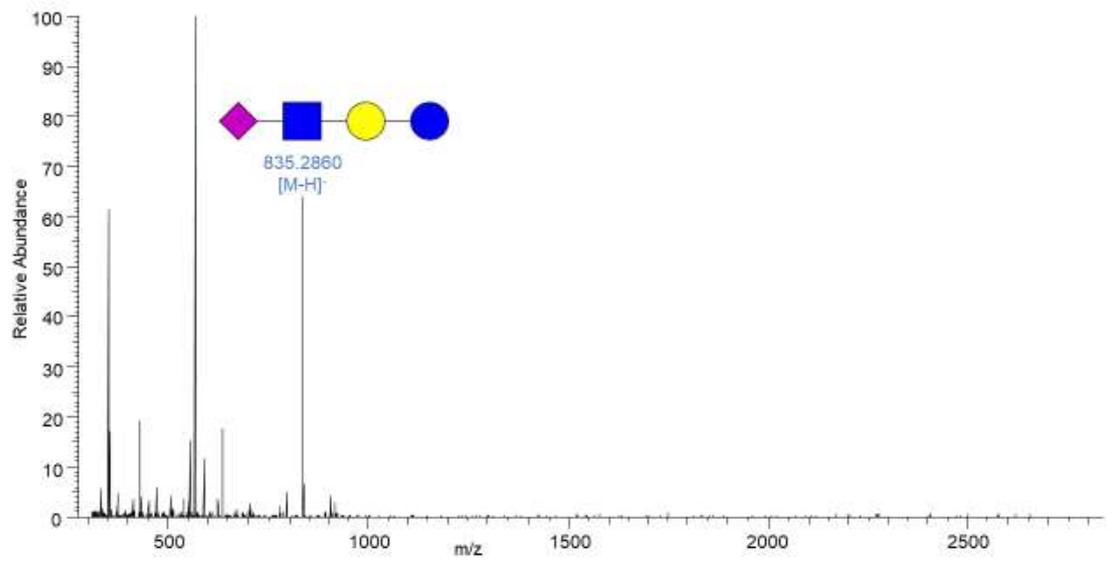
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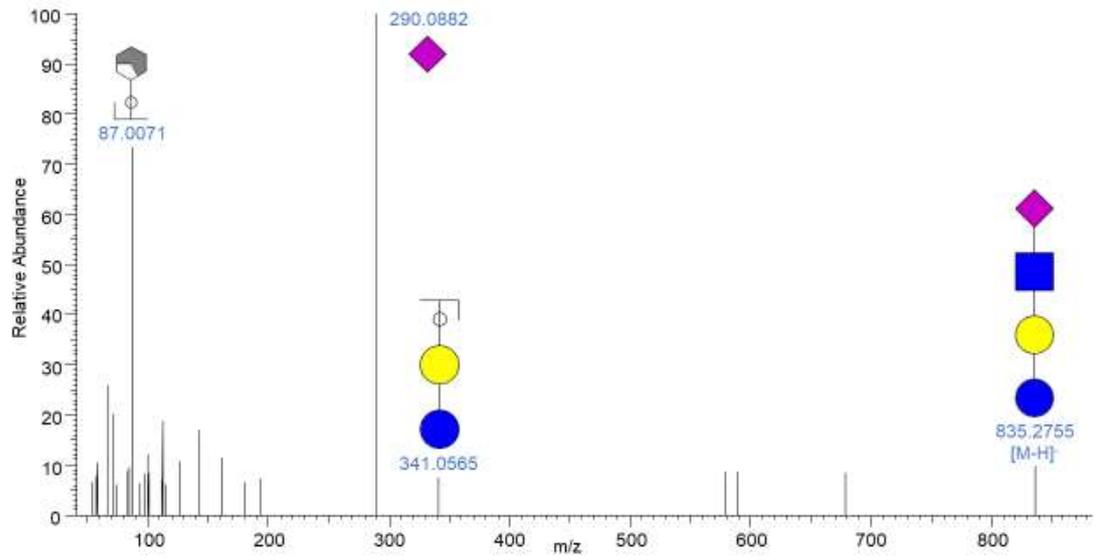
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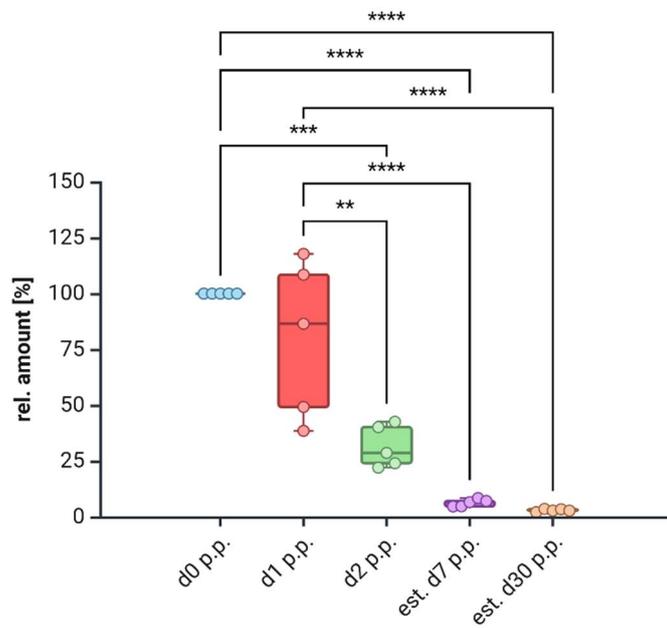
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(RT 11.0 min)**

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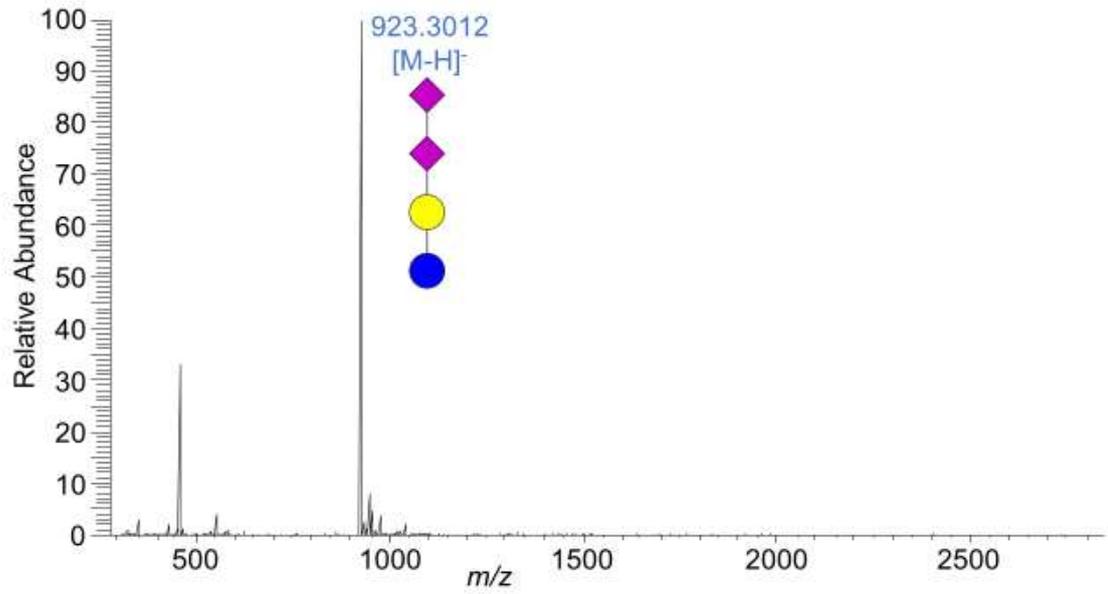
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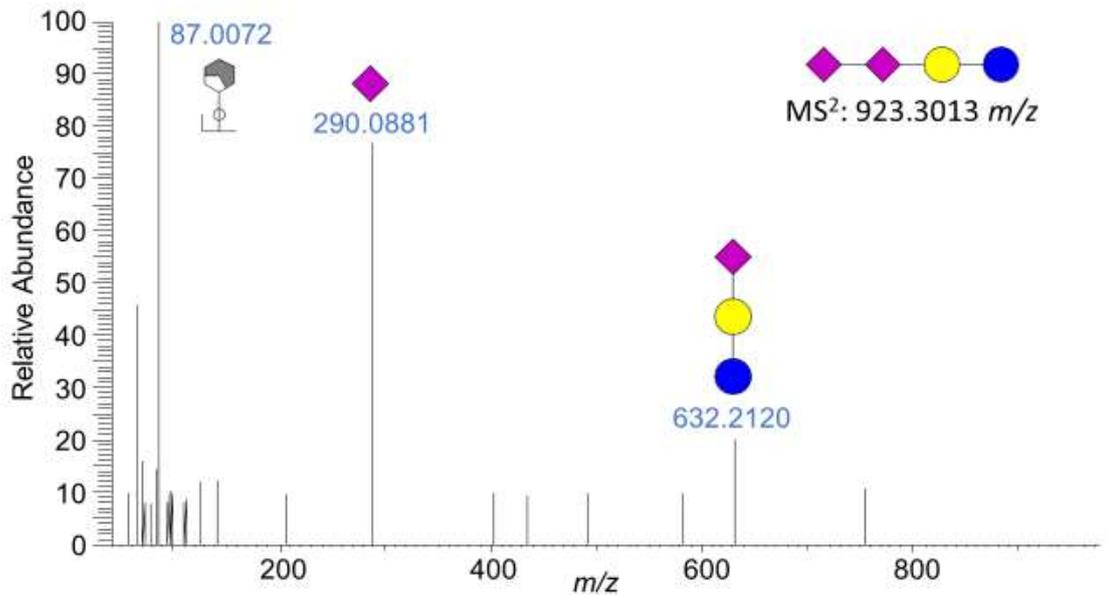
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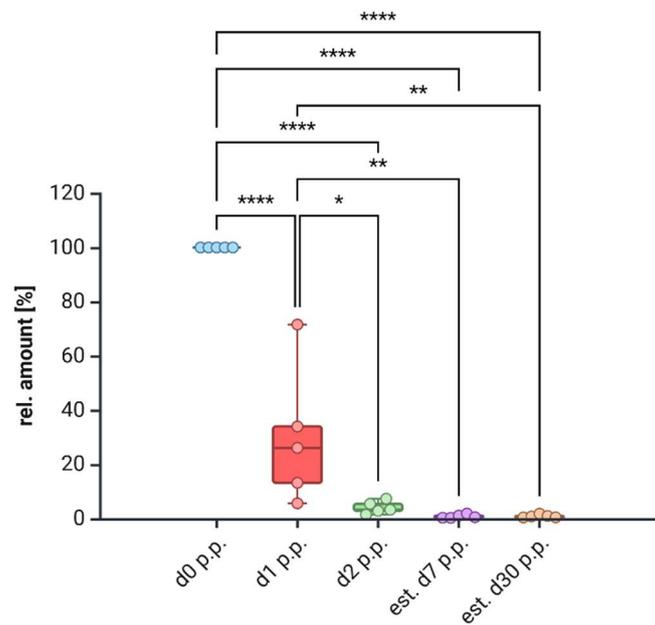
**#17
(RT 7.0 min)
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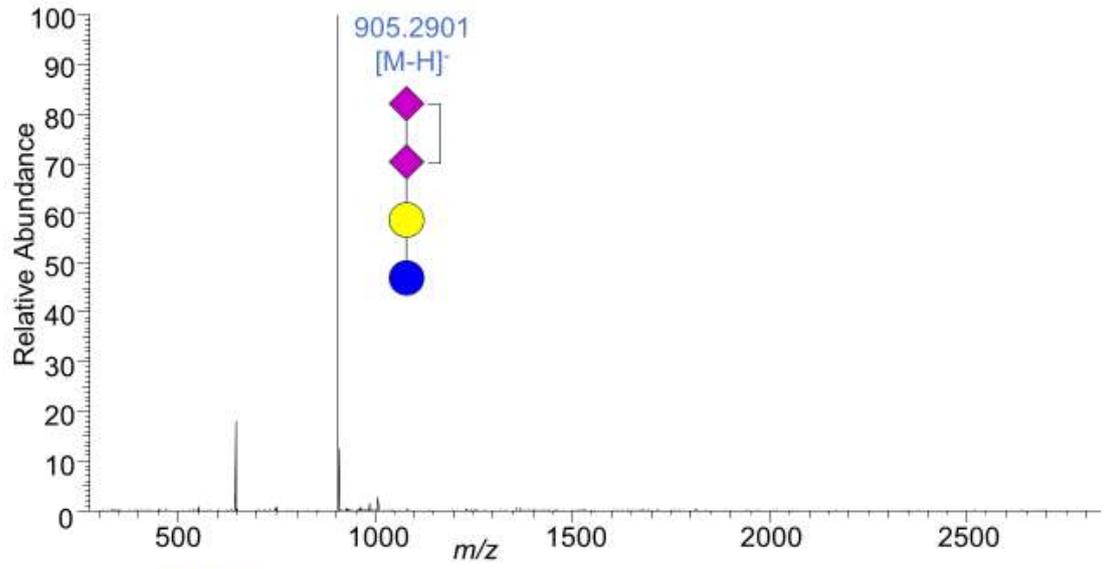
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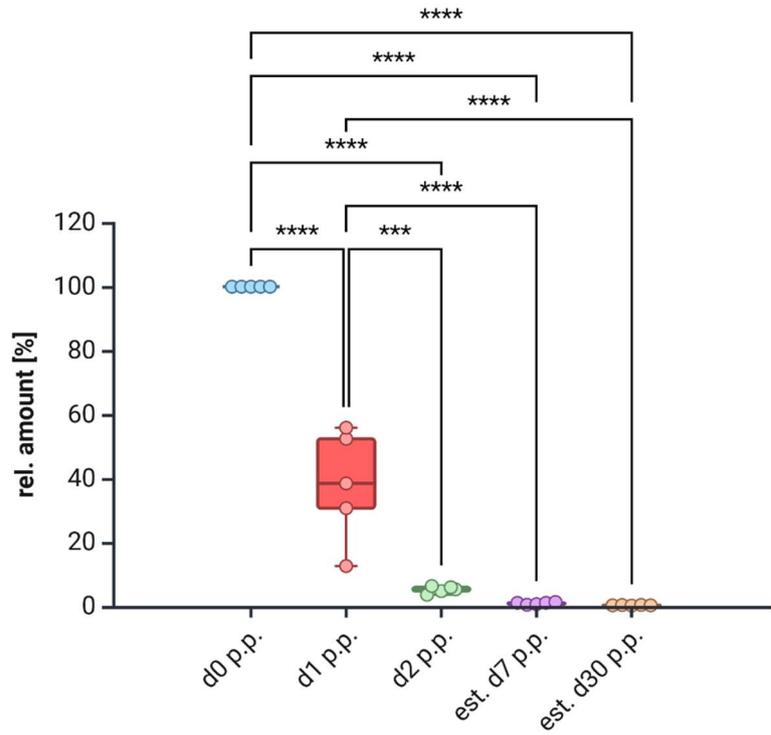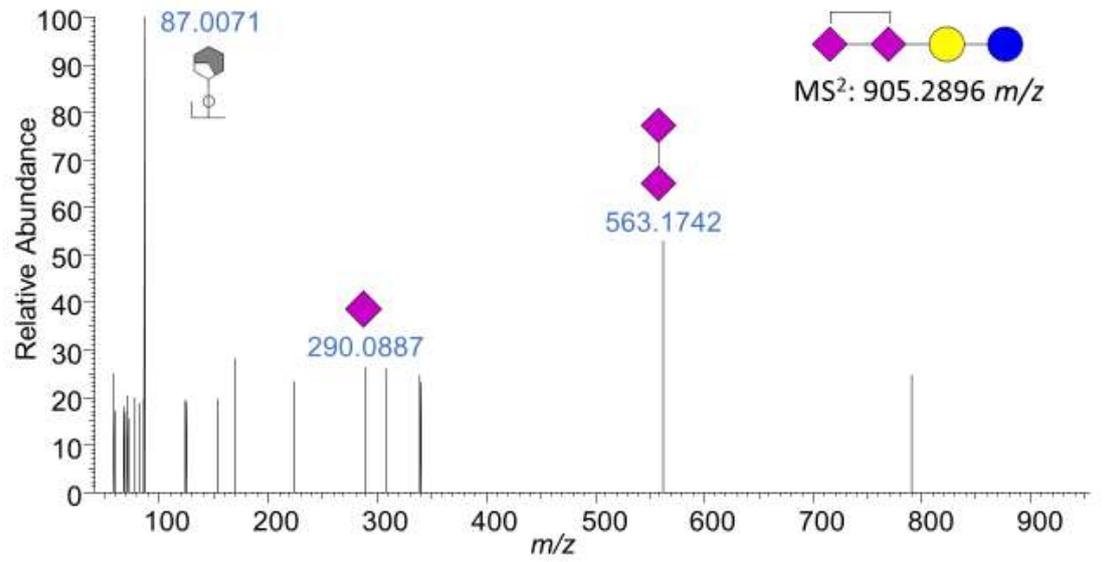
**#18
(RT 11.5 min)
A**

