Table S1 Primers used for cloning components of *Ct*Xyl5A and for constructing mutants

Protein	Primers	Sequence (5'-3')
*Ct*GH5	Forward	CTCGCTAGCAGCCCGCAACGTGGCCGG
	Reverse	CACCTCGAGGTCATAATACGAAACCCC
*Ct*GH5-CBM6	Forward	CTCGCTAGCCCGCAACGTGGCCGG
	Reverse	CACCTCGAGTATCGGAGAAAGTTC
*Ct*CBM6	Forward	CTCGCTAGCACGGATTCGGTGAATG
	Reverse	CACCTCGAGTATCGGAGAAAGTTC
E171	Forward	TTGTATGAAATACACAATGCGCCTGTGGCATGGGGA
	Reverse	TCCCCATGCCACAGGCGCATTGTGTATTTCATACAA
E279	Forward	CCTGCTTTATGACTGCGTATGCCGGAGGTGC
	Reverse	GCACCTCCGGCATACGCAGTCATAAAGCAGG
W242A	Forward	GGCGGTTACAATGTCGGAGCGATTTCGGAAGGAGAATC
	Reverse	CATTCTCCTTCCGAAATCGCTCCGACATTGTAACCGCC
W242A	Forward	CTGCCTGCTACCGGAGGTGCTCAGACTTGGATACA
	Reverse	TGTAGTCCAAGTCTGAGCACCTCCGGTAGCAGGCAG

Table S2 Crystal and structure resolution statistics

	***Ct*GH5-CBM6 SeMet**	***Ct*GH5-CBM6 Wt native**
Data collection		
Space group	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions		
a, *b*, *c* (Å)	69.26, 75.82, 106.11	69.12, 75.55, 105.97
Resolution (Å)	61.66-2.20 (2.32-2.20)	36.74-1.47 (1.55-1.47)
R_{merge}	0.098(0.372)	0.081 (0.639)
$I / \sigma I$	16.9 (5.8)	13.9 (2.8)
Completeness (%)	99.0 (98.3)	100 (99.9)
Redundancy	7.2 (7.4)	7.0 (6.6)
Anomalous completeness (%)	99.2 (98.5)	
Anomalous redundancy	3.8 (3.8)	
Refinement		
No. reflections	-	90100 (4744)
R_{work} / R_{free}	-	0.146 / 0.166
No. Atoms		
Protein	-	3782
Ligand / Ion	-	41
Water	-	636
B-factor		
Protein	-	16
Ligand / Ion	-	32
Water	-	33
R.m.s.[b] deviations		
Bond lengths (Å)		0.014
Bond angles (°)		1.40

Table S3 ^{1}H and ^{13}C chemical shifts of the NMR resonances of Fraction 1

Sugar residues	H-1/C-1	H-2/C-2	H-3/C-3	H-4/C-4	H-5/C-5[a]	H-5'/C-5[a]
β-D-Xylp	4.618	3.392	3.736	3.809	4.073	3.387
(reducing)	96.6	74.8	77.8	73.8	63.0	
α-D-Xylp	5.169	3.680	3.906	nd	3.840	3.770
(reducing)	92.4	71.8	77.7		59.4	
α-L-Araf (α)	5.342	4.164	3.911	4.273	3.799	3.718
	107.9	80.8		84.9	61.5	
α-L-Araf (β)	5.391	4.169	3.911	4.283	3.799	3.718
	107.9	80.8		84.9	61.5	
α-L-Araf	5.327	4.181	3.961	4.182	3.815	3.706
(nonreducing)	108.4	80.8		84.1	61.5	
3-linked-β-D-Xylp	4.465	3.401	nd	nd	nd	nd
(nonreducing)	101.7	75.3				
4-linked-β-D-Xylp	4.453	3.280	3.54	3.761	nd	nd
(nonreducing)	101.7	73.0	73.8	76.5		
terminal–β-D-Xylp	4.438	3.241	3.42	3.597	nd	nd
(nonreducing)	101.7	73.0	75.8	69.3		

a. The axial proton on C5 of Xylp residues is designated H5 and the equatorial proton is designated H5'. The stereochemistry (*pro*-R *vs. pro*-S) of H5 and H5' of Ara*f* residues is not specified.

