

Supporting Information

Differentiation and Relative Quantitation of Disaccharide Isomers by MALDI-TOF/TOF Mass Spectrometry

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Supporting Information

Calibration curves.

The calibration curves are built by plotting the ratio of α_A to α_B against the ratio of two isomer concentration c_A to c_B , which can be interpreted as

$$\frac{\alpha_A}{\alpha_B} = n \times \frac{C_A}{C_B}$$

The ratio of c_A to c_B was known, and the ratio of α_A to α_B can be calculated from the LIFT spectrum, as

$$R_{mix} = R_A \alpha_A + R_B \alpha_B = R_A \alpha_A + R_B (1 - \alpha_A)$$

Then $\alpha_A = \frac{R_{mix} - R_B}{R_A - R_B}$ and $\alpha_B = \frac{R_{mix} - R_A}{R_B - R_A}$ was obtained, the equation can be converted to

$$\frac{\alpha_A}{\alpha_B} = \frac{R_{mix} - R_B}{R_A - R_{mix}}$$

The standard variations are calculated using the error propagation formula. To calculate the standard variation of α values, the equation can be displayed as

$$\alpha = \frac{b}{c}$$

where $\alpha = \frac{\alpha_A}{\alpha_B}$, $b = R_{mix} - R_B$, $c = R_A - R_{mix}$, then the standard variation relationship are

$$\frac{S_\alpha^2}{\alpha^2} = \frac{S_b^2}{b^2} + \frac{S_c^2}{c^2}$$

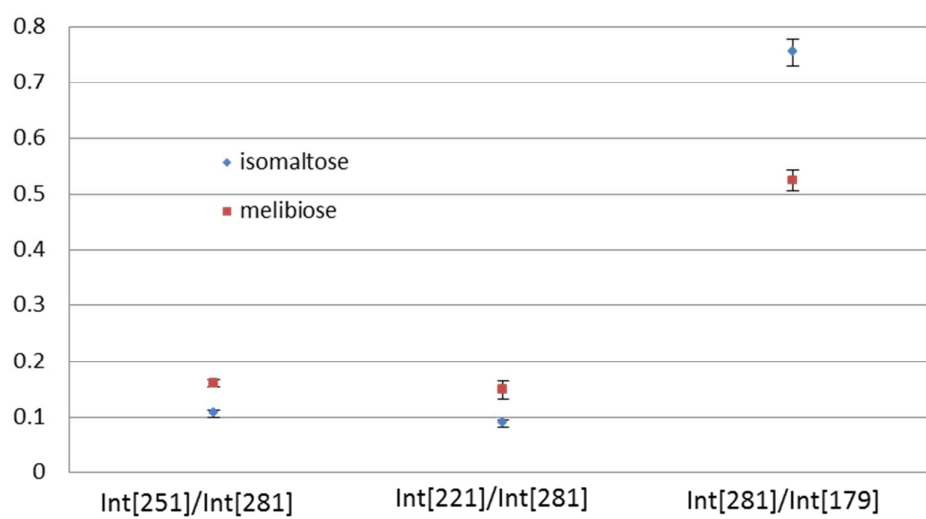


Figure S1. Discrimination of compositional isomers, isomaltose and melibiose. Error bars show the standard deviation of eight measurements.