B

C

#18
(RT 8.2 min)

A

921.2864
[M-H]⁻

B

87.0072

170.0453

290.0898

579.1691

MS²: 921.2865 *m/z*
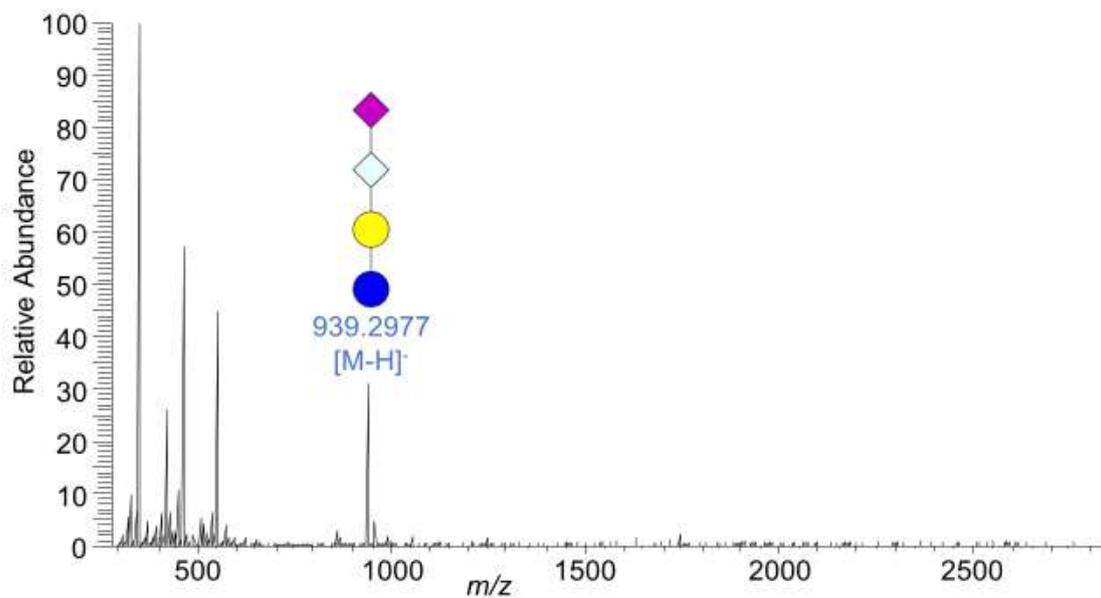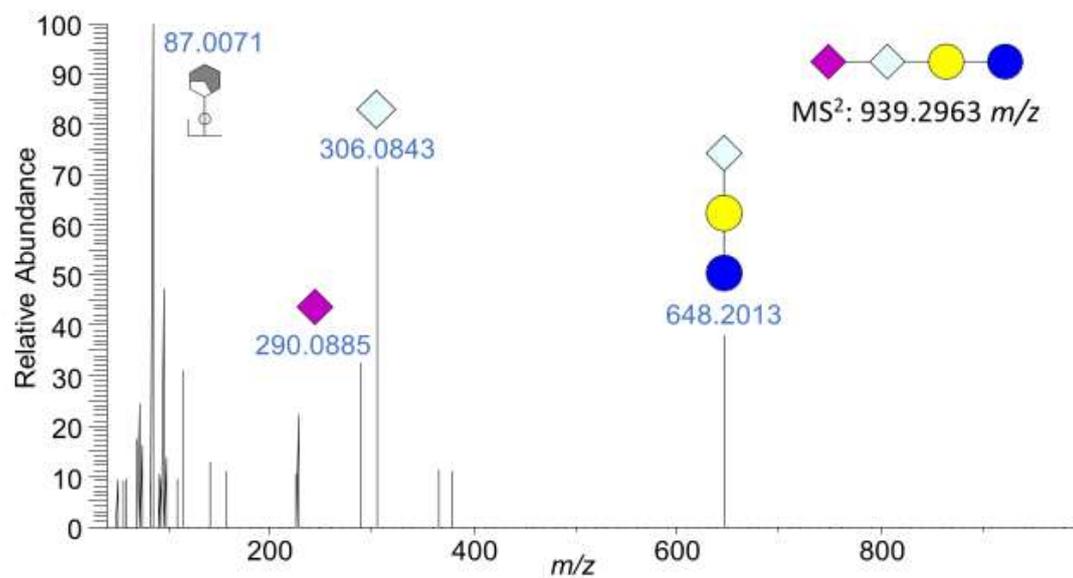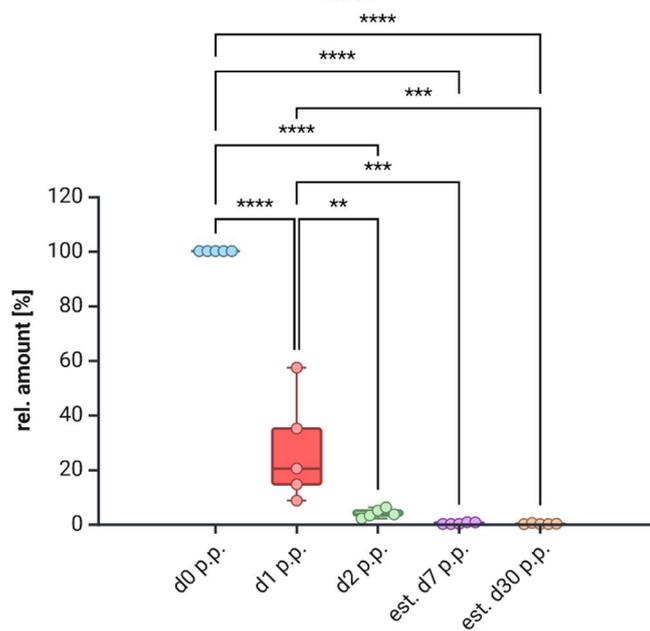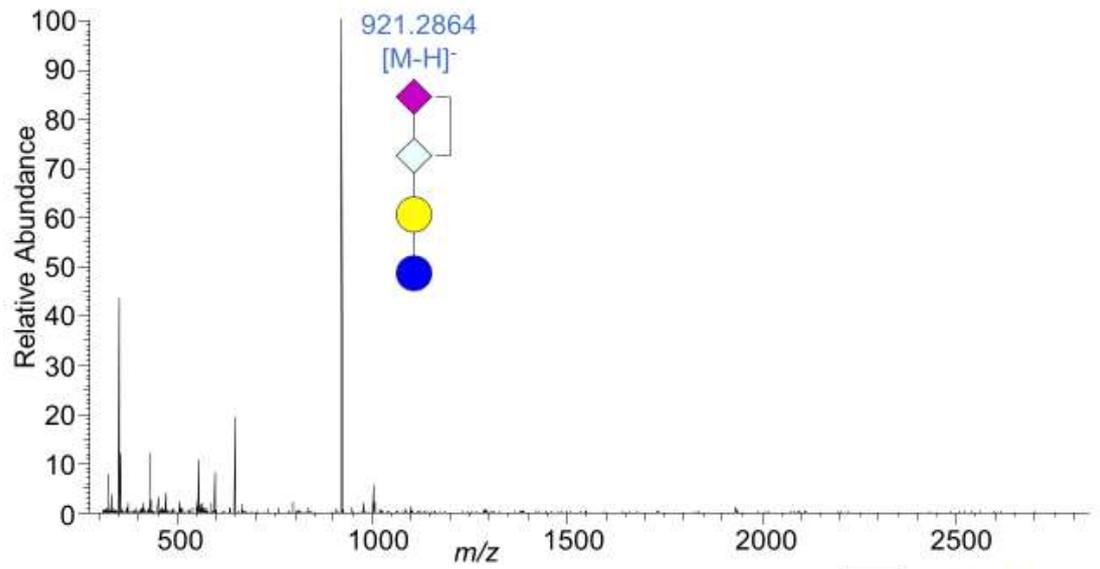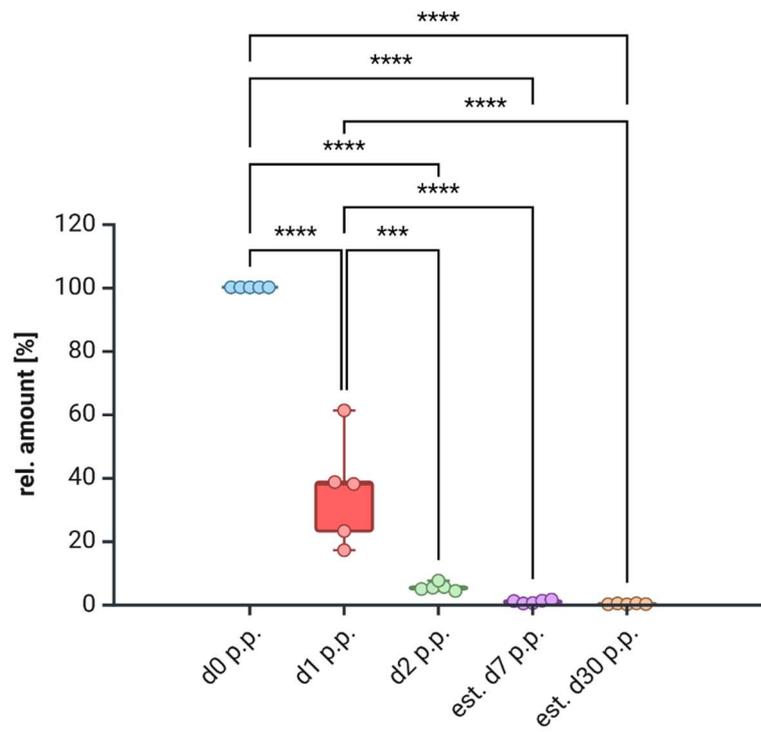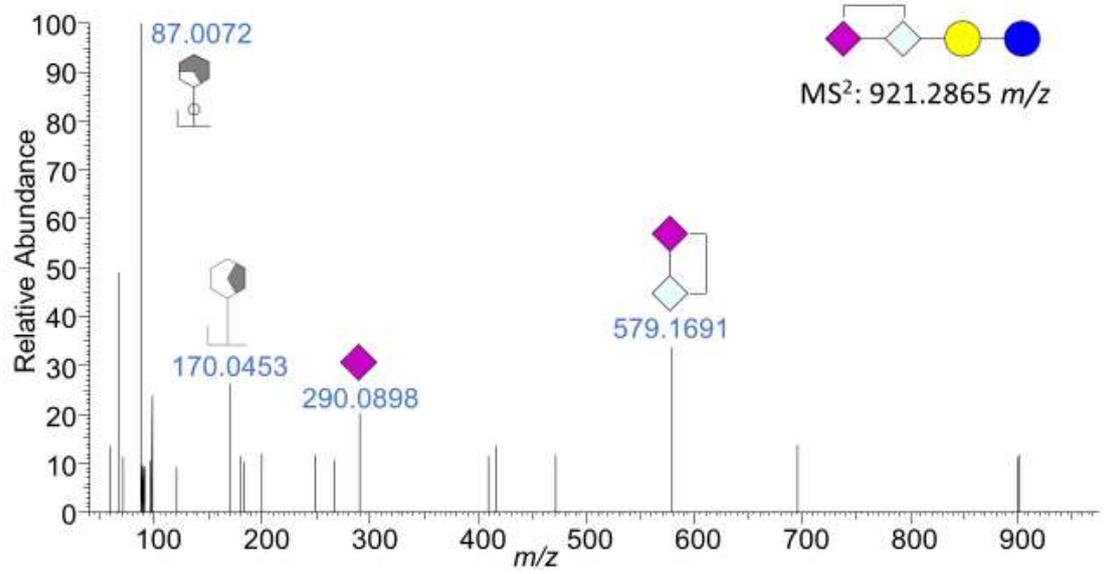
#18
(RT 12.7 min)
A

