

Supplementary Information

A simple tandem mass spectrometry method for structural identification of pentose oligosaccharide

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(A) Relative abundances measured by NMR spectroscopy

Table S1. Relative abundances (in %) of pentose isomers in D₂O measured by NMR spectroscopy.⁵³

Carbohydrate	Temp (°C)	α-Pyranose (%)	β-Pyranose (%)	α-Furanose (%)	β-Furanose (%)	Acyclic carbonyl form(%)
Arabinose ⁴²	31	60	35.5	2.5	2	0.03
Xylose ⁴²	31	36.5	63	<1	<1	0.02

(B) Branching ratio of cross-ring dissociation and dehydration at the reducing end

For the disaccharides or oligosaccharides with 1→4 or 1→5 linkage at the reducing end, the cross-ring dissociation mechanism predicts the loss of neutral $m=62$ if the O1 atom of the sugar at the reducing end is ¹⁸O labelled. Figure S1 shows that the cross-ring fragments are dominated by the loss of neutral $m=62$. In addition to the cross-ring dissociation, dissociation mechanism predicts that dehydration mainly occurs by transferring an H atom to O1 of the sugar at the reducing end, followed by breaking the C1-O1 bond. Figure S1 shows that the dehydration products mainly result from the loss of neutral $m=20$, consisting to the mechanism. The branching ratios, B , of fragment ions from the loss of ¹⁸O labelled neutrals are calculated using the following equation.

$$B = \frac{\text{Fragment ions from labeled neutral loss}}{\text{Fragment ions from labeled and unlabeled neutral losses}}$$

For example, the branching ratio of dehydration reaction of lithiated ¹⁸O labeled β-Xylp-(1→4)-Xylp is:

$$\frac{I_{(m/z\ 271)}}{(I_{(m/z\ 271)} + I_{(m/z\ 273)})}$$

The branching ratios of fragment ions from the loss of ¹⁸O labelled neutrals, as listed in Table 2, suggests dissociation follows the proposed mechanism closely.

