

Distinguishing galactoside isomers with MS-IR

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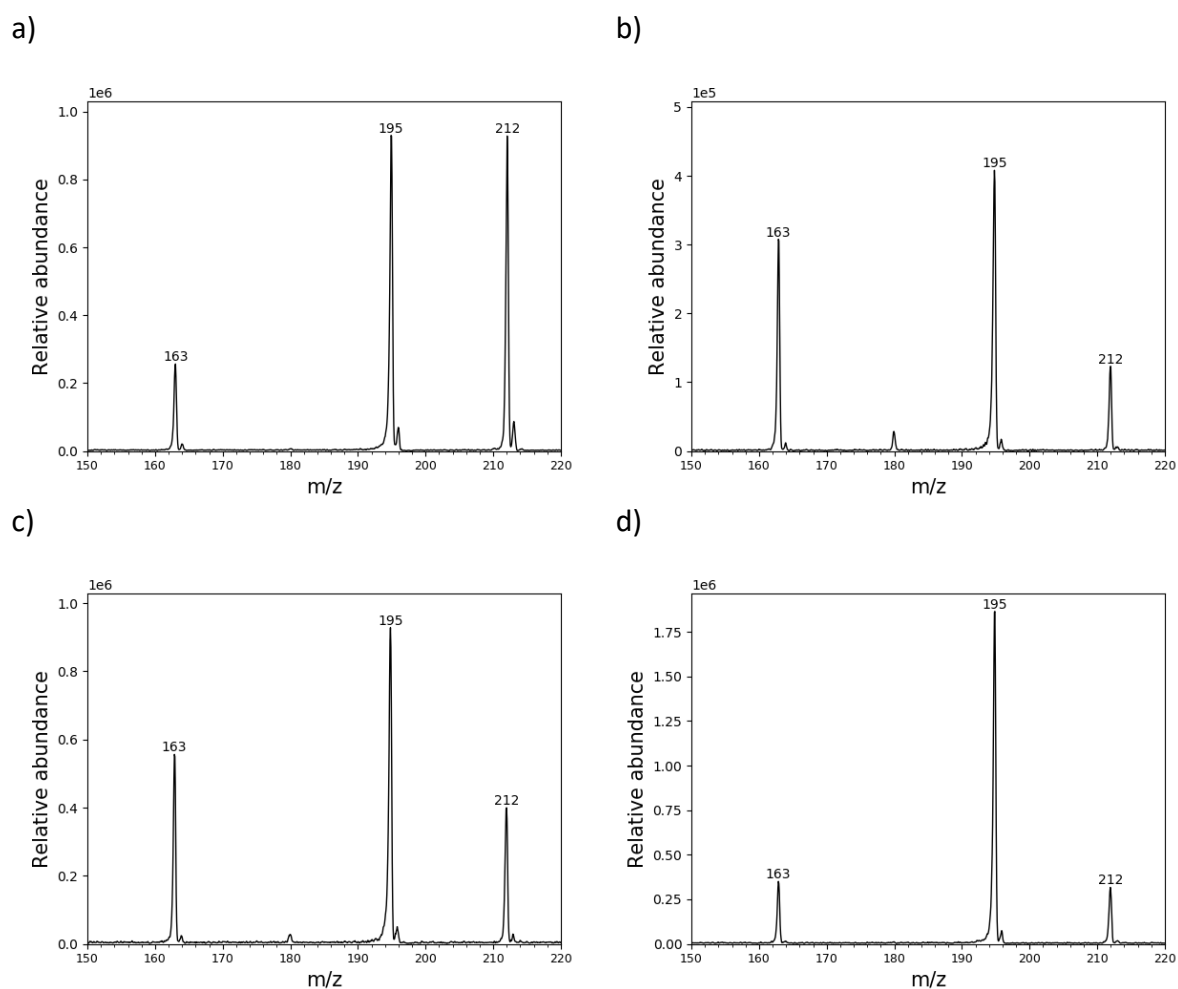


Fig. S1: MS/MS spectra obtained by CID for NH_4^+ adducts of galactose monosaccharides: 1-methyl- α -D-galactofuranose (a), 1-methyl- β -D-galactofuranose (b), 1-methyl- α -D-galactopyranose (c), and 1-methyl- β -D-galactopyranose (d).