B

C

**#18
(RT 8.5 min)
A**

921.2869
[M-H]⁻

B

87.0072

MS²: 939.2968 *m/z*

579.1716
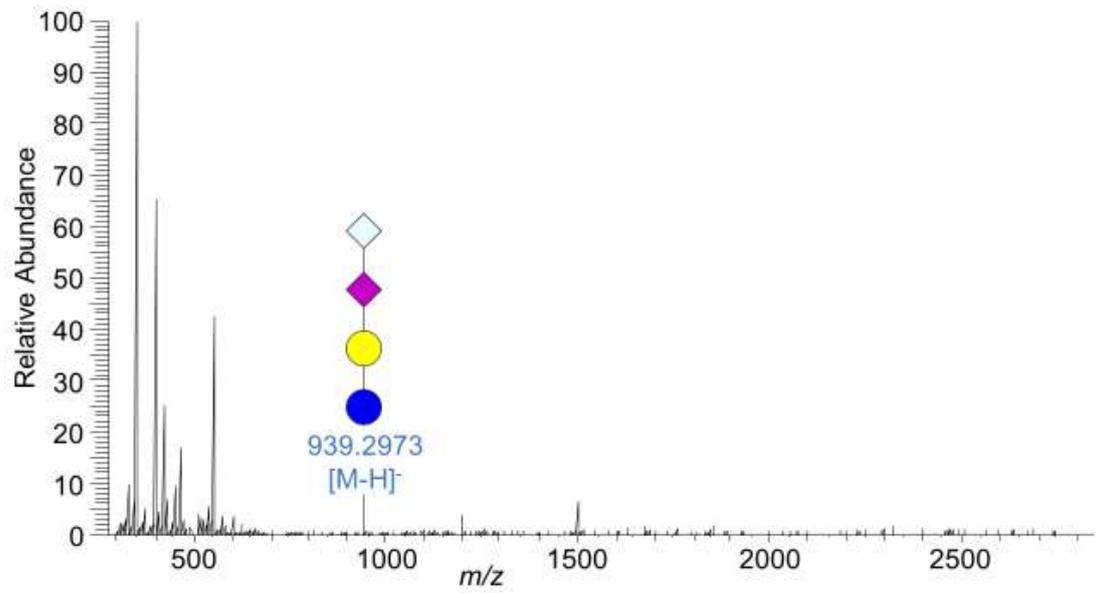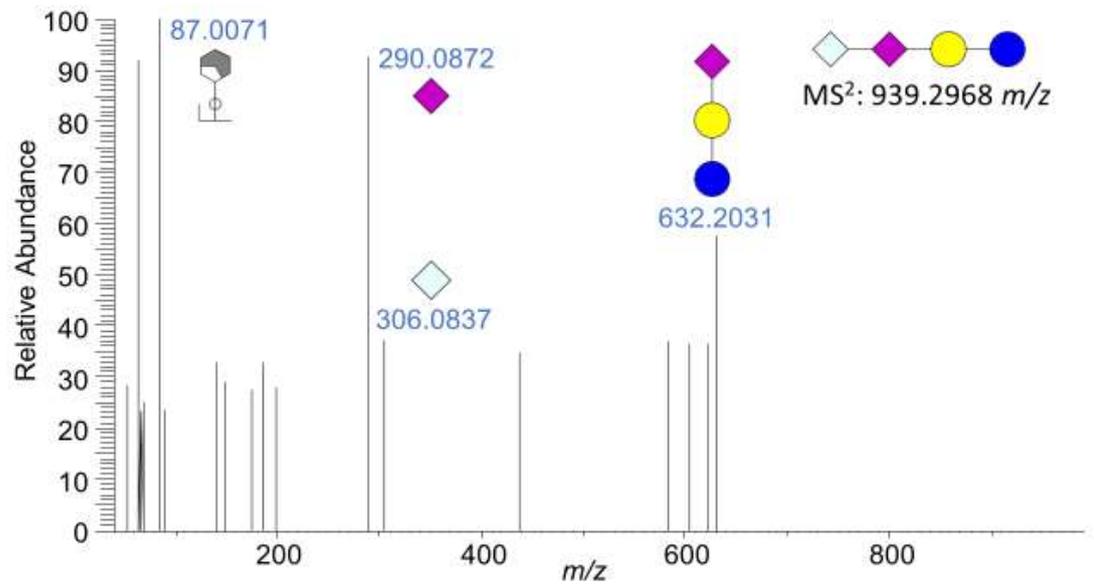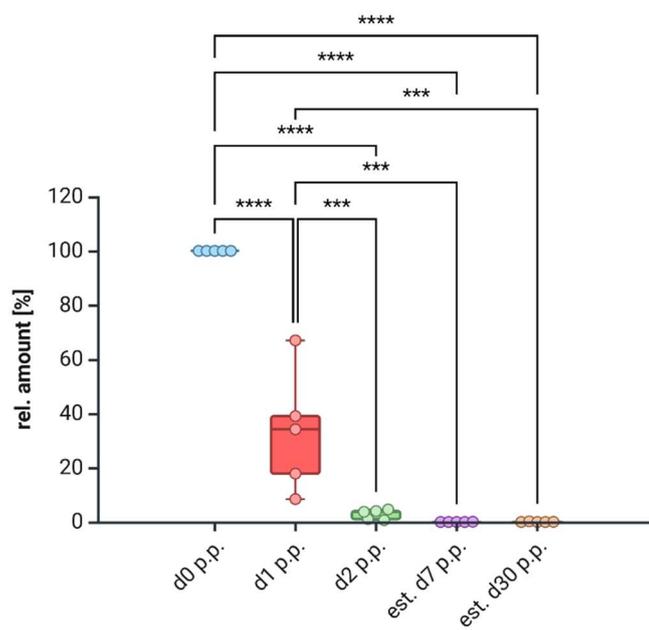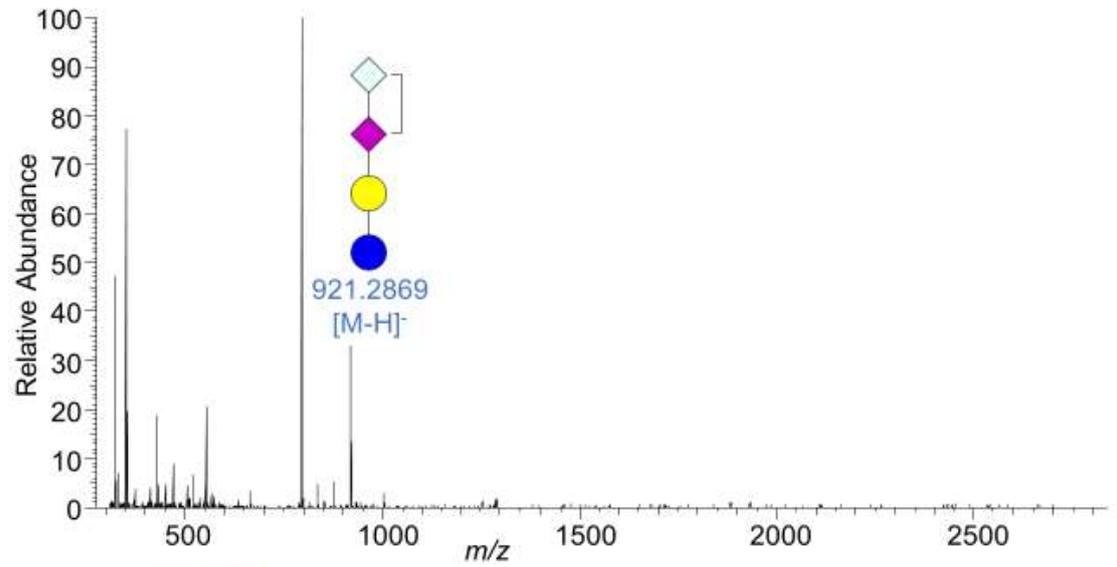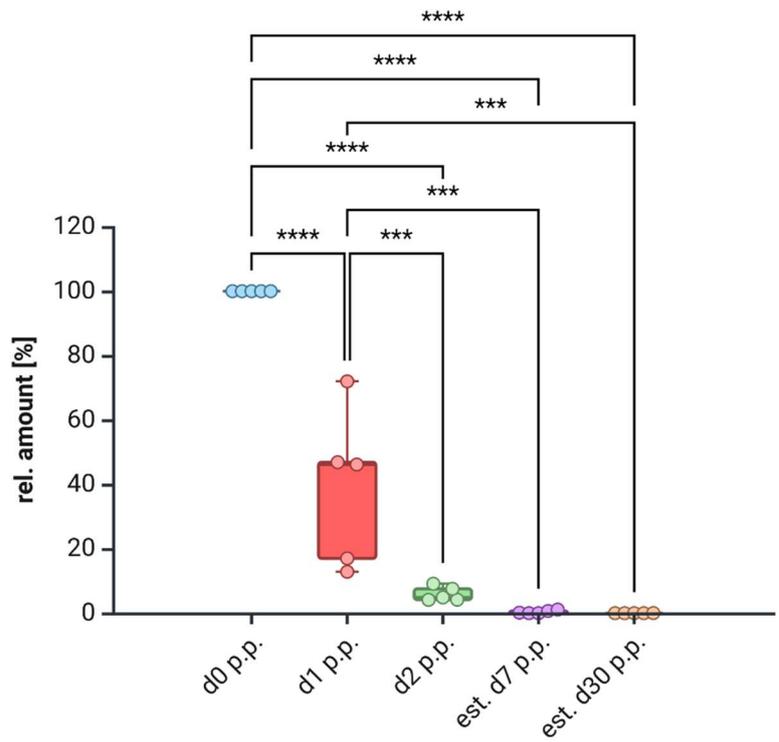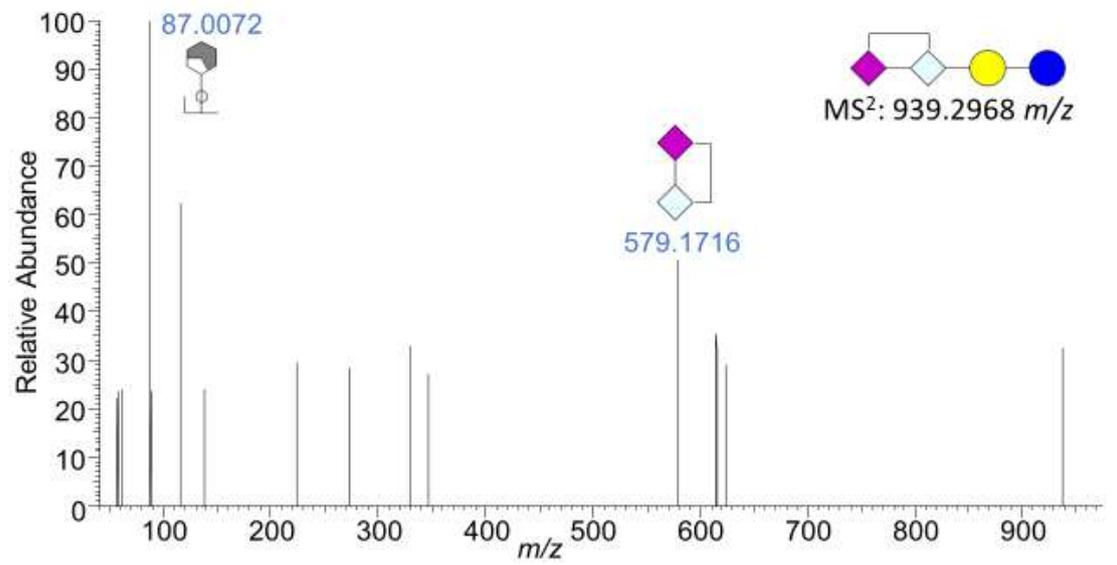
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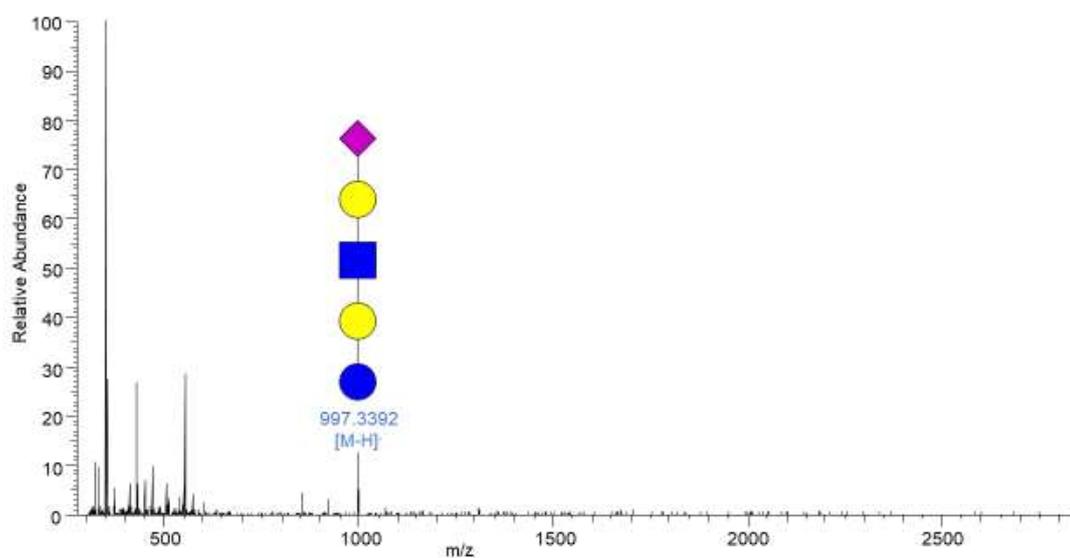
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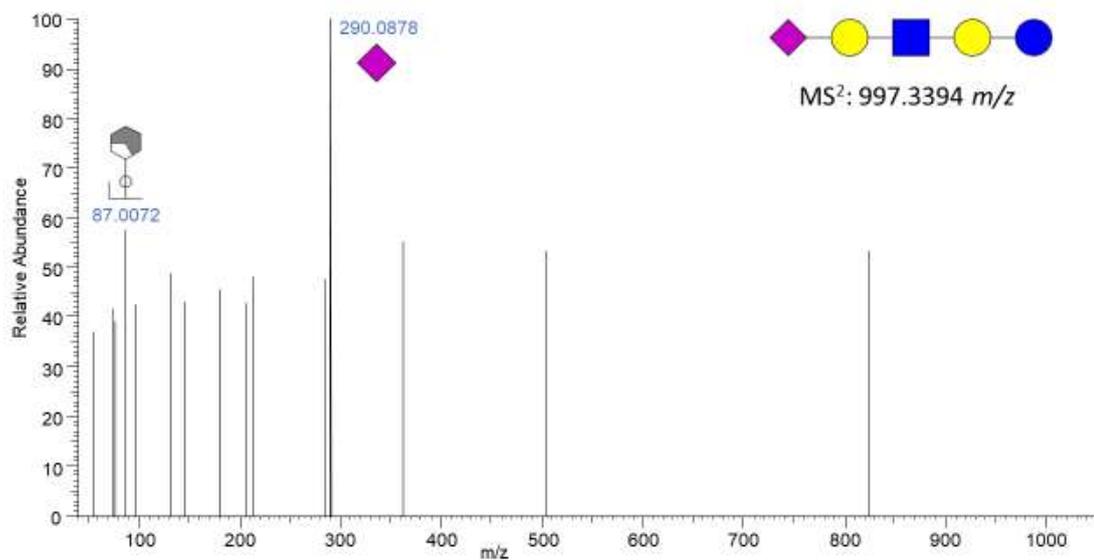
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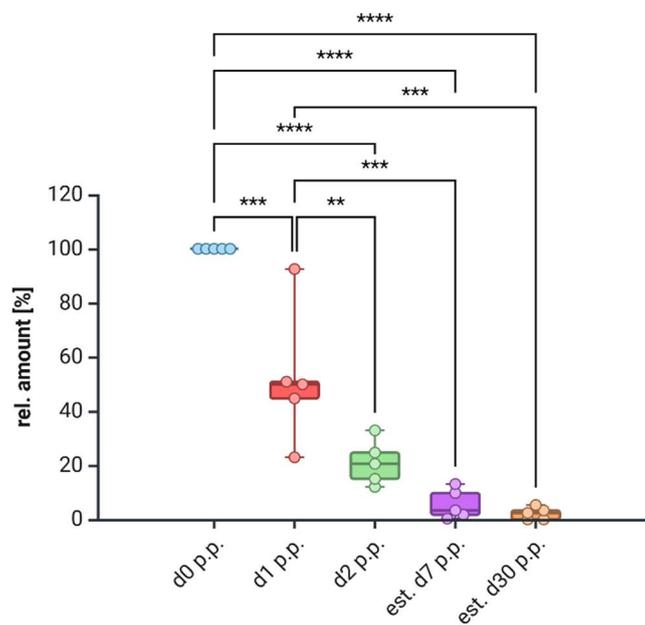
**#19
(RT 12.1 min)**