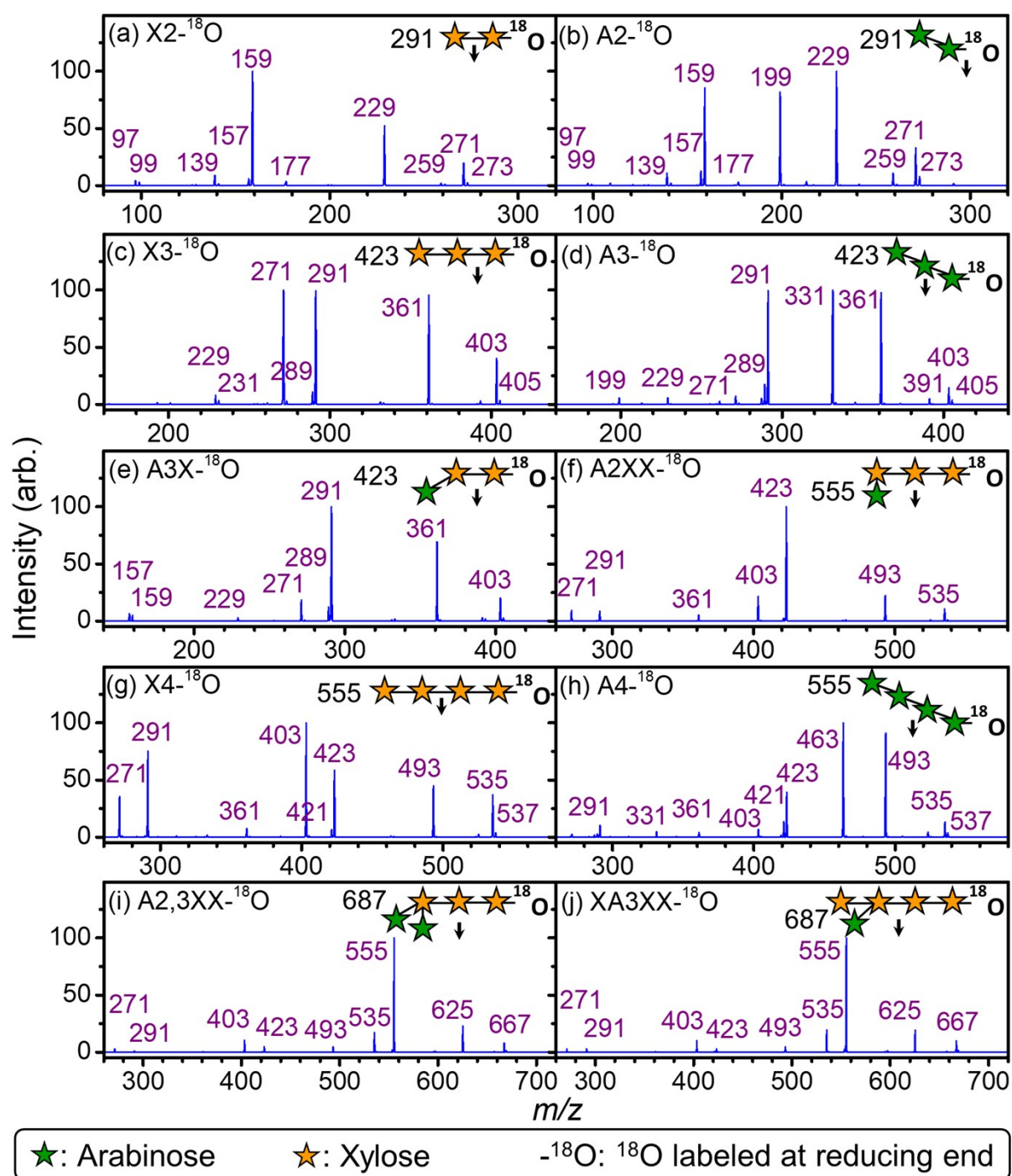


Figure S1. CID spectra of ^{18}O labeled (at O1 of the sugar at the reducing end) of pentose disaccharide and oligosaccharides. (a): X2 (β -Xylp-(1 \rightarrow 4)-Xylp); (b) A2 (α -Araf-(1 \rightarrow 5)-Araf); (c): X3 (β -Xylp-(1 \rightarrow 4)- β -Xylp-(1 \rightarrow 4)-Xylp); (d): A3 (α -Araf-(1 \rightarrow 5)- α -Araf-(1 \rightarrow 5)-Araf); (e): A3X (α -Araf-(1 \rightarrow 3)- β -Xylp-(1 \rightarrow 4)-Xylp); (f): A2XX (α -Araf-(1 \rightarrow 2)- β -Xylp-(1 \rightarrow 4)- β -Xylp-(1 \rightarrow 4)-Xylp); (g): X4 (β -Xylp-(1 \rightarrow 4)- β -Xylp-(1 \rightarrow 4)- β -Xylp-(1 \rightarrow 4)-Xylp); (h): A4 (α -Araf-(1 \rightarrow 5)- α -Araf-(1 \rightarrow 5)- α -Araf-(1 \rightarrow 5)-Araf); (i): A2,3XX (α -Araf-(1 \rightarrow 2)-[α -

Araf-(1→3)]-β-Xylp-(1→4)-β-Xylp-(1→4)-Xylp); (j): XA3XX (β-Xylp-(1→4)-[α-Araf-(1→3)]-β-Xylp-(1→4)-β-Xylp-(1→4)-Xylp).