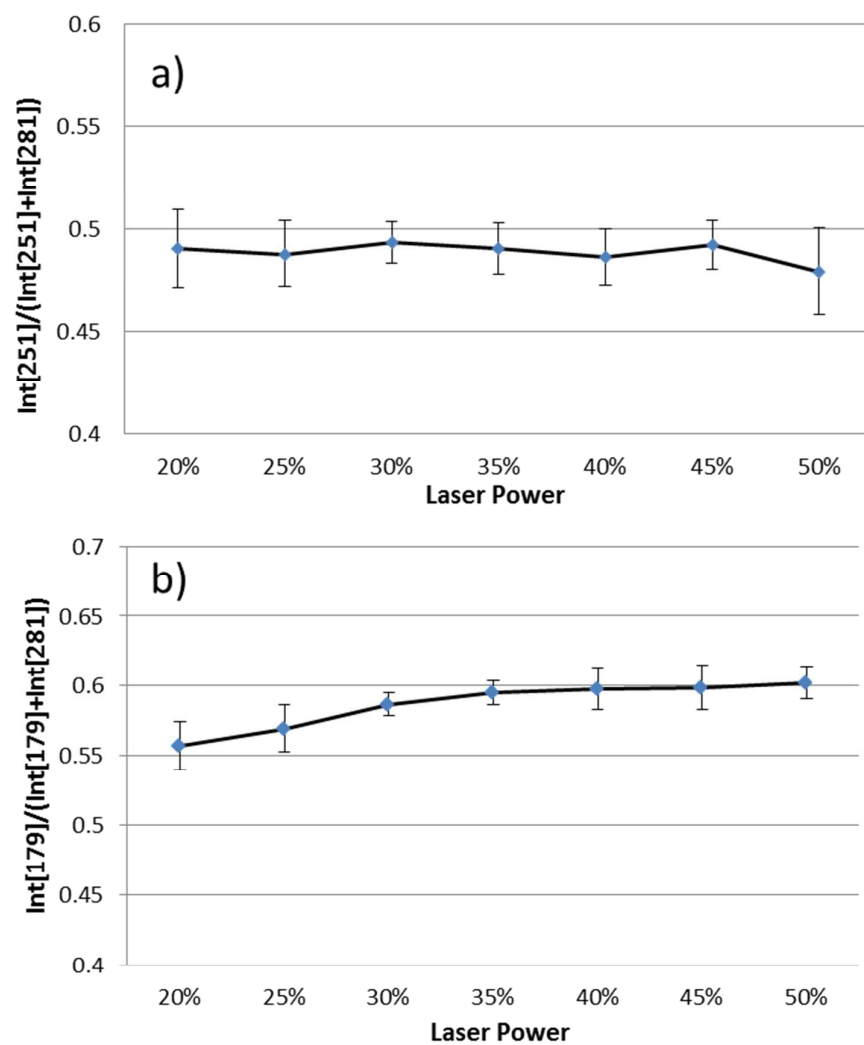


Figure S2: Relative peak intensity (RPI) as a function of laser power in the case of (a) gentiobiose and (b) isomaltose. It was shown that the RPI is fairly stable regardless of laser power.

CID of disaccharides by MALDI-Q-FTICR. The low energy CID of disaccharides was done by MALDI-Q-FTICR (9.4T Solarix, Bruker). The instrument settings were for collision energy (8.5V), isolation window (5 m/z), laser power (65%), laser shots (50), plate offset (-100V), deflector plate (-180V).