A

B

C

**#21
(RT 13.9 min)
A**

B

MS^2: 579.1916 *m/z*
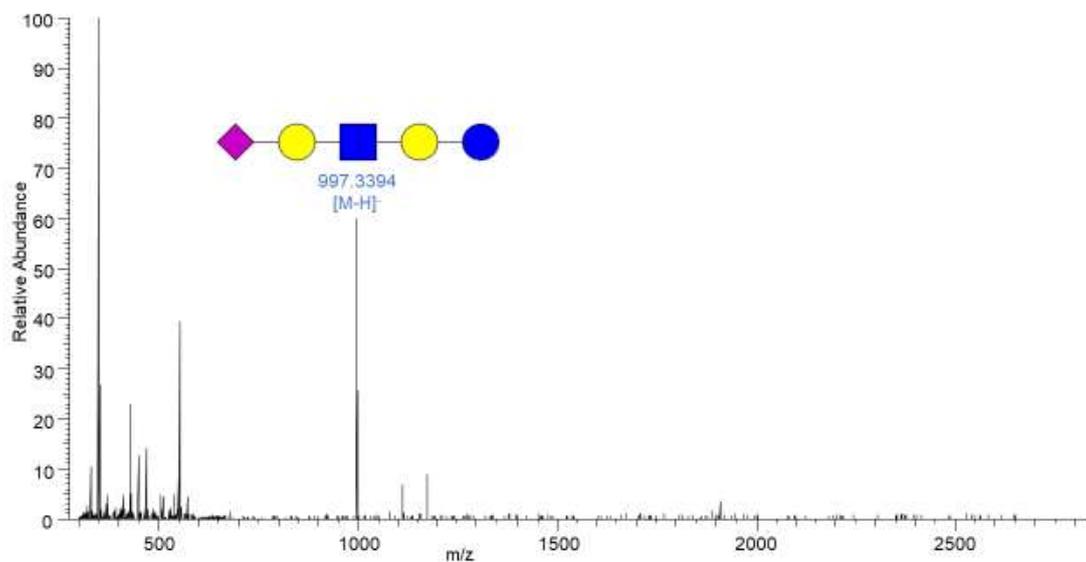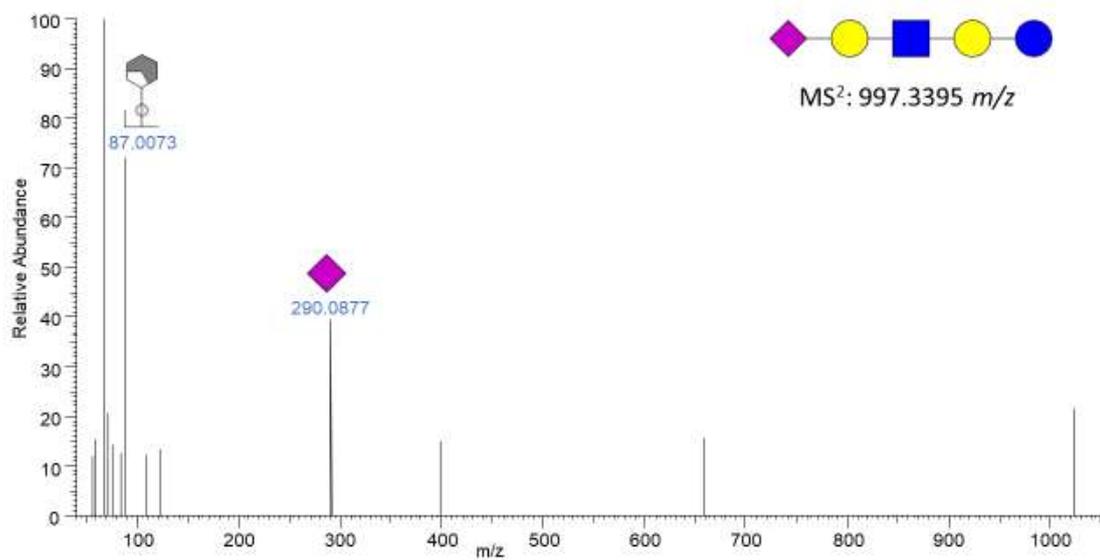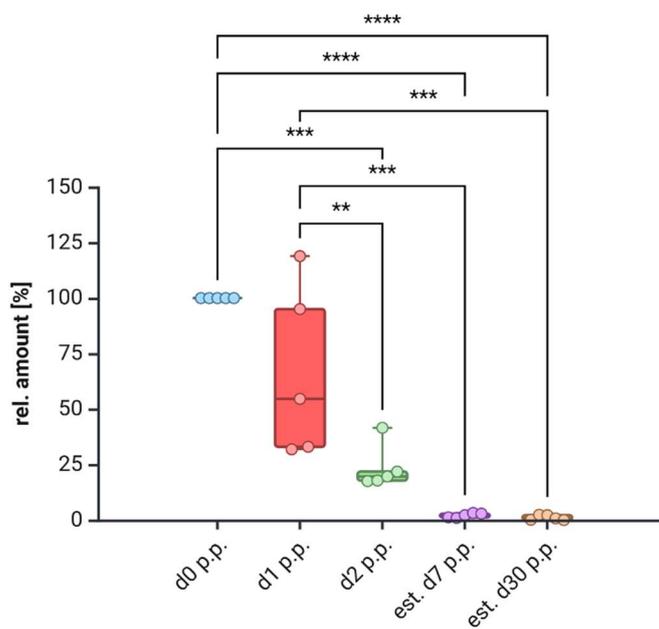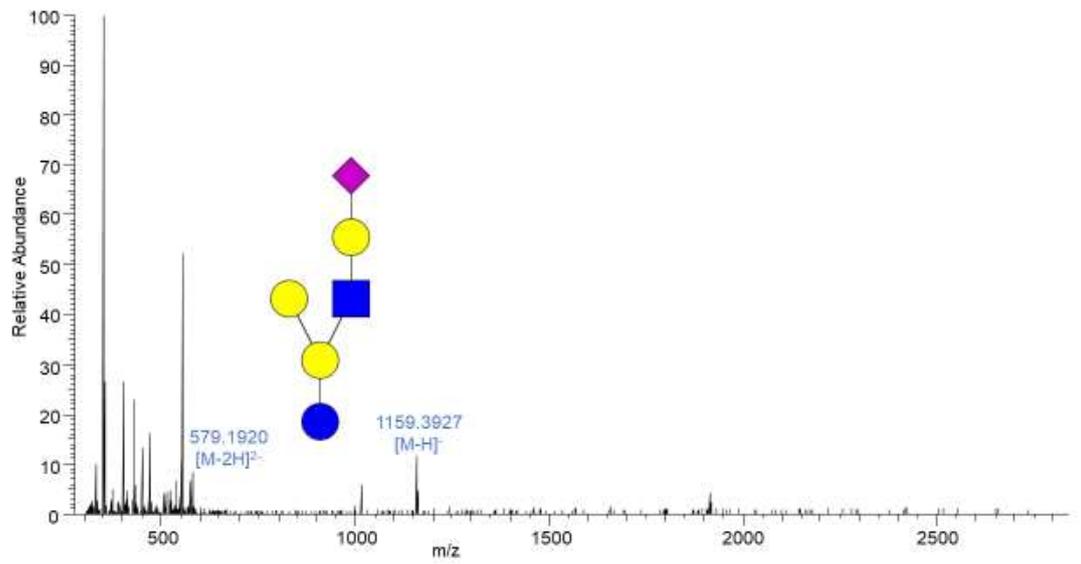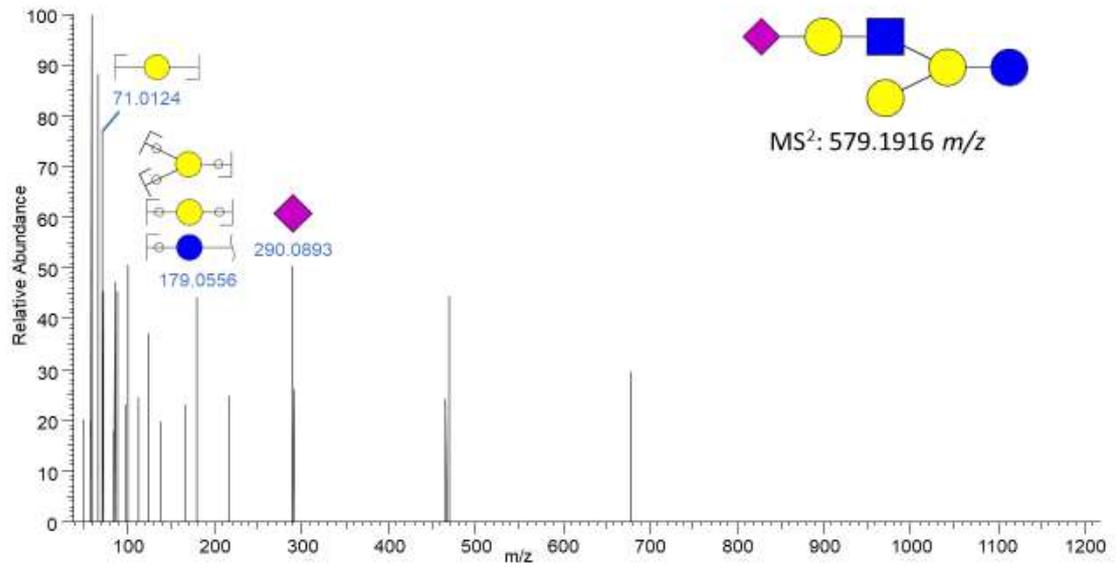
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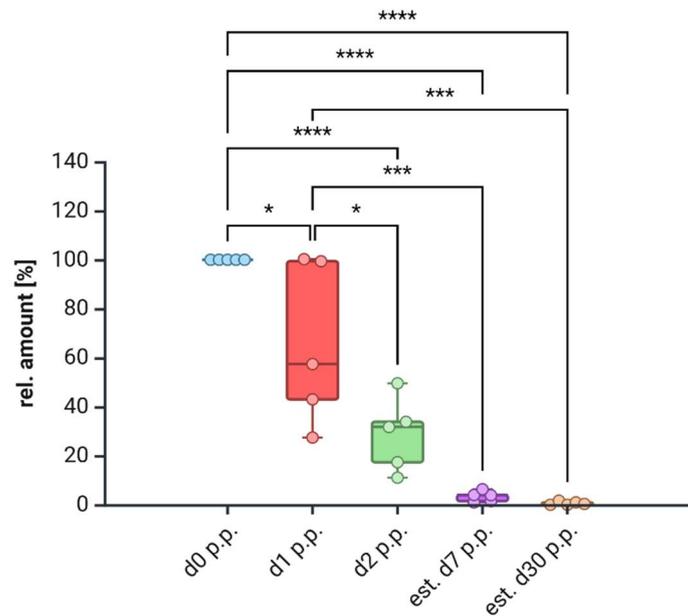
#21
(RT 15.4 min)
A