AOX	Linkage at Reducing end	Dehydration	Cross ring	Cross ring
		$\frac{I_{(20u\ loss)}}{(I_{(20u\ loss)} + I_{(18u\ loss)})}$	$\frac{I_{(62u\ loss)}}{(I_{(62u\ loss)} + I_{(60u\ loss)})}$	$\frac{I_{(92u\ loss)}}{(I_{(92u\ loss)} + I_{(90u\ loss)})}$
X2	1→4	90%	99%	
A2	1→5	80%	99%	99%
X3	1→4	92%	99%	
A3	1→5	79%	99%	99%
A3X	1→4	88%	98%	
A2XX	1→4	91%	99%	
X4	1→4	90%	99%	
A4	1→5	78%	100%	99%
A23XX	1→4	84%	99%	
XA3XX	1→4	86%	99%	

Table 2. Branching ratios of fragment ions from the loss of ¹⁸O labelled neutrals. The precursor ions are the lithiated disaccharides and oligosaccharides displayed in Figure S1.

(C) Additional examples

The application to determine the structures of xylose in β-Xylp-(1→4)-β-Xylp-(1→4)-Xyl, α-Araf-(1→4)-β-Xylp-(1→4)-Xyl, and α-Xylp-(1→4)-Glc are illustrated in Figure S2, and the spectral similarity scores are shown Table S3. All the monosaccharides can be predicted correctly.