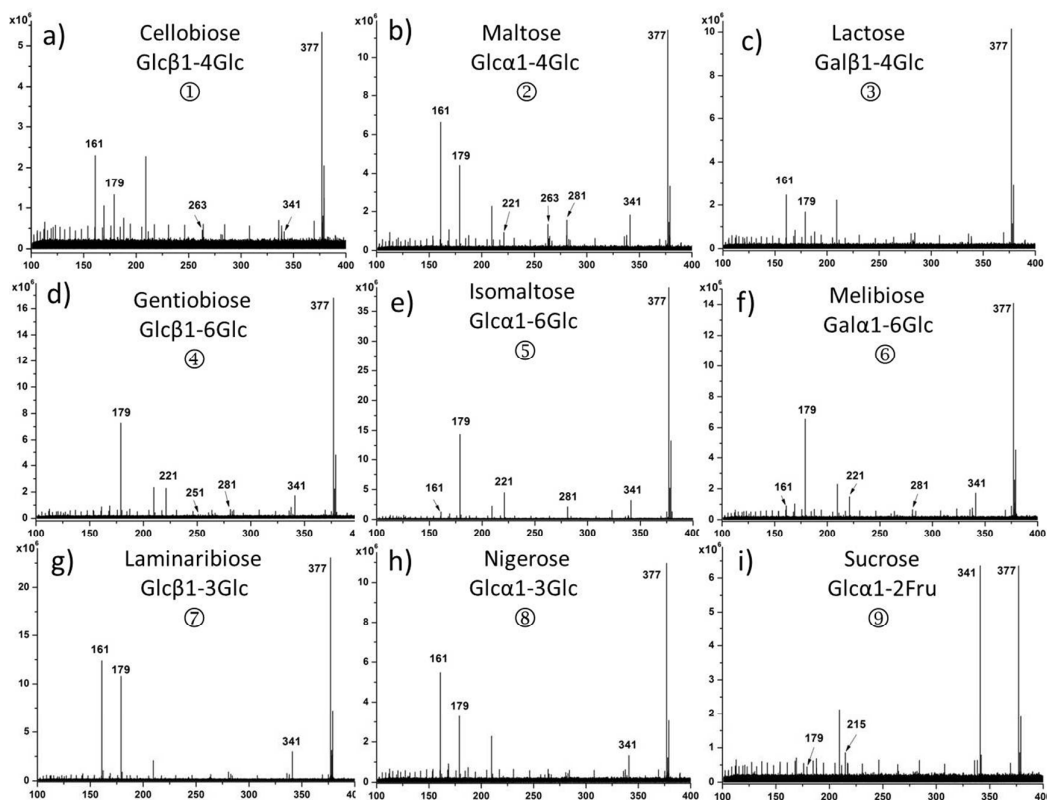


Figure S3. Tandem mass spectra of nine disaccharides acquired by MALDI-Q-FTICR. Most of the unlabeled peaks in the spectra were electronic noise. The correct peaks of product ions can be identified according to the high resolution mass spectra and the isotopic peaks.

Limit of detection. The ratio of solution concentration is not comparable to the ratio of gas phase quantity, because of that the detecting and fragmenting efficiencies of disaccharide isomers were different. We determine the limit of detection of tandem MS of these disaccharide isomers considering the most intensive peak ($S/N > 3$), most of which are around $0.1 \mu\text{M}$. Because of the coffee ring effect in MALDI-MS, it was not applicable to compare the detection efficiency directly from a single spectrum. We compare the most intensive product ion of these disaccharides at various concentrations, it can be estimated that the detecting sensitivity of tandem MS was ranked for the studied disaccharide isomer pairs as cellobiose > lactose, isomaltose > maltose, isomaltose > gentiobiose and maltose ~ sucrose. When one low abundance isomer was very low, i.e. c_A/c_B is lower than 0.1, it may not be detectable in the presence of one high abundance isomer and not contribute to the spectra of the binary mixture. We determine the calibration curve of the four studied disaccharide isomers in this situation that when c_A/c_B is lower than 0.1 or larger than 10. Five mixture of disaccharide isomers (A:B in 100:1, 100:2.5, 100:5, 100:7.5 and 100:10) were prepared. The case of that maltose was very low in the mixture of sucrose and maltose was analyzed in Fig 5. The calibration curve was built as described in the

main text. The results showed that the situations that the gas phase ratio was not proportional to the solution molar ratio were found in two cases that when lactose was very low in the mixture of lactose and cellobiose and when gentiobiose was very low in the mixture of gentiobiose and isomaltose (data not shown). It was found that the linearity of the calibration curve holds in the mixture of isomaltose and maltose when isomer ratio was lower than 0.1 (Fig.S4 b and c). However, the fitting equations were different from each other, indicating the linearity may not hold through 1:100 to 100:1. The performance of quantitation was not only affected by the limit of detection of the disaccharides, but also the fragment ions selected to build the calibration curve. When the compositional isomers, cellobiose and lactose, were analyzed in the situation, the linearity hold only when cellobiose was the low abundance one (Figure S4a). The fitting equation was similar with that in Fig.2. This phenomenon may attribute to that the detection efficiency of cellobiose was higher than that of lactose and the fragments selected were the lower abundant ones (m/z 263 and 281). The similar phenomenon was found for the configurational isomers, gentiobiose and isomaltose (Figure S4d) and the maltose-sucrose isomer pair (Figure S4e and Fig 5).