SUPPLEMENTAL INFORMATION

Figure S1 ESI-MS of the pentasaccharides in Fraction 1
The pentasaccharides in Fraction 1 were analyzed by ESI-MS^n. *Panel A* shows the fragmentation of the *m/z* 725 ion which comprises the pentasaccharides. *Panel B, C, D, E* and *F* show the fragmentation patterns of the 711 (MS^3), 537 (MS^4), 391 (MS^5), 377 (MS^5) and 363 (MS^5) ions, respectively. The masses of Y-ions are indicated unless otherwise stated.

Figure S2 The structure of the pentasaccharides generated by *Ct*Xyl5A
Based on the data displayed in **Figure S1**, the structures of the pentasaccharides in Fraction 1 were identified. The sugars labelled P can be Ara*f* or Xyl*p*. The dotted arrow between sugar linkages shows the fragmentation site and the ion identified. Arrows pointing at sugars (but did not link two sugars together) identified hydroxyl groups that were not methylated as they comprised a glycosidic linkage in a parental ion. Xylol is the reducing end xylose that has been reduced to its alditol form by $NaBH_4$.

Figure S3 ESI-MS of the trisaccharides in Fraction 1
The trisaccharides in Fraction 1 were analyzed by ESI-MS^n. *Panel A* shows the fragmentation of the *m/z* 565 ion (MS^2), which comprises the trisaccharides. *Panel B* shows the fragmentation pattern of the *m/z* 391 ion (MS^3) generated from the *m/z* 565 ion.

Figure S4 The structure of the trisaccharides generated by *Ct*Xyl5A
Based on the data displayed in **Figure S3**, the structures of the trisaccharides in Fraction 1 were identified. The sugars labelled P can be Ara*f* or Xyl*p*. The data showed that the oligosaccharide ions coloured green were present, while those coloured red were not evident. The solid arrows between oligosaccharides showed the conversion of one oligosaccharide into another, through ESI-MS fragmentation. Dotted arrows indentified theoretical MS-mediated oligosaccharide conversions that did not occur in these analyses. The dotted arrows between sugar linkages in the oligosaccharides show the fragmentation site and the ion identified. Arrows pointing at sugars (but did not link two sugars together) within oligosaccharides identified hydroxyl groups that were not methylated as they comprised a glycosidic linkage in a parental ion. Xylol is the reducing end xylose that has been reduced to its alditol form by $NaBH_4$.

Figure S5 Temperature optimum of *Ct*GH5 and *Ct*GH5-CBM6 The two enzymes were assayed in 50 mM sodium phosphate buffer, pH 7.0, containing 50 nM enzyme and 1 mg/ml wheat arabinoxylan. The reactions were monitored for 10 min by assaying for reducing sugar release.