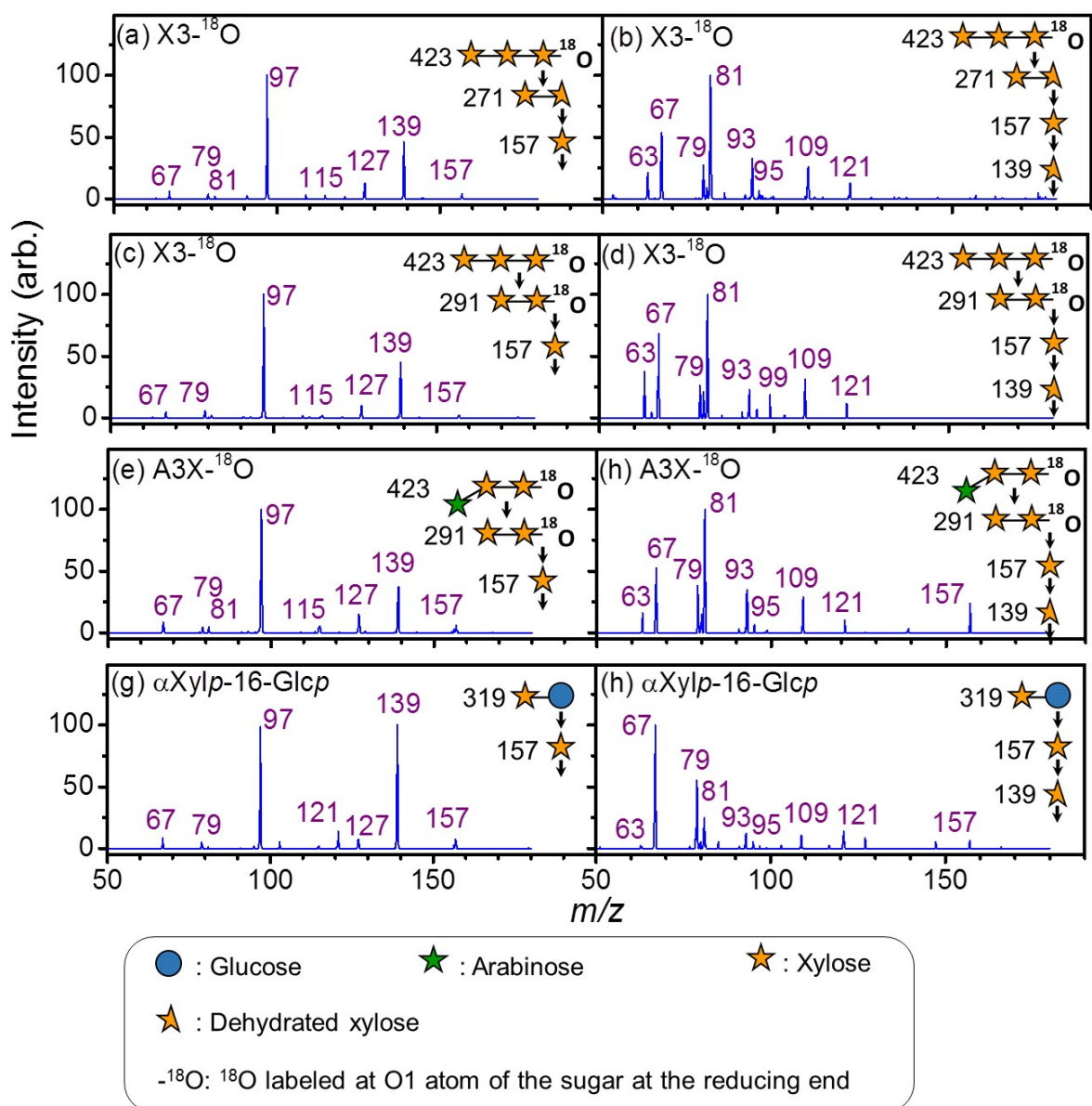


Figure S2. CID spectra of lithiated ¹⁸O-labeled X3 (β -Xylp-(1 \rightarrow 4)- β -Xylp-(1 \rightarrow 4)-Xylp-¹⁸O) (a)-(d), ¹⁸O-labeled A³X (α -Araf-(1 \rightarrow 3)- β -Xylp-(1 \rightarrow 4)-Xylp-¹⁸O) (e) and (f), and α -Xylp-(1 \rightarrow 6)-Glc p (g) and (h).

Fig.	α Arap	β Arap	α Araf	β Araf	β Xylf	α Xylf	β Xylp	α Xylp
S2(a)/S2 (b)	77/83	53/57	84/83	71/88	76/83	63/61	99/98	79/59
S2(c)/S2(d)	74/82	54/59	81/82	69/87	74/82	64/62	98/98	79/60
S2(e)/S2(f)	73/83	50/59	80/84	67/87	72/84	59/61	95/97	75/60
S2(g)/S2(h)	90/41	72/87	85/44	86/45	91/42	82/89	82/58	97/98

Table S3. The spectral similarity scores R of spectra of m/z 157 and m/z 139 shown in Figure S2. The number before and after slash denote the scores R of spectra of m/z 157 and 139 shown in Figure S2, respectively. The correct assignments are highlighted in red.

The applications to determine the structures of arabinose in α -Araf in α -Araf-(1 \rightarrow 5)-Araf, α -Araf-(1 \rightarrow 5)- α -Araf-(1 \rightarrow 5)-Araf, and α -Araf-(1 \rightarrow 3)- β -Xylp-(1 \rightarrow 4)-Xylp are shown in Figure S3, and the spectral similarity scores are shown in Table S3. The similarity scores show α -Arap and β -Xylf are near that of α -Araf, indicating they are not easy to be distinguished. This is consistent to the results shown in Table 1.