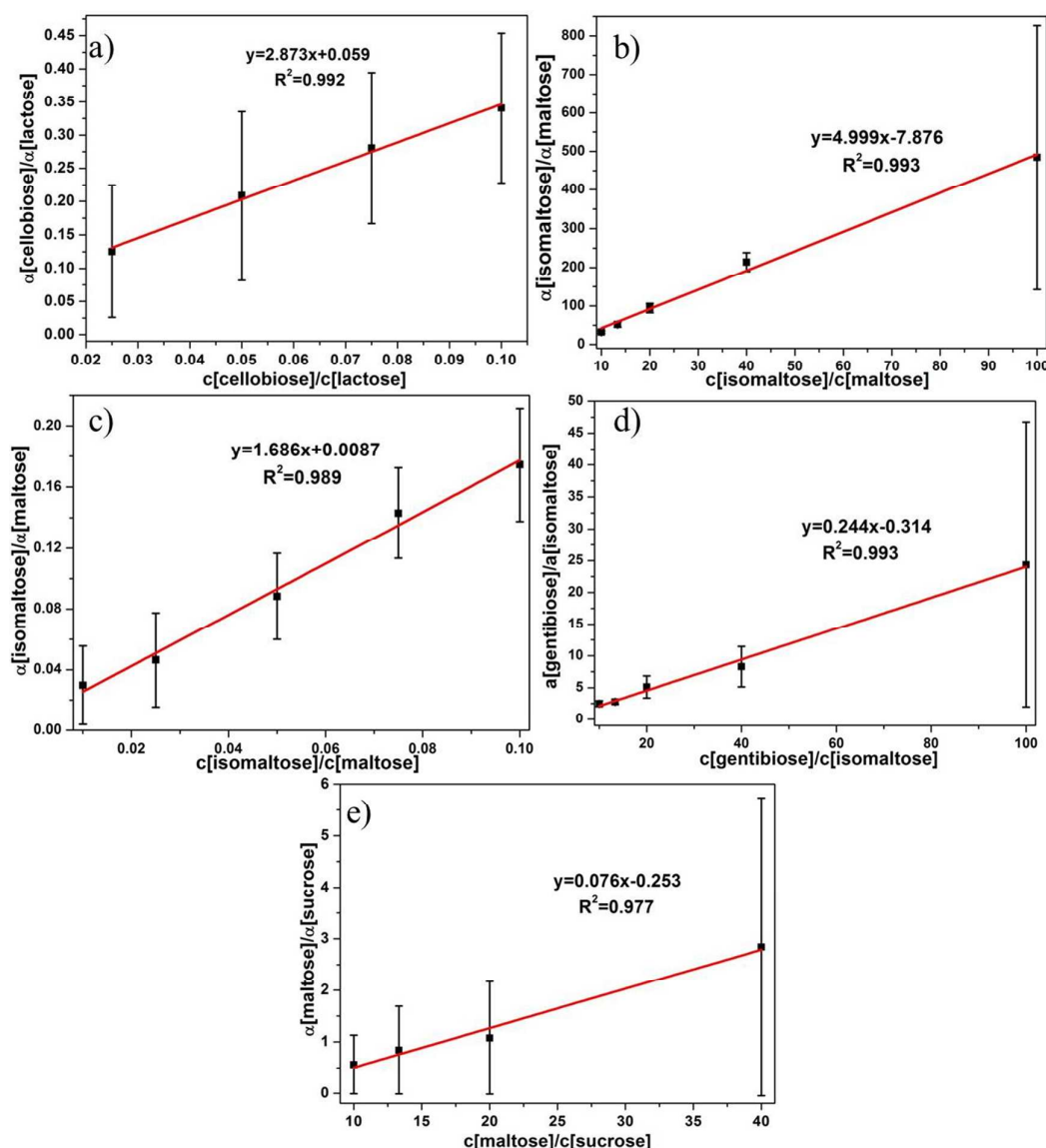


Figure S4. Limit of detection of disaccharide isomers when fraction of one isomer is very low. a) when the ratio of gentiobiose to isomaltose is larger than 10; b) when the relative ratio of isomaltose to maltose is larger than 10; c) when the ratio of isomaltose to maltose is lower than 0.1; d) when the ratio of