B

C

#22
(RT 12.7 min)

A

B

MS^2: 1200.4187 m/z

C

#22
(RT 14.0 min)
A

B

MS^2: 1200.4147 m/z

C

#23
(RT 16.7 min)

A

B

MS²: 660.2186 *m/z*
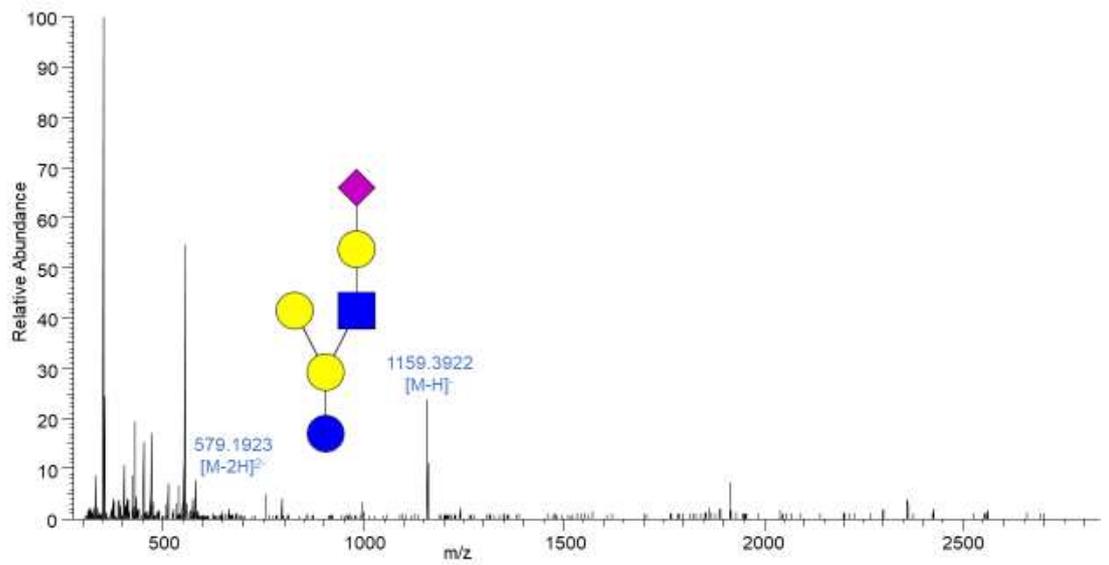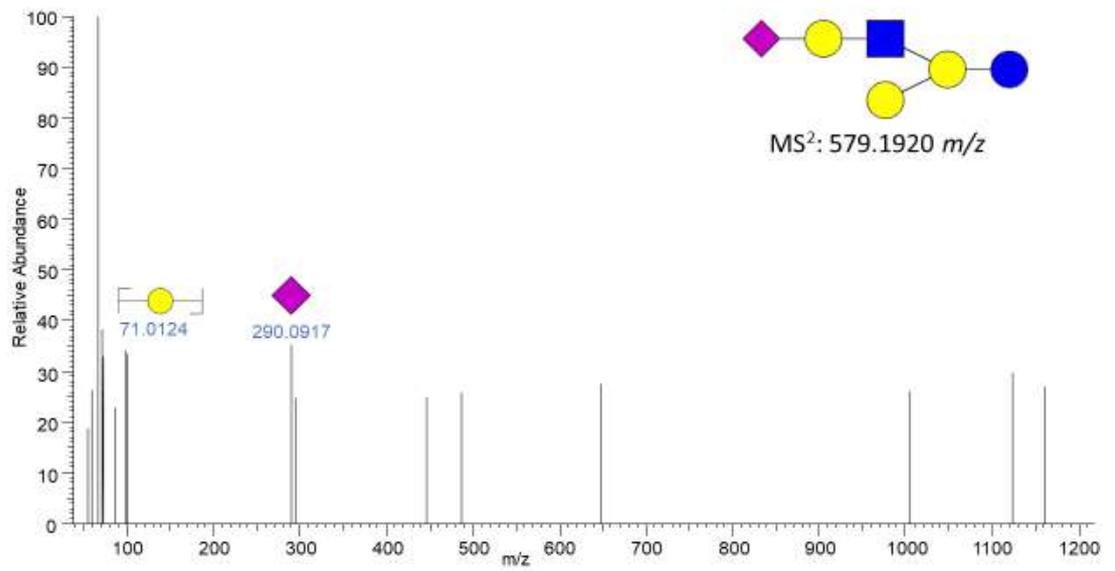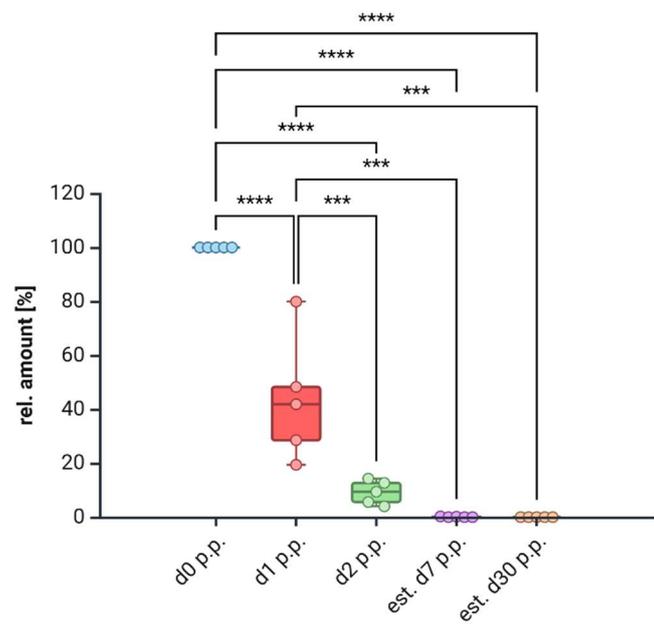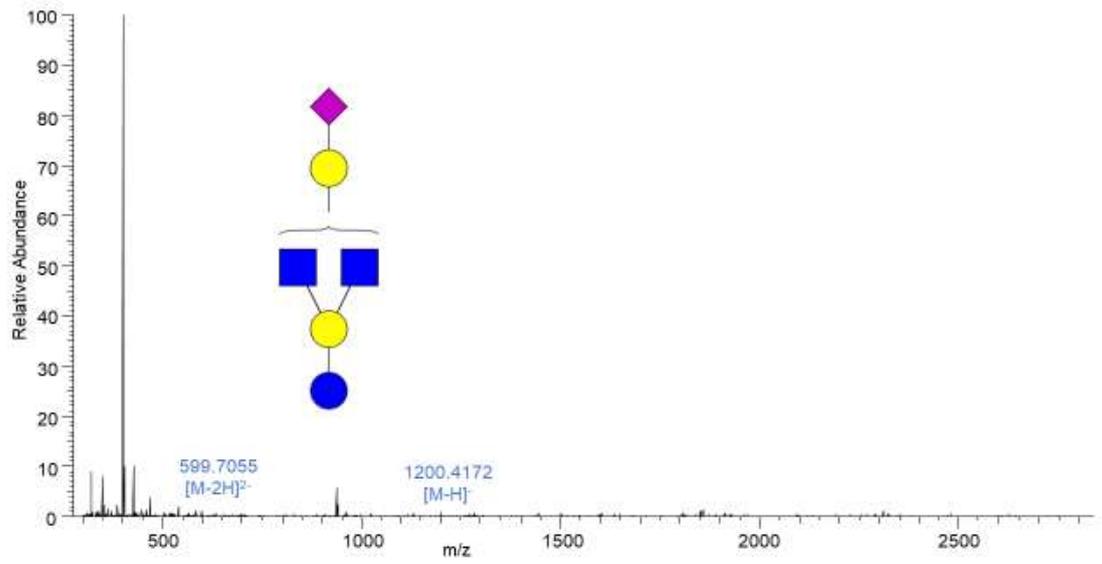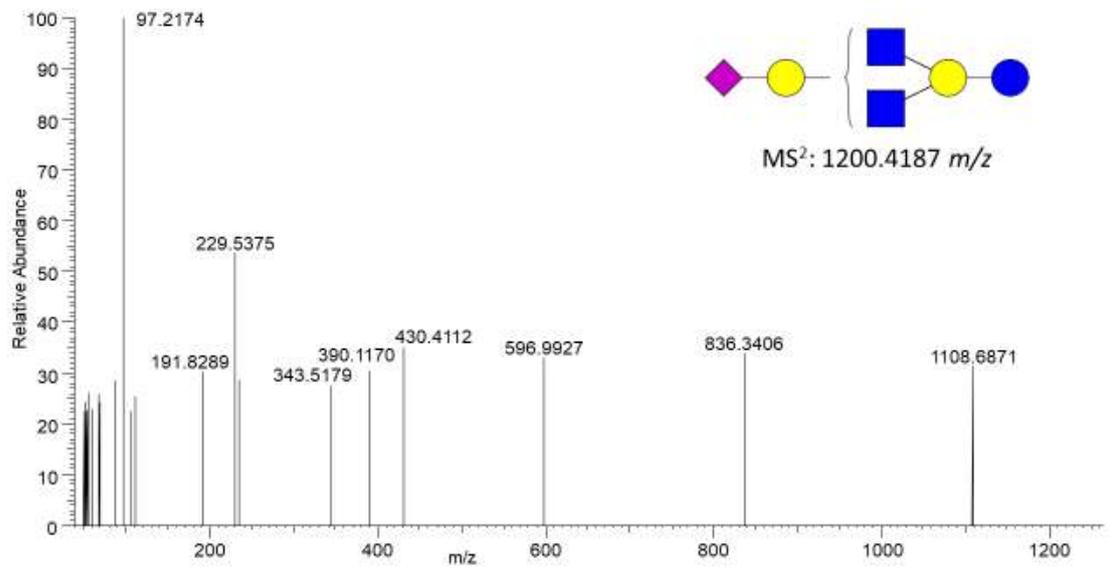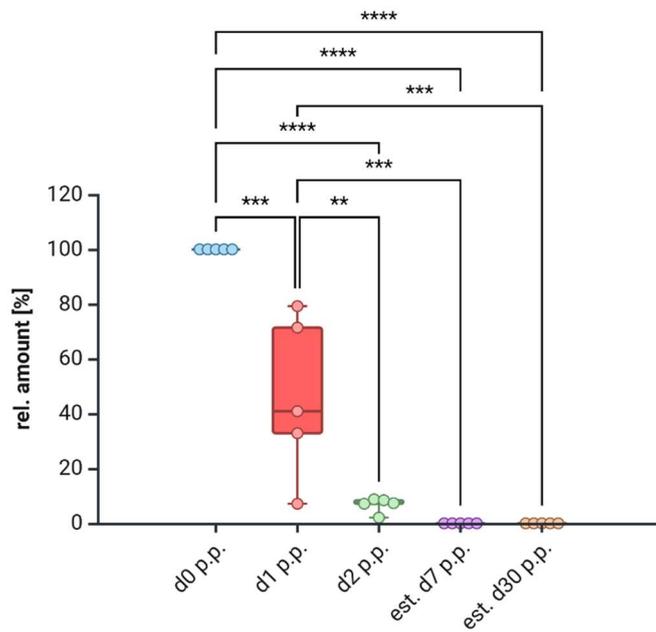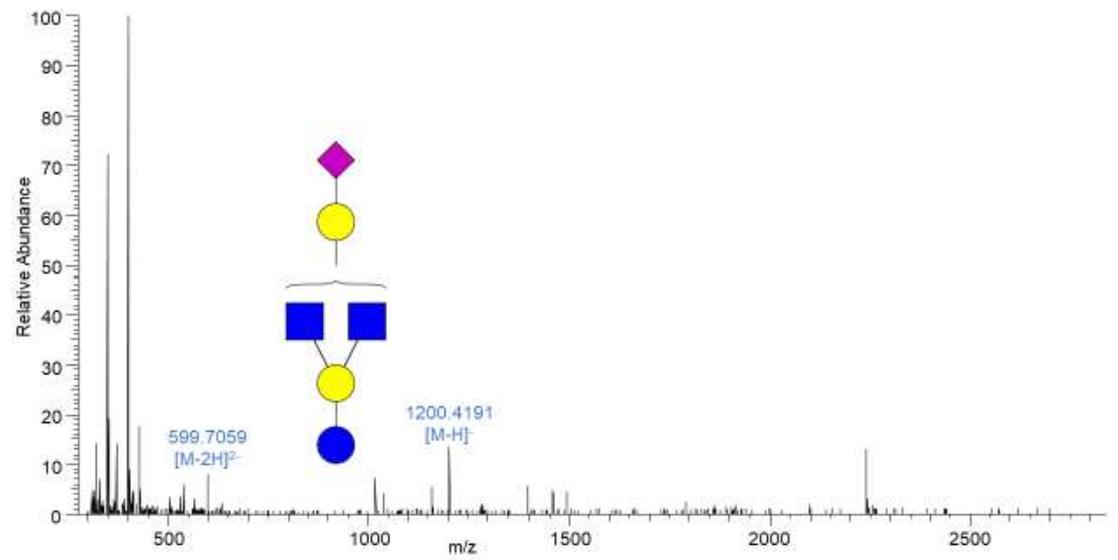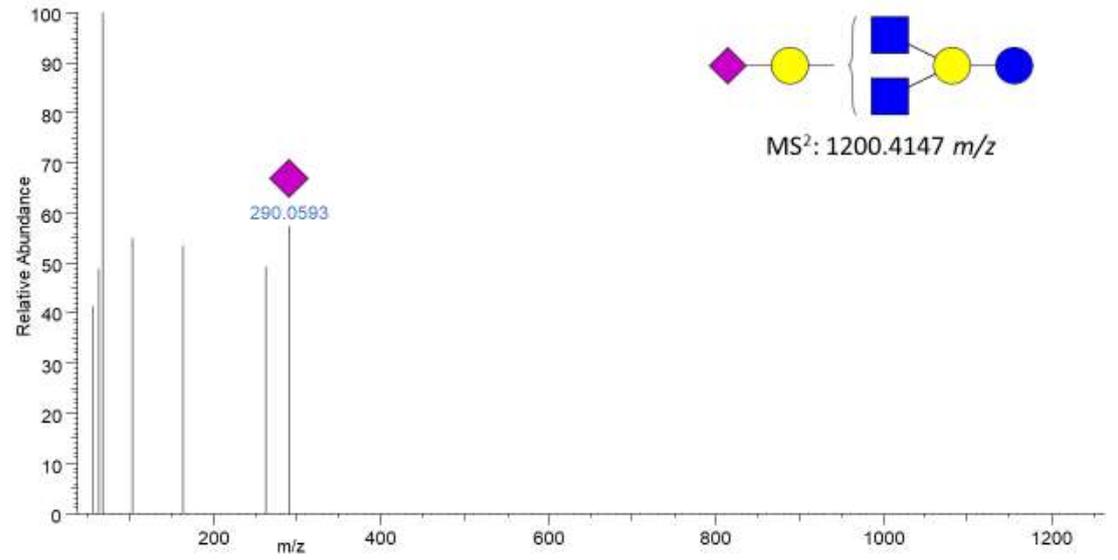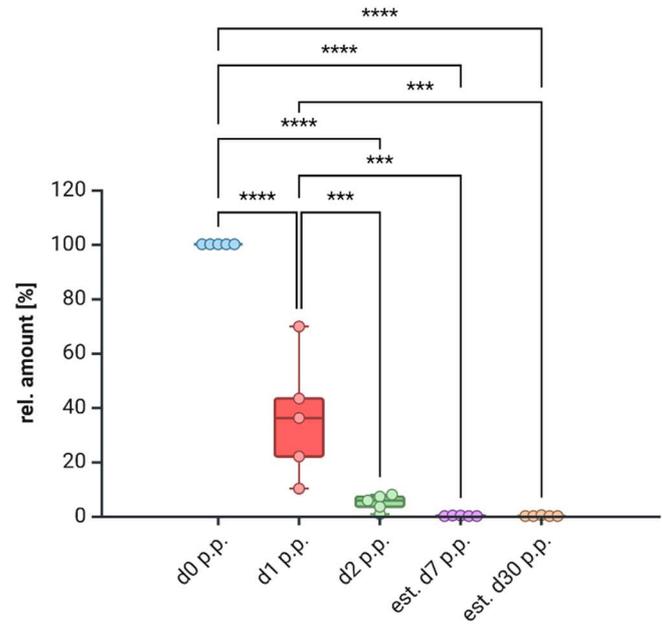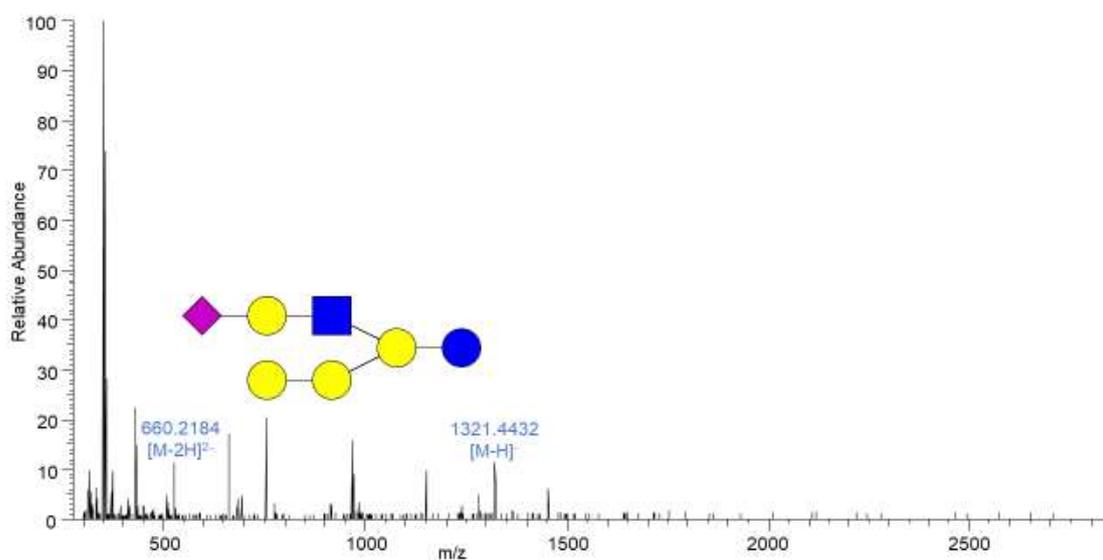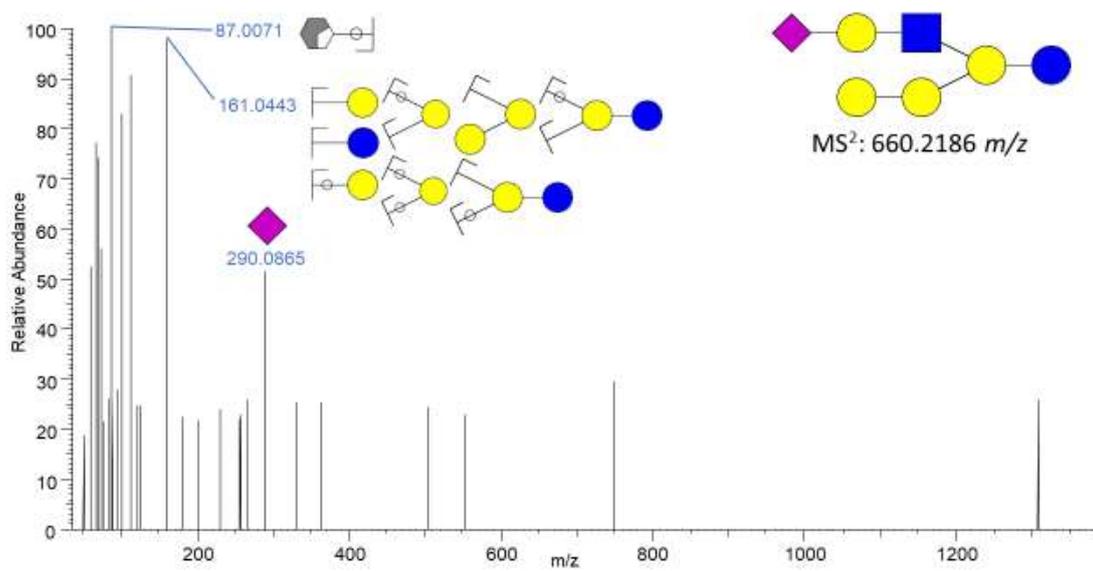
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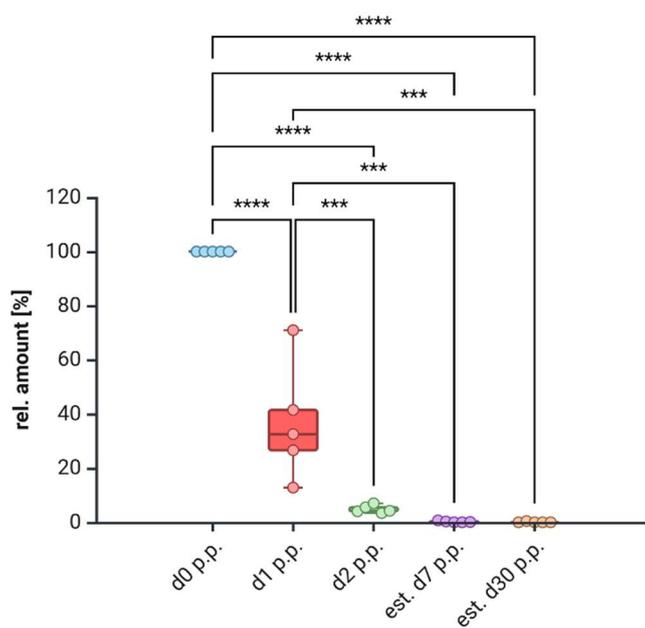
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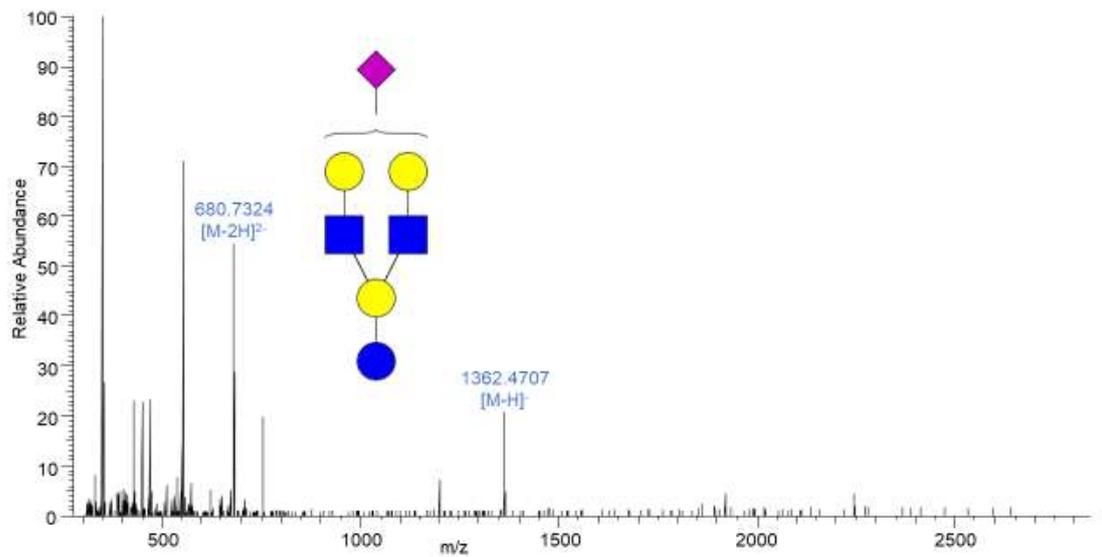
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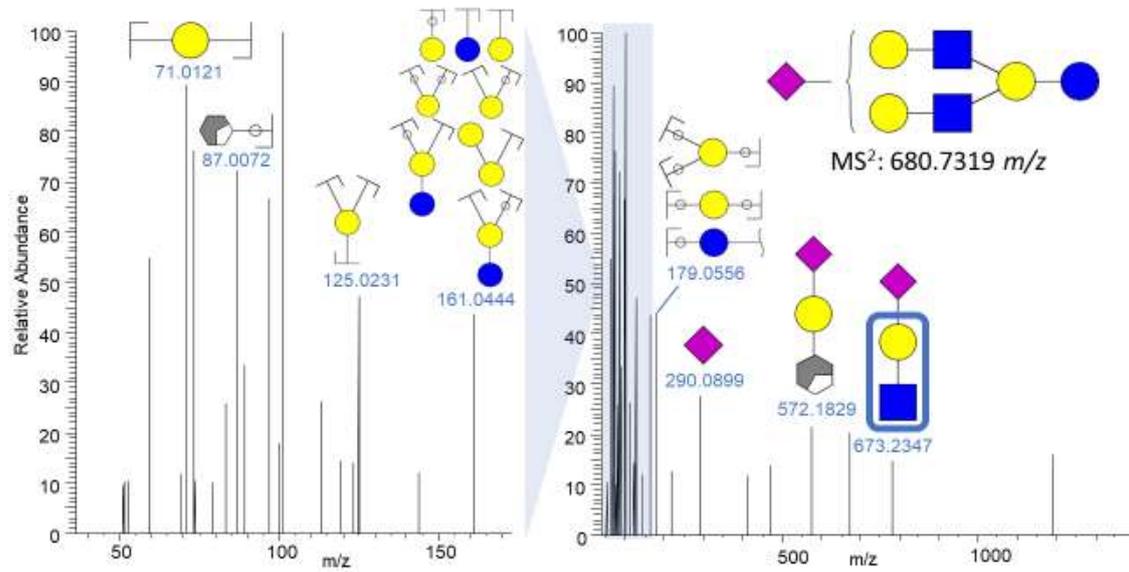
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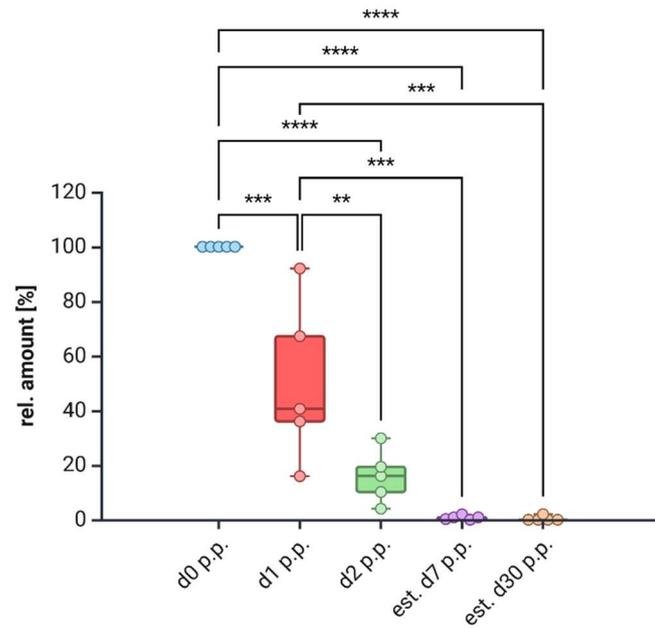
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(RT 20.3 min)
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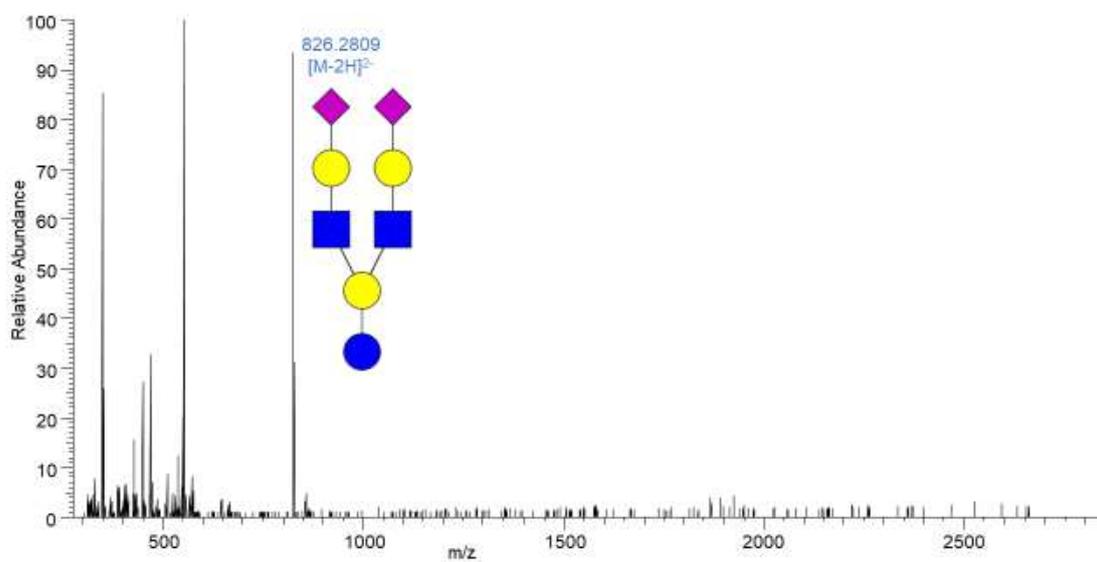