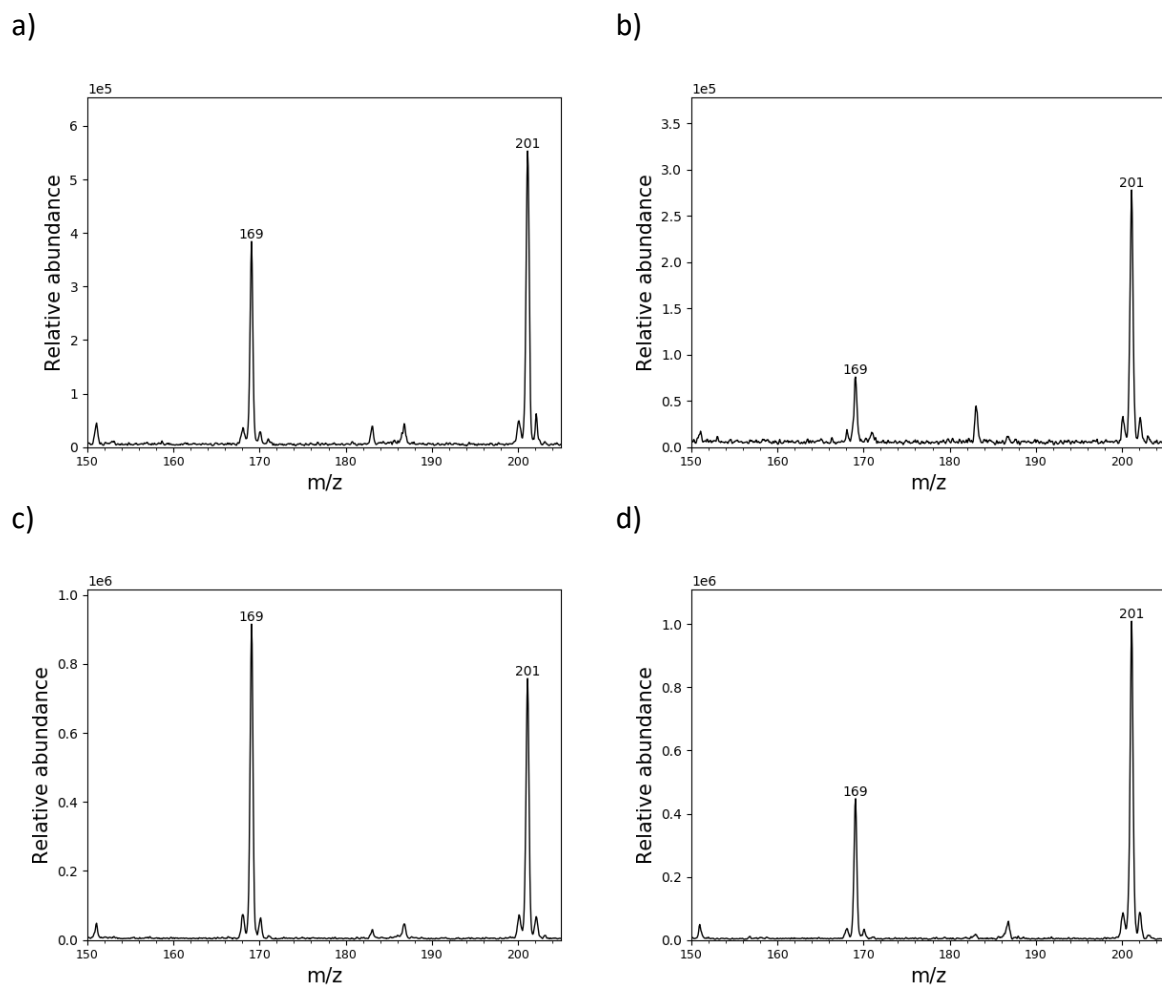


Fig. S2: MS/MS spectra obtained by CID for Li⁺ adducts of galactose monosaccharides: 1-methyl- α -D-galactofuranose (a), 1-methyl- β -D-galactofuranose (b), 1-methyl- α -D-galactopyranose (c), and 1-methyl- β -D-galactopyranose (d).

Ions were detected at m/z 201 for the lithium adducts. Under collision-induced dissociation (CID), the lithium adducts yielded one main fragment at m/z 169 corresponding to a neutral loss of 32 u.