cellobiose to lactose lower than 0.1; e) when the ratio of maltose to sucrose is larger than 10.

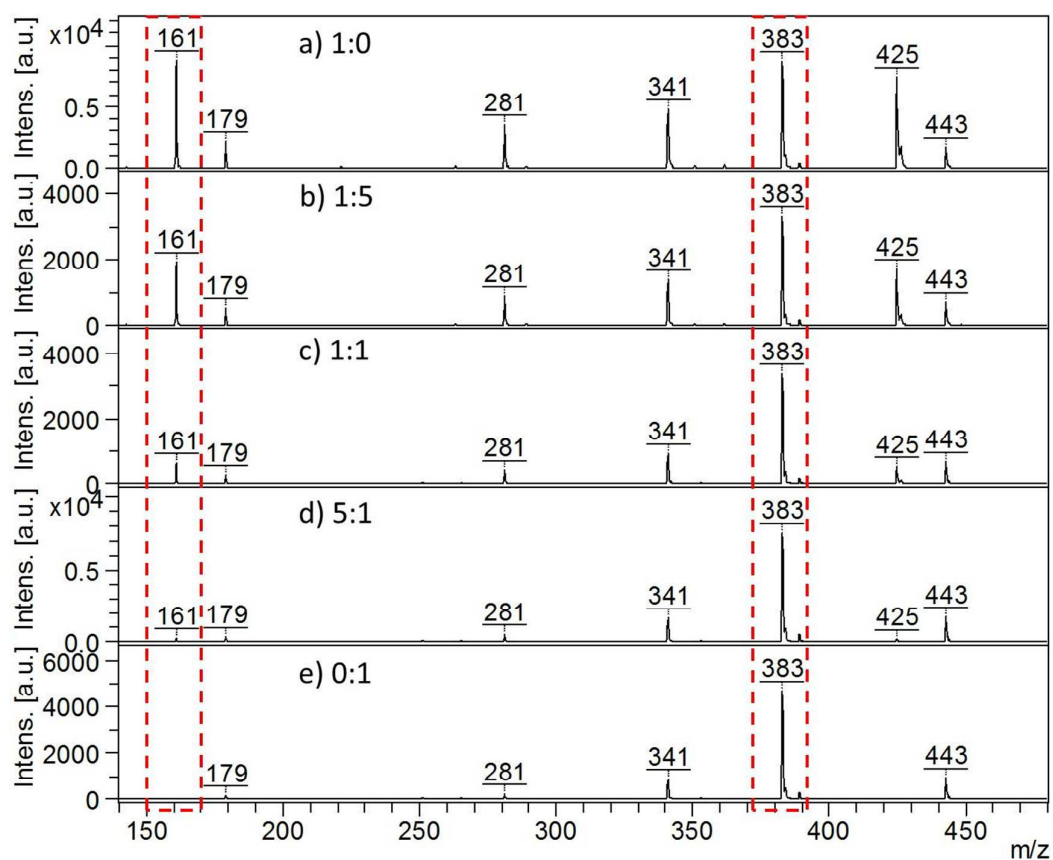


Figure S5. MALDI-LIFT-TOF/TOF spectra of the mixtures of trisaccharide isomers, maltotriose and isomaltotriose. The ratio of maltotriose to isomaltotriose in the binary mixtures are a) 1:0, b) 1:5, c) 1:1, d) 5:1 and e) 0:1.