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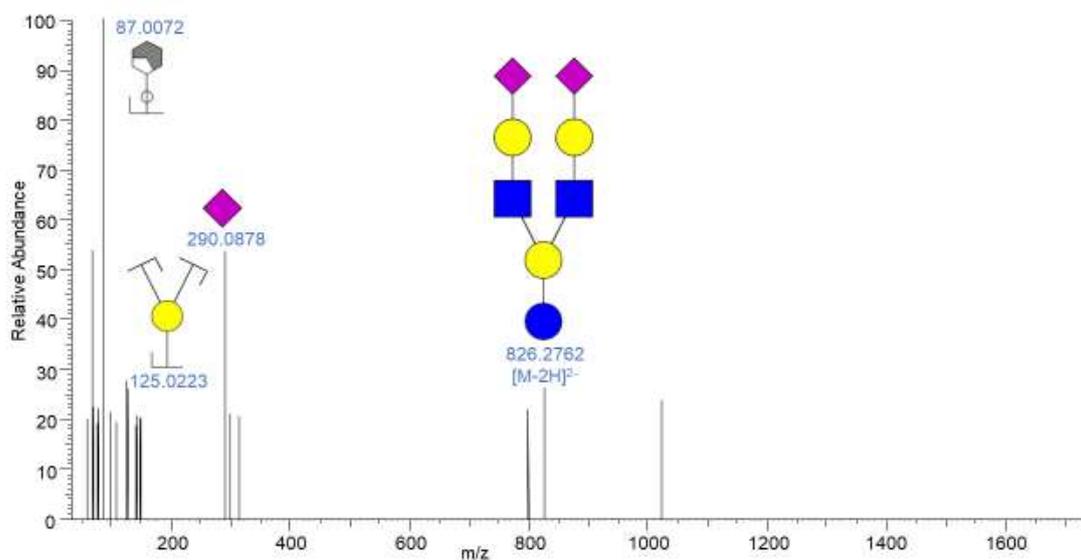
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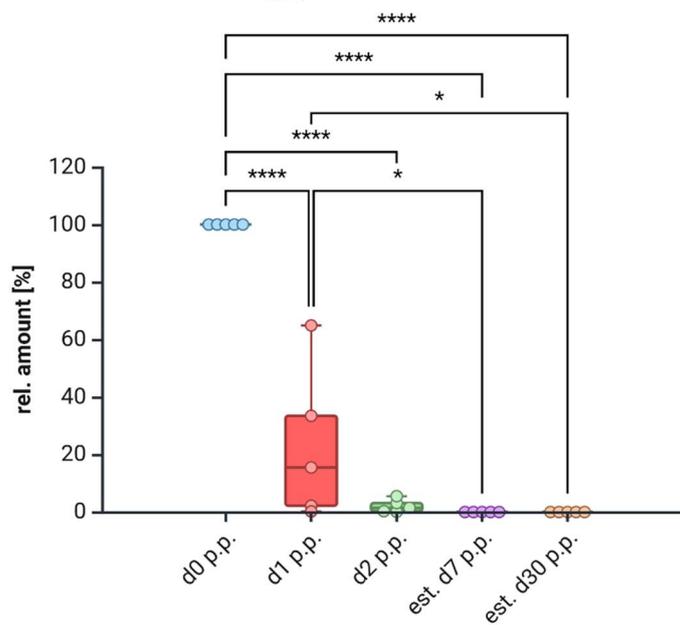
Suppl. Figure 1: LC-MS data of all detected sialylated bMOs in Holstein-Friesian cows during early lactation. A) MS spectra of sialylated bMOs at the corresponding retention time showing the deprotonated molecular ions. B) Corresponding MS^2 spectrum for each sialylated bMO. C) The ratios of the peak areas of the sialylated bMOs were determined (n= 5 animals) and the Box & Whisker plots (median; min to max) are shown to illustrate the distribution of the corresponding bMo over the course of lactation. The statistical analysis and graphs were computed using BioRender.com. Significant differences are given: *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$, ****: $p<0.0001$. Extracted from the ThermoFisher Scientific Software Freestyle. The bMO structures were visualized with GlycoWorkbench 2 [43]. Selected spectra and/or graphs of Suppl. Figure 1 (#6, #8, #17, and #18) are also shown in Figures 4, 5, 6, 8 in the main part of this publication. Blue circle, glucose; yellow circle, galactose; blue square, N-acetylglucosamine; purple diamond, N-acetylneuraminic acid; light blue diamond, N-glycolylneuraminic acid.