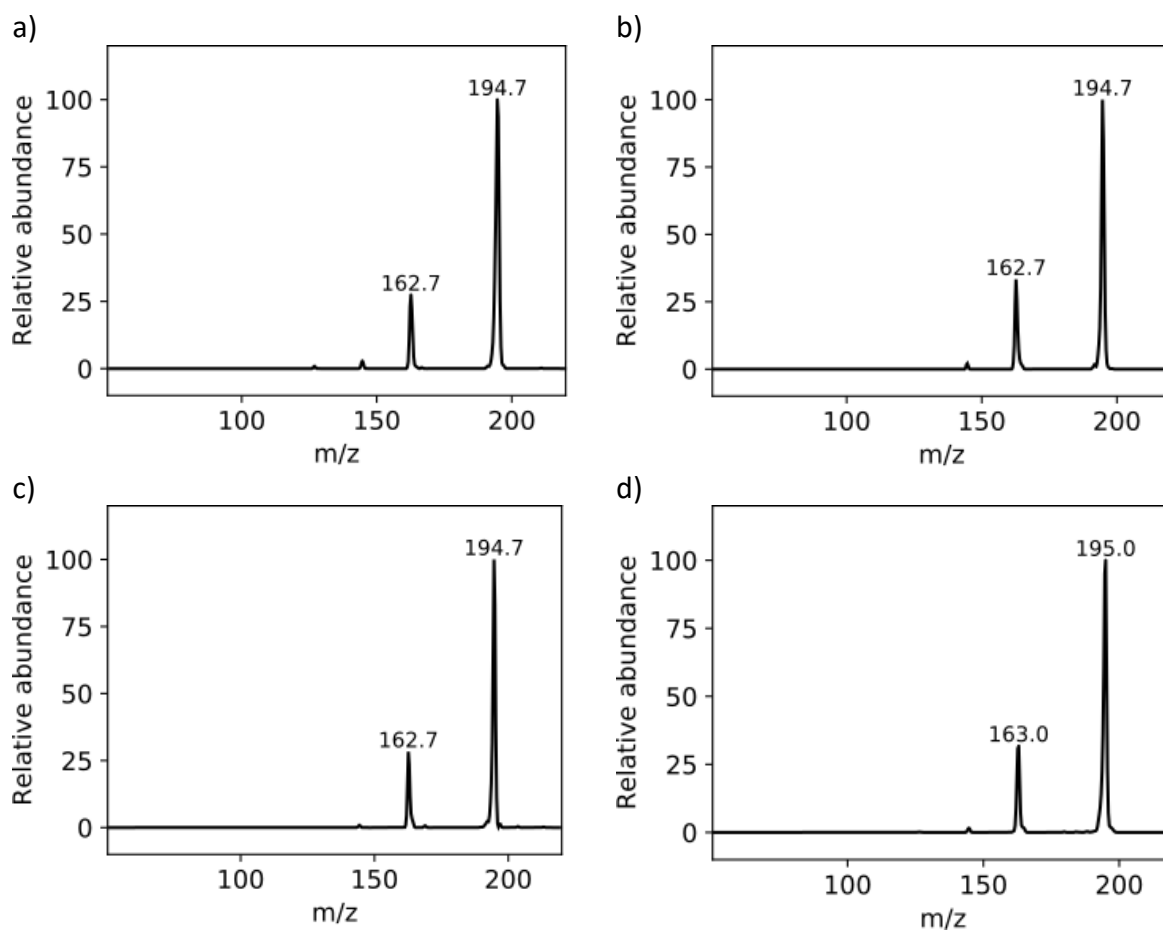


Fig. S3: MS/MS spectra obtained by CID for protonated galactose monosaccharides: 1-methyl- α -D-galactofuranose (a), 1-methyl- β -D-galactofuranose (b), 1-methyl- α -D-galactopyranose (c), and 1-methyl- β -D-galactopyranose (d).

Protonated galactose are obtained by fragmentation of ammonium adducts. Under collision-induced dissociation (CID), protonated ions yielded one main fragment at m/z 163 corresponding to a neutral loss of 32 u.