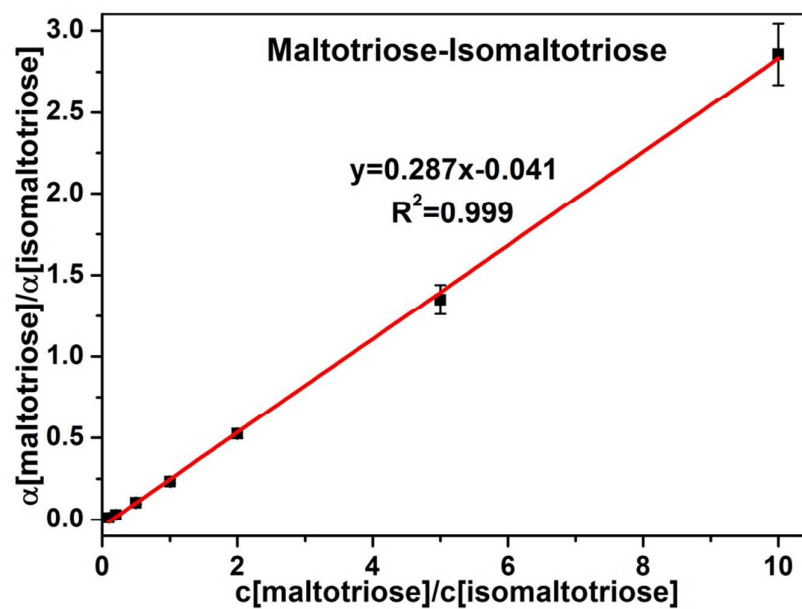


Figure S6. Calibration curve of trisaccharide isomers.