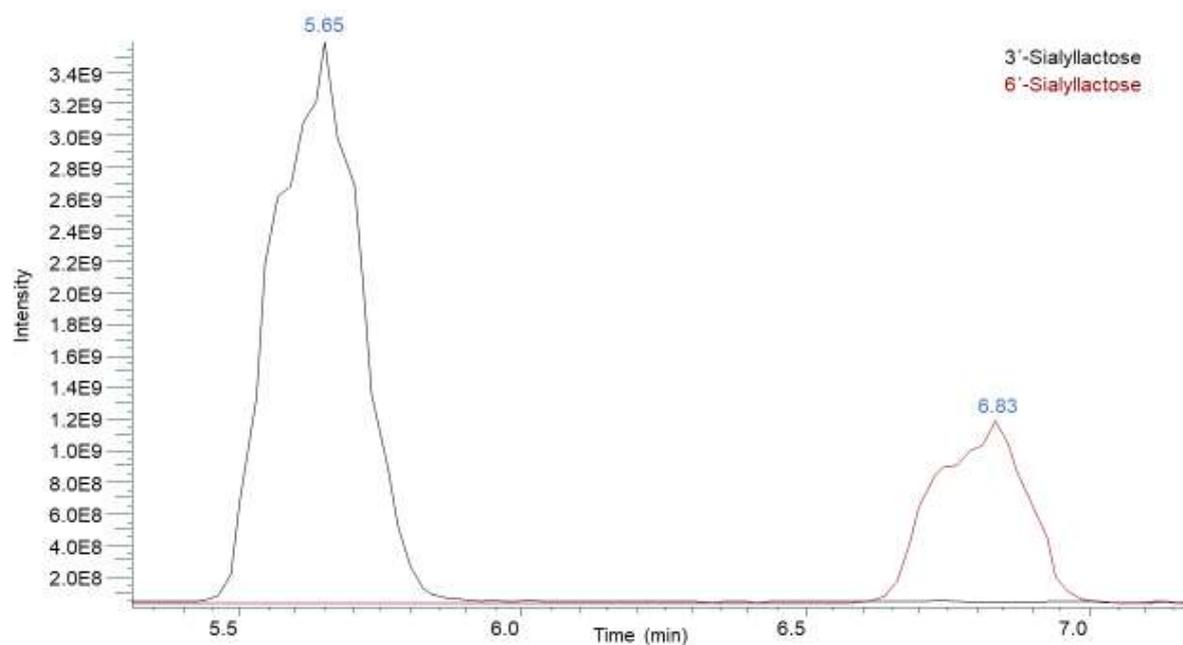
Suppl. Figure 2: Extracted ion chromatogram of 3′-SL (black) and 6′-SL (red) standards showing the respective retention time of both isomers. Extracted from the ThermoFisher Scientific Software Freestyle.
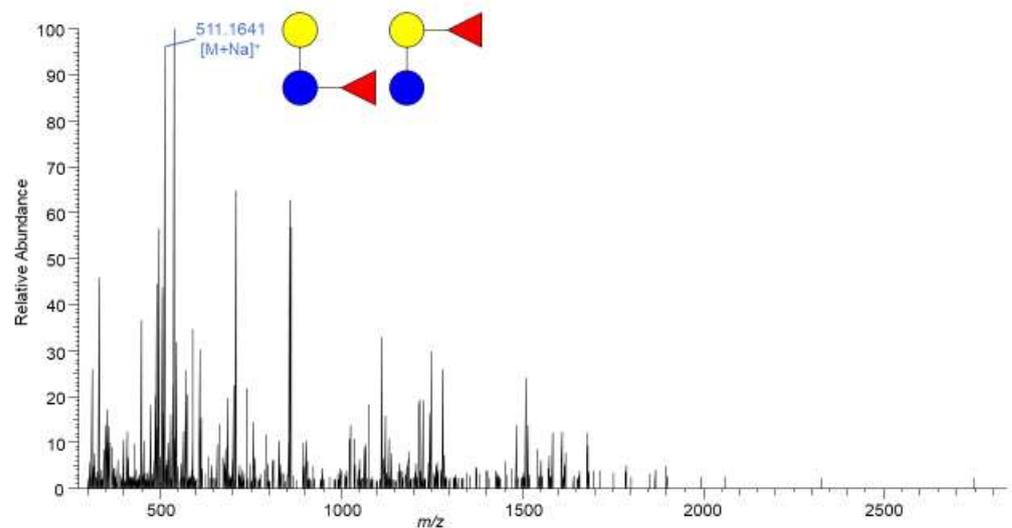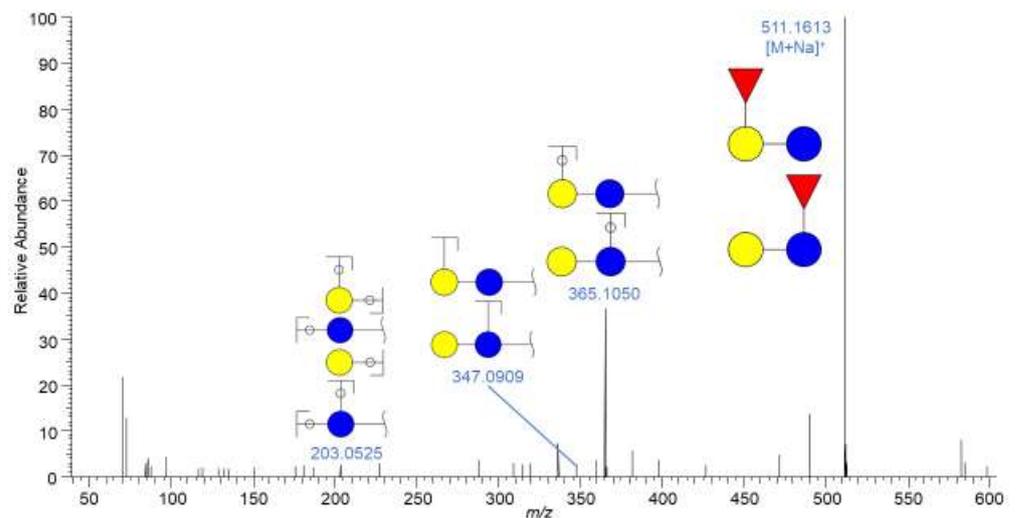
Suppl. Figure 3: LC-MS analysis of fucosyllactose (FL). A) MS spectrum of FL at 4.55 min showing the sodium-adduct [M+Na]$^+$ and B) the corresponding MS^2 spectrum. Extracted from the ThermoFisher Scientific Software Freestyle. The bMO structures were computed with GlycoWorkbench 2 [43]. Blue circle, glucose; yellow circle, galactose; red triangle, fucose.
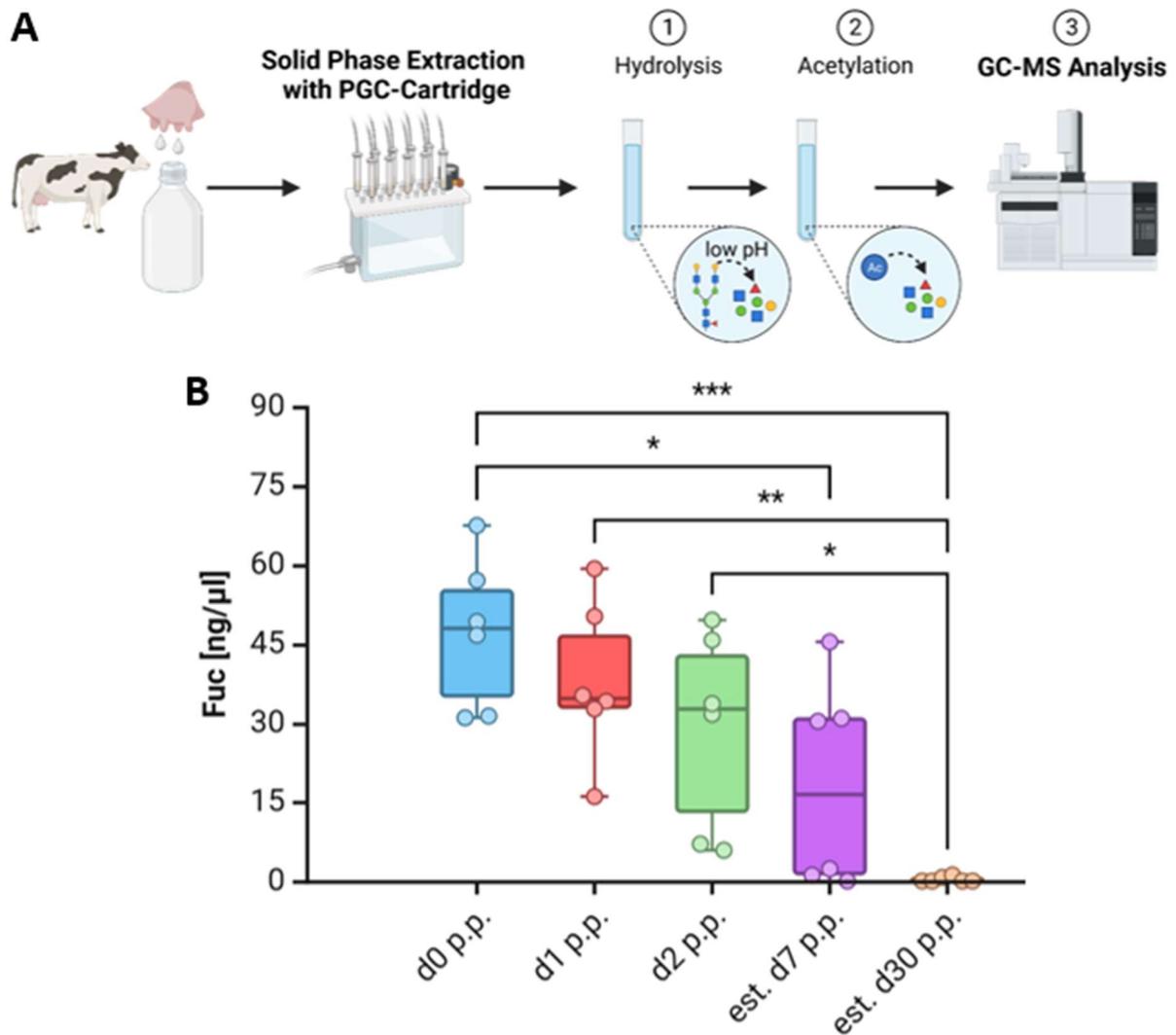
Suppl. Figure 4: Quantification of fucose (Fuc). A) Scheme of the alditol acetate strategy for Fuc quantification using GC-MS. The bMOs were isolated from defatted milk using PGC cartridges. The monosaccharides of the enriched bMOs were released by hydrolysis and the