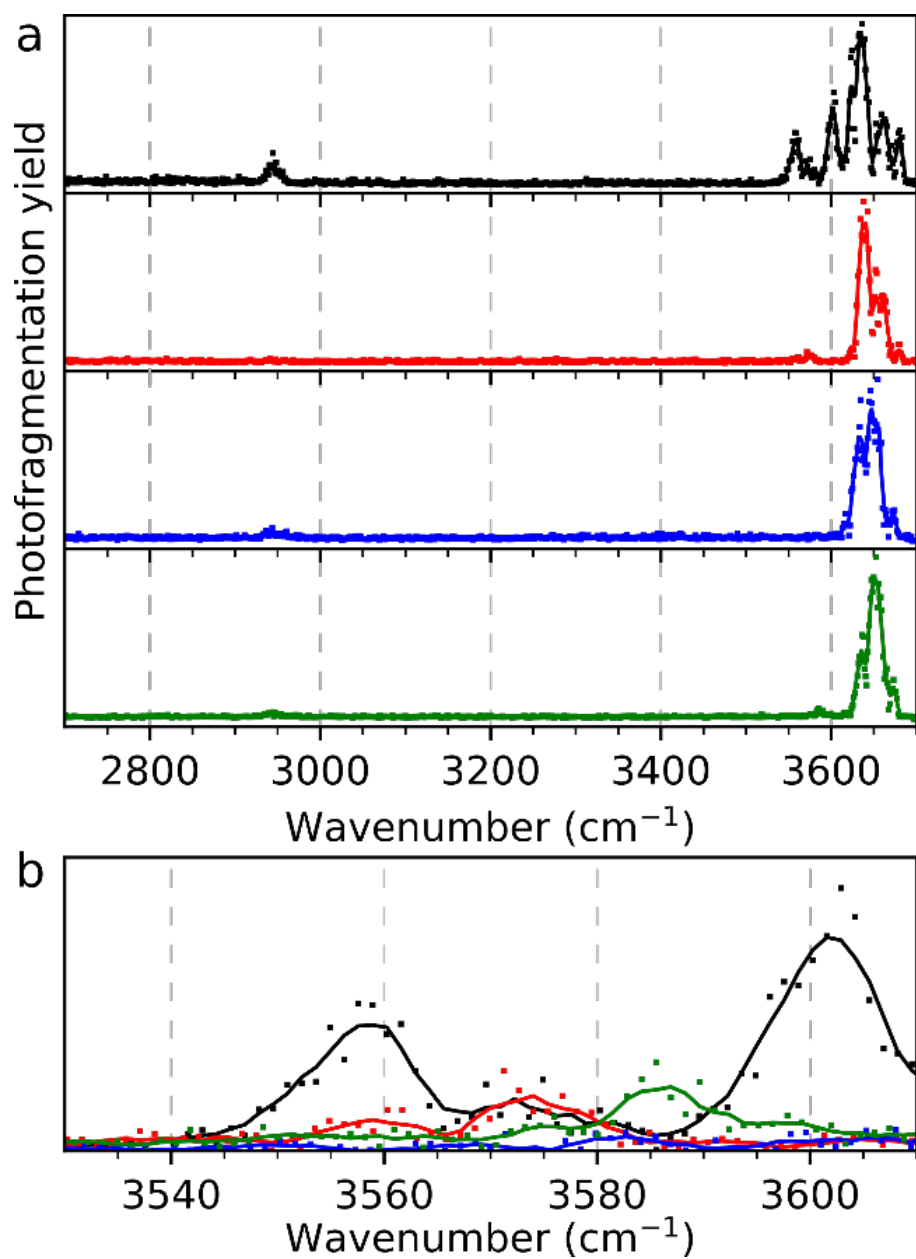


Fig S4: (a) IRMPD spectra of Li⁺ adducts of galactosides: methyl- α -D-galactopyranoside (**1**, black), methyl- β -D-galactopyranoside (**2**, red), methyl- α -D-galactofuranoside (**3**, blue), and methyl- β -D-galactofuranoside (**4**, green). (b) Expansion of the [3530, 3610] cm⁻¹ range. Black trace was scaled down by twofold.