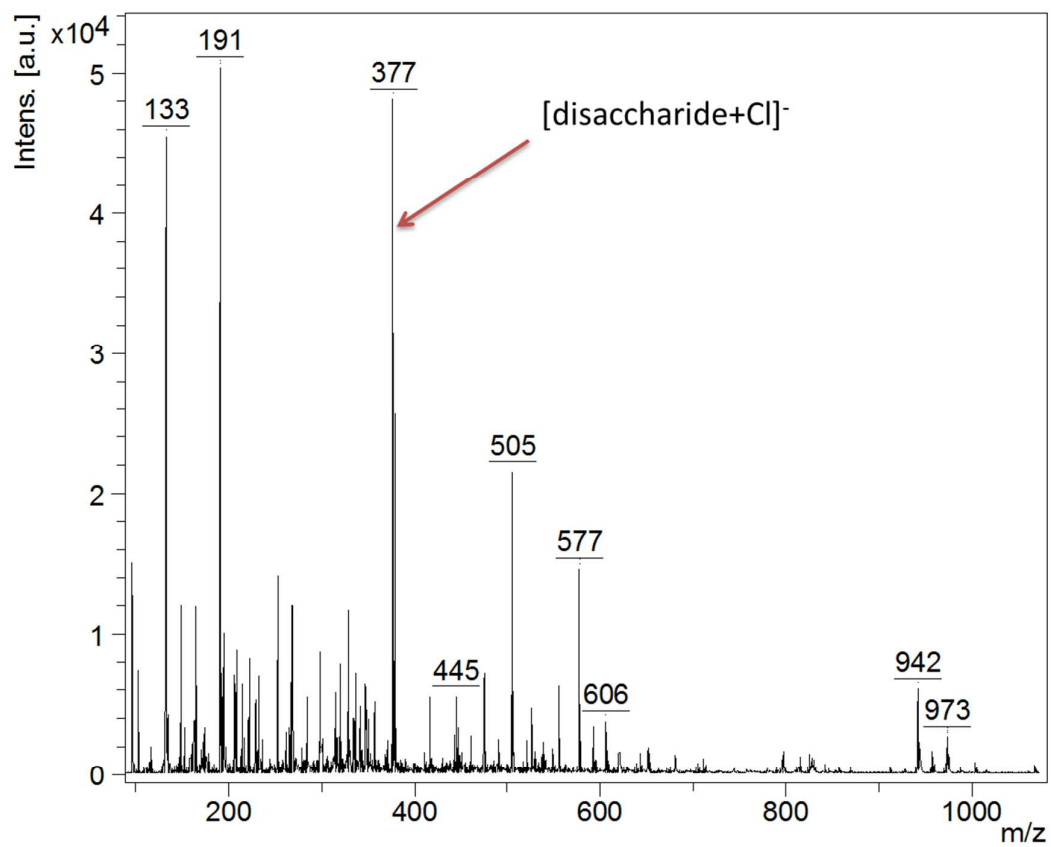


Figure S7. Mass spectrum of the Medicago leaf extracts.