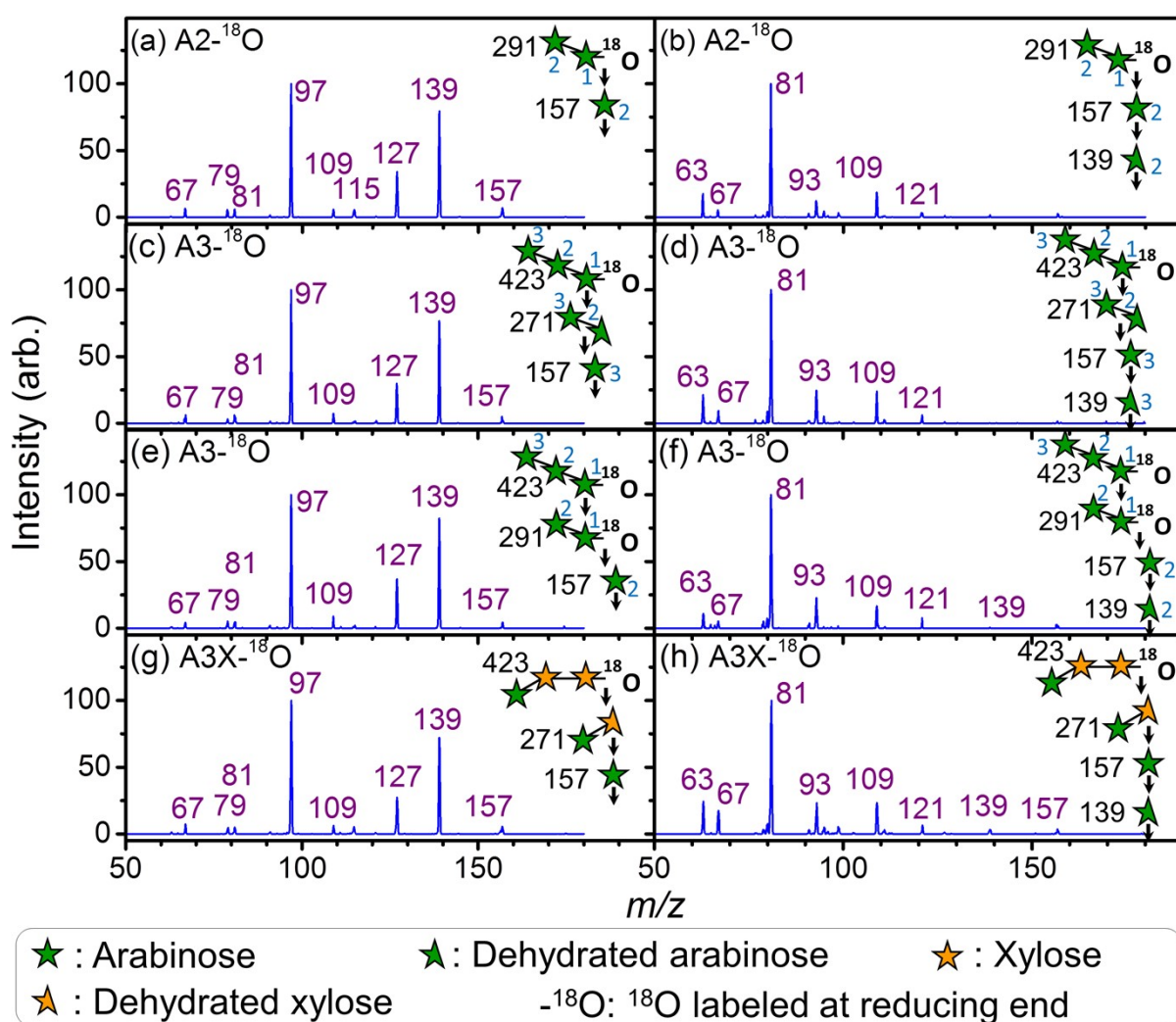


Figure S3. CID spectra of lithiated ¹⁸O-labeled A2 (α -Araf-(1 \rightarrow 5)-Araf) (a), (b), ¹⁸O-labeled A3 (α -Araf-(1 \rightarrow 5)- α -Araf-(1 \rightarrow 5)-Araf-¹⁸O) (c)-(f), and ¹⁸O-labeled A3X (α -Araf-(1 \rightarrow 3)- β -Xylp-(1 \rightarrow 4)-Xylp-¹⁸O) (g), (h).

Fig.	α Arap	β Arap	α Araf	β Araf	β Xylf	α Xylf	β Xylp	α Xylp
S3(a)/S3(b)	97/97	59/41	96/97	79/92	95/97	69/45	79/79	84/43
S3(c)/S3(d)	96/99	59/42	97/98	79/93	95/98	69/46	80/81	84/44
S3(e)/S3(f)	95/97	58/43	95/97	77/91	94/96	68/46	79/80	83/44
S3(g)/S3(h)	90/97	57/44	97/95	77/97	89/97	67/47	87/85	82/46

Table S4. Spectral similarity scores R of CID spectra of m/z 157 and m/z 139 shown in Figure S3. The number before and after slash denote the scores R of spectra of m/z 157 and 139 shown in Figure S3, respectively. The correct assignments are highlighted in red, and both scores higher than 90 are highlighted in green.