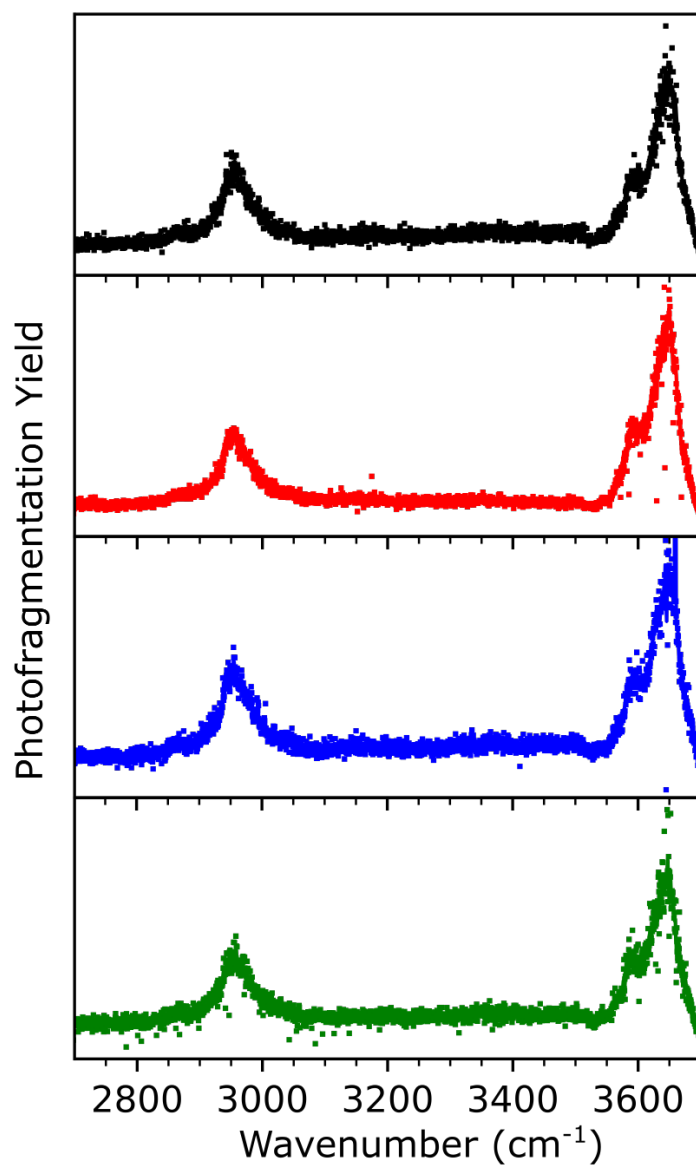
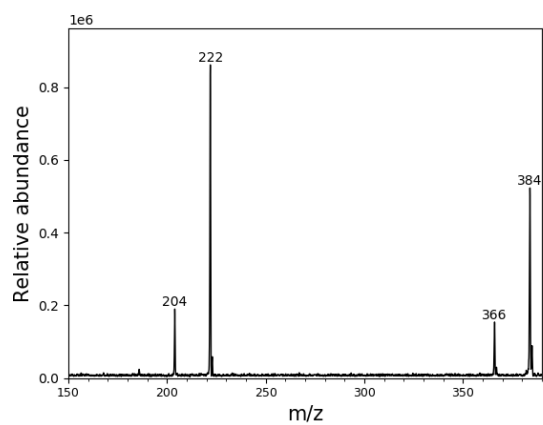


Fig S5: IRMPD spectra of m/z 195 fragments of methyl- α -D-galactopyranoside (**1**, black), methyl- β -D-galactopyranoside (**2**, red), methyl- α -D-galactofuranoside (**3**, blue), and methyl- β -D-galactofuranoside (**4**, green).

The IRMPD spectra of the protonated monosaccharides feature very similar spectra and cannot be used for diagnostic.

a)



b)

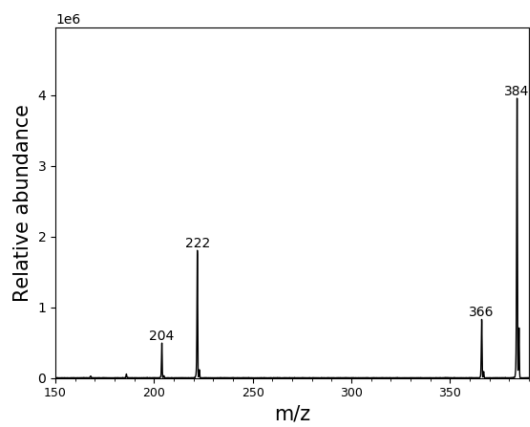
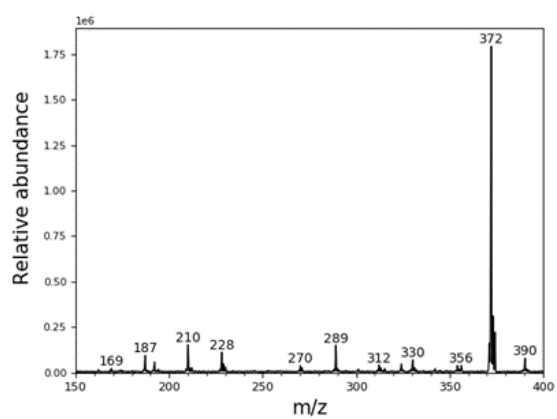