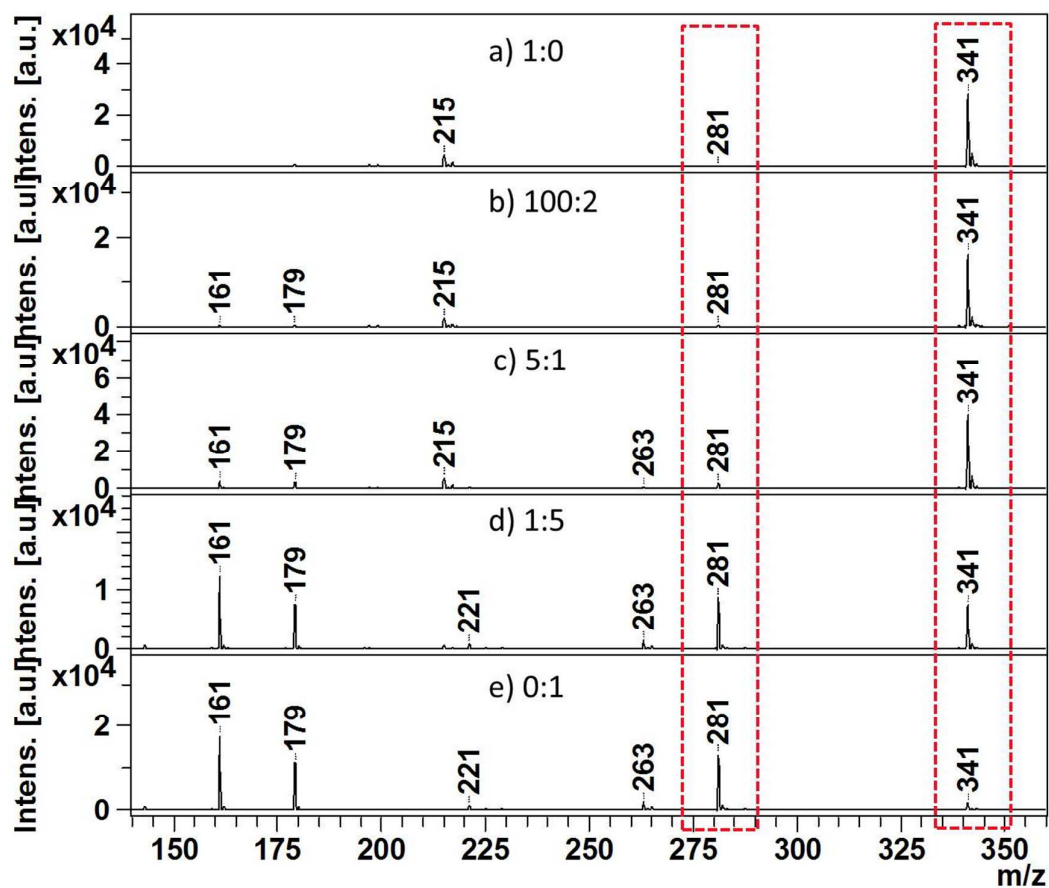


Figure S8. MALDI-LIFT-TOF/TOF spectra of the mixtures of sucrose and maltose. The ratio of sucrose to maltose in the mixture are a) 1:0, b) 100:2, c) 5:1, d) 1:5 and e) 0:1. The fragments m/z 281 and 341 were selected to build the calibration curve.

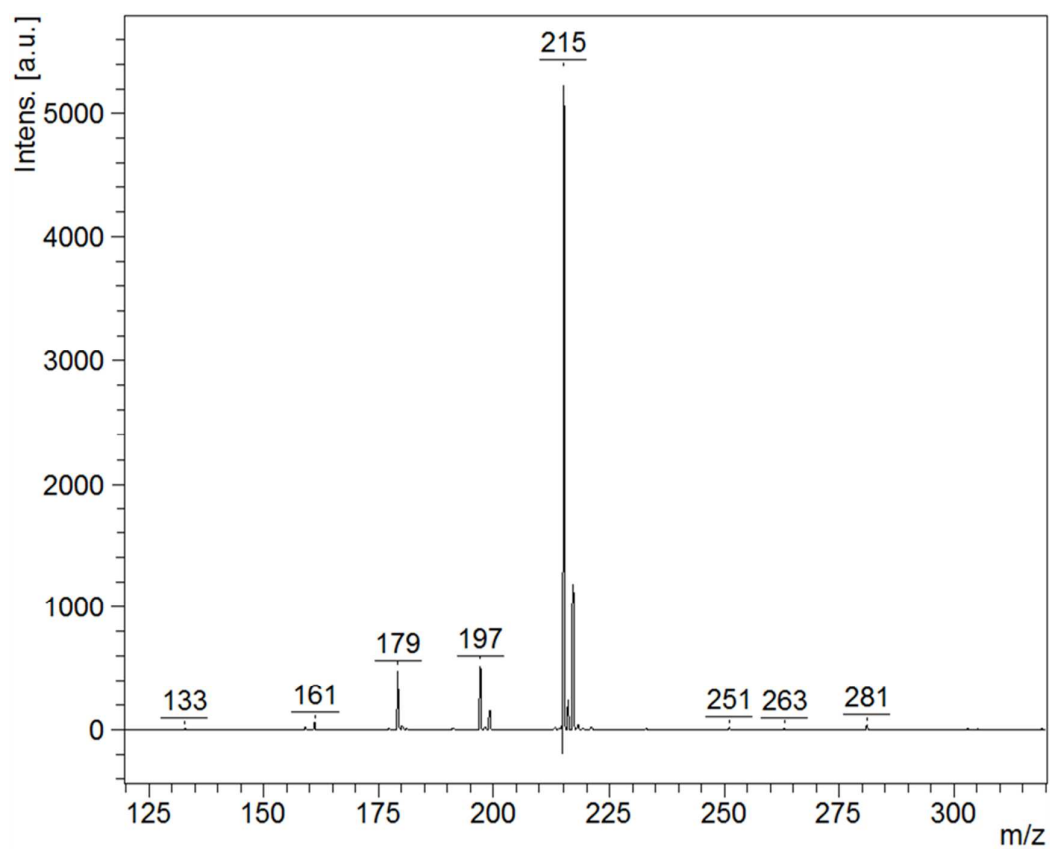


Figure S9. MALDI-LIFT spectrum of m/z 377 in the extract of *Medicago* stem.