resulting monosaccharides were reduced and acetylated for the GC-MS analysis. Created with BioRender.com. B) Box & Whisker plot (median; min to max) shown for Fuc concentrations during early lactation (n=6 animals). The statistical analysis and graph were computed using BioRender.com. Significant differences are given: *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$.
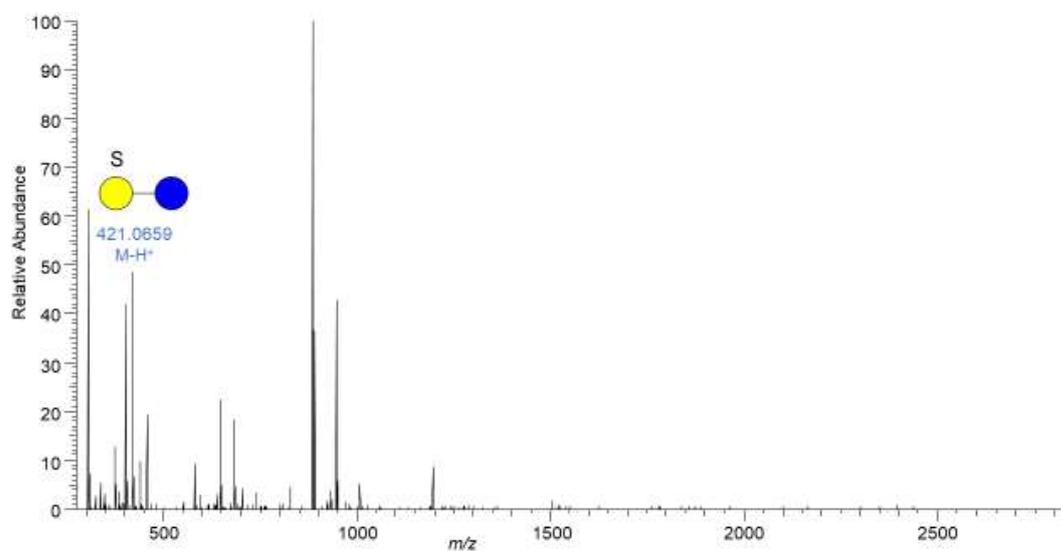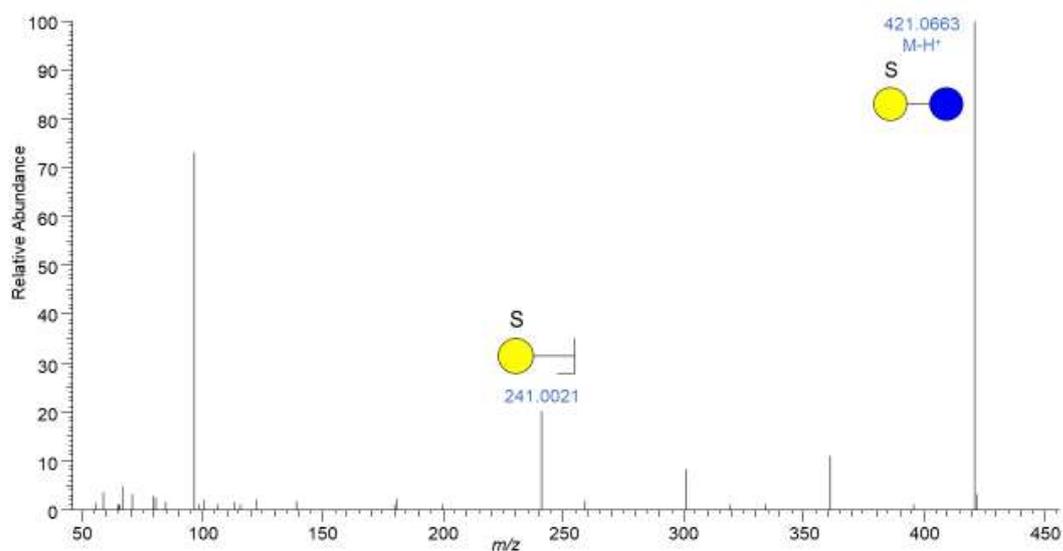
Suppl. Figure 5: LC-MS Analysis of sulfated lactose. A) MS spectrum at 3.06 min showing the deprotonated molecular ion [M-H]$^-$. B) Corresponding MS^2 spectrum at 3.05 min showing a fragment ion corresponding to a sulfated hexose. The bMO structures were computed with GlycoWorkbench 2 [43]. Blue circle, glucose; yellow circle, galactose.
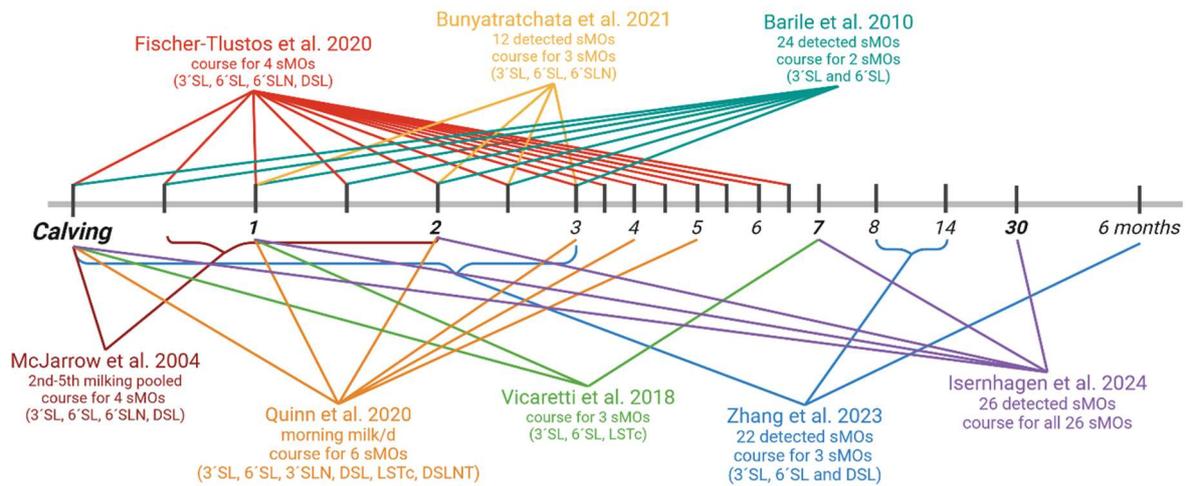
Suppl. Figure 6: Sampling Timeline in previous studies and the outlined analysis. Following references were used for this comparison: [28, 54, 80, 87-90] SL: sialyllactose; SLN: sialyllactosamine; DSL: disialyllactose; LST: Sialyl-lacto-N-tetraose; DSLNT: Disialyl-lacto-N-tetraose. Created with BioRender.com.