Fig. S6: MS/MS spectra obtained by CID for protonated of galactose-containing disaccharides: Gal β (1-4)GlcNAc (a) and Galp β (1-4)GlcNAc (b).

a)



b)

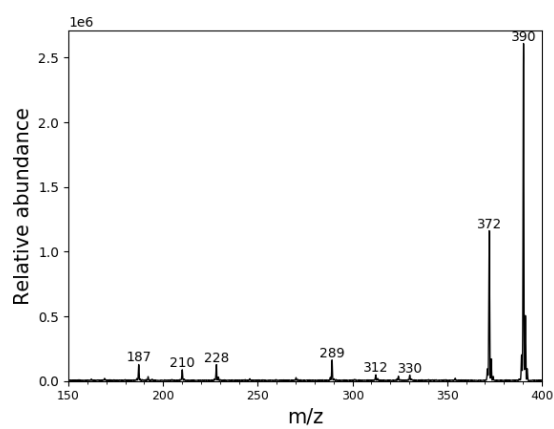


Fig. S7: MS/MS spectra obtained by CID for Li⁺ adducts of galactose-containing disaccharides: Gal β (1-4)GlcNAc (a) and Galp β (1-4)GlcNAc (b).

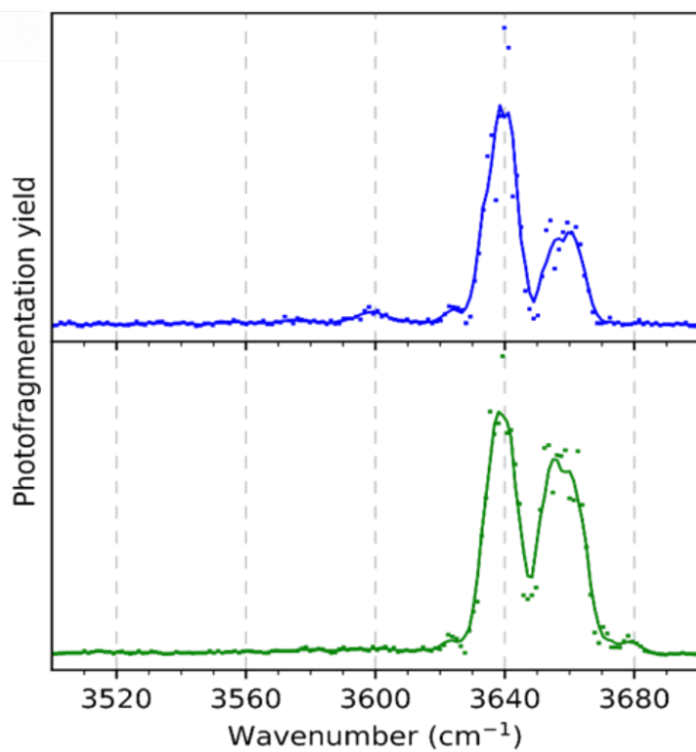
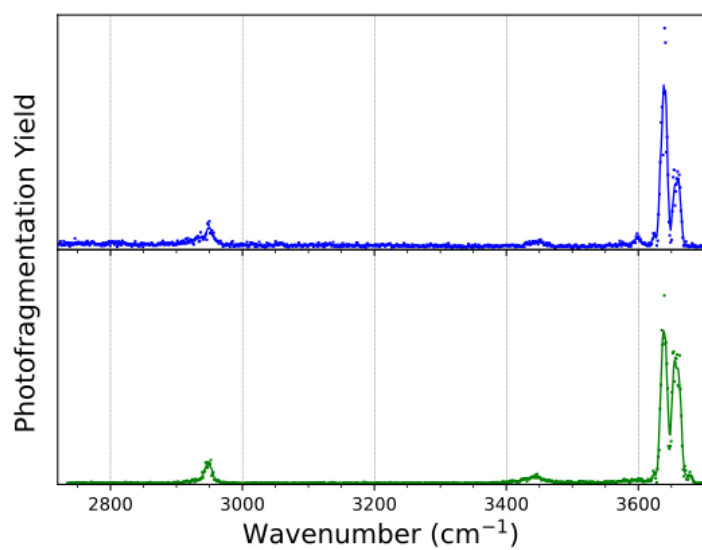


Fig. S8: IRMPD spectra of Li^+ adducts of galactose-containing disaccharides. $\text{Gal}\beta(1-4)\text{GlcNAc}$ (green) and $\text{Galp}\beta(1-4)\text{GlcNAc}$ (blue)

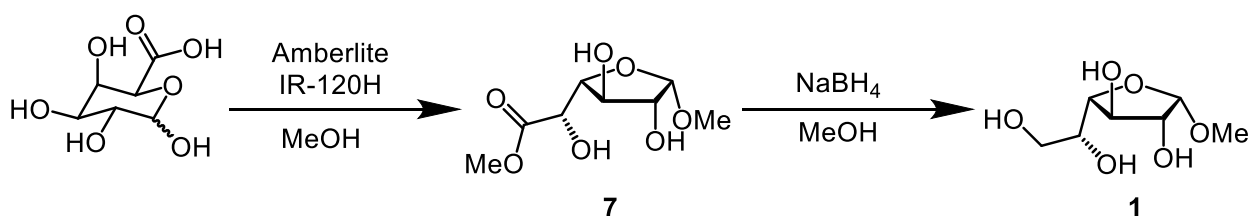
In the OH spectral range $[3500, 3700] \text{ cm}^{-1}$, compounds **5** and **6** are dominated by a doublet at 3640 cm^{-1} and at 3660 cm^{-1} .

Samples

Samples of methyl α -D-galactopyranoside, methyl- β -D-galactopyranoside, β -D-Galp-(1,4)-D-GlcpNAc, and β -D-Galf-(1,4)-D-GlcpNAc were purchased from Carbosynth. Samples of methyl α -D-galactofuranoside and methyl β -D-galactofuranoside were synthesized by the methods detailed below.

General Synthetic Methods

All reagents were purchased from Sigma-Aldrich Co. and used as received. Methanol (MeOH) was distilled from magnesium. Flash chromatography was performed using silica gel 60, 230-450 mesh (Sorbent Technologies). Analytical thin-layer chromatography (TLC) was carried out on EM Science TLC plates precoated with silica gel 60 F254 (250- μ m layer thickness). Visualization of TLC was accomplished using a UV lamp and charring with a p-anisaldehyde solution. ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were obtained at 400 or 600 MHz and 100 or 150 MHz, respectively, using a Bruker Avance 400 or Avance 600 spectrometer. Chemical shifts are reported relative to residual solvent signals; (CD_3OD): ^1H : δ 3.32, ^{13}C : δ 49.00; (D_2O): ^1H : δ 4.79. Peak multiplicity is reported as singlet (s), doublet (d), doublet of doublets (dd), triplet (t), and multiplet (m).



Methyl-(methyl α -D-galactofuranosid)uronate (7)

Synthesized from D-galacturonic acid according to reported literature.¹ Amberlite IR-120H (1 g) was rinsed and washed with MeOH before being suspended in methanol (20 mL). D-Galacturonic acid (1.00 g, 5.15 mmol) was added and reaction stirred at room temperature for 2 days. The solution was filtered, concentrated under reduced pressure, and purified by silica gel chromatography (49:1 EtOAc:toluene) to afford compound 7 (311 mg, 23%) as a pale oil. The physical data matched previously reported values.¹

^1H NMR (600 MHz, D_2O): δ 4.87 (d, J = 4.7 Hz, 1H), δ 4.43 (d, J = 3.8 Hz, 1H), δ 4.29 (t, J = 8.0 Hz, 1H), δ 4.13-4.17 (m, 2H), δ 3.82 (s, 3H), δ 3.43 (s, 3H).

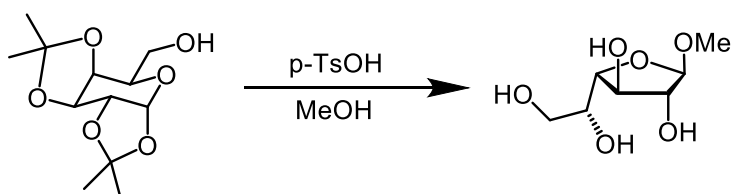
^{13}C NMR (150 MHz, D_2O): δ 174.24, 102.49, 82.19, 76.38, 73.69, 70.78, 56.05, 53.47

Methyl α -D-galactofuranoside (**1**)

Compound **7** (311mg, 1.4mmol) was dissolved in MeOH (10 mL). NaBH_4 (160 mg, 4.23 mmol) was added in 40 mg portions every half hour. After last portion was added, the solution stirred for an additional 3 hours at room temperature. Reaction was then deionized by passing through Amberlite IR-120H and then concentrated under reduced pressure. Crude mixture was then purified by silica gel chromatography (100:7 EtOAc:MeOH) to afford **1** (84 mg, 31%) as a pale oil. The physical data matched previously reported values.¹

^1H NMR (400 MHz, D_2O): δ 4.92 (m, 1H), δ 4.16 (m, 2H), δ 3.80-3.84 (m, 1H), δ 3.73-3.77 (m, 2H), δ 3.60-3.66 (dd, $J = 7.9, 12.7$ Hz, 1H), δ 3.47 (s, 3H).

^{13}C NMR (100 MHz, D_2O): δ 103.80, 82.98, 78.06, 76.12, 74.43, 63.99, 57.07



Methyl β -D-galactofuranoside (**2**)

Synthesized from 1,2:3,4-di-O-isopropylidene- α -D-galactopyranose following similar reported methods.²⁵ The protected 1,2:3,4-di-O-isopropylidene- α -D-galactopyranose (800 mg, 3.07 mmol) and p-toluenesulfonic acid monohydrate (500 mg, 2.63 mmol) was dissolved in 20 mL dry methanol under N_2 atmosphere and refluxed for 6 hours. Triethylamine was added to quench reaction. Reaction was concentrated under reduced pressure and purified by silica gel chromatography (4:1 EtOAc:MeOH) to afford **2** (158 mg, 26%) as a pale oil. The physical data matched previously reported values.¹

^1H NMR (600 MHz, CD_3OD): δ 4.77 (d, $J = 1.6$ Hz, 1H), δ 4.02 (dd, $J = 3.8, 6.4$ Hz, 1H), δ 3.92-3.94 (m, 2H), δ 3.72-3.74 (m, 1H), δ 3.63-3.65 (m, 2H), δ 3.38 (s, 3H).

^{13}C NMR (150 MHz, CD_3OD): δ 110.51, 84.42, 83.20, 78.80, 72.49, 64.48, 55.27

Mass spectrometry

MS analysis is performed using a commercial mass spectrometer Thermofinnigan LCQ Classic coupled with an electrospray ionization source (ESI) and equipped with a 3D

ion trap. MS/MS analysis is carried out by collisional induced dissociation (CID) on mass-selected ions using a helium buffer. All samples were prepared in 50 μM $\text{H}_2\text{O}/\text{MeOH}$ (50:50) solutions. Protonated $\beta\text{-D-Gal-(1,4)-D-GlcpNHAc}$ ions were readily obtained. Lithium chloride LiCl (0.1%) or ammonia NH_3 (0.1%) was added to the solutions in order to promote Li^+ and NH_4^+ adducts, respectively. No further purification was required.

MS/IR analysis

The setup used to perform MS/IR analysis was previously described.^{22,26} In short, the mass spectrometer was customized to allow laser irradiation of trapped ions. Laser pulses of 10 ns duration, at 10 mJ / pulse with a repetition rate of 10 Hz are delivered by a YAG-pumped tunable OPO/OPA IR laser system (LaserVision) in the range of 2700–3700 cm^{-1} . The laser beam is injected inside the modified 3D ion trap where ions of interest are stored for 800 ms. Upon sequential resonant photon absorption, a molecular vibration is excited. Relaxation occurs through intramolecular energy redistribution leading to fragmentation via InfraRed Multiple Photon Dissociation (IRMPD). The IRMPD spectroscopic scheme allows obtaining the vibrational fingerprint of m/z selected ions in the range of OH, NH and CH vibrations (mid-IR region) by monitoring the photofragmentation yield as a function of the wavenumber, where the photofragmentation yield is calculated using the following variation of the

Beer-Lambert formula: $-\log \frac{I_p}{I_p + \sum I_f}$

where I_p is the intensity of the precursor ion and I_f is the intensity of the photofragments. The IRMPD spectrum of the precursor ion is retrieved by monitoring its photofragmentation yield as a function of the wavelength. The data points shown thorough the manuscript are averaged 3 times and a trend line (5-points Fourier Transform rolling averaging) is added to guide the eye.

Additional References

(1) Bordoni, A.; Lima, C.; Mariño, K.; de Lederkremer, R. M.; Marino, C. Facile Synthesis of Methyl α - and β -d-[6-3H]Galactofuranosides from d-Galacturonic Acid. Substrates for the Detection of Galactofuranosidases. *Carbohydrate Research* 2008, 343 (10–11), 1863–1869. <https://doi.org/10.1016/j.carres.2008.02.019>.