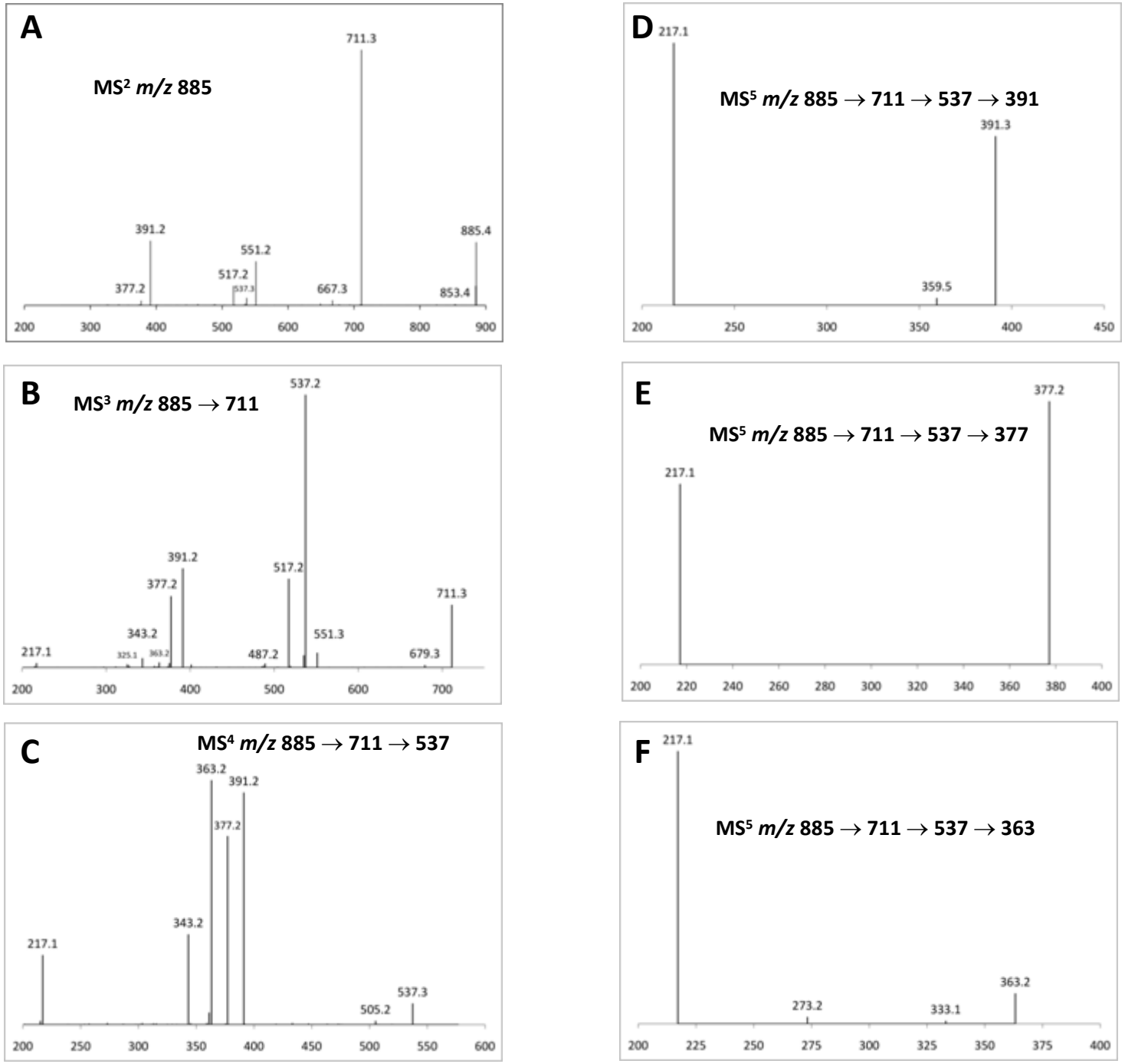

A
MS² m/z 885
711.3
391.2
885.4
551.2
517.2
377.2
667.3
853.4
200 300 400 500 600 700 800 900
B
MS³ m/z 885 → 711
537.2
391.2
517.2
377.2
711.3
343.2
551.3
217.1
487.2
679.3
200 300 400 500 600 700
C
MS⁴ m/z 885 → 711 → 537
363.2
391.2
377.2
343.2
217.1
537.3
505.2
200 250 300 350 400 450 500 550 600
D
217.1
MS⁵ m/z 885 → 711 → 537 → 391
391.3
359.5
200 250 300 350 400 450
E
377.2
MS⁵ m/z 885 → 711 → 537 → 377
217.1
200 220 240 260 280 300 320 340 360 380 400
F
217.1
MS⁵ m/z 885 → 711 → 537 → 363
363.2
273.2
333.1
200 225 250 275 300 325 350 375 400

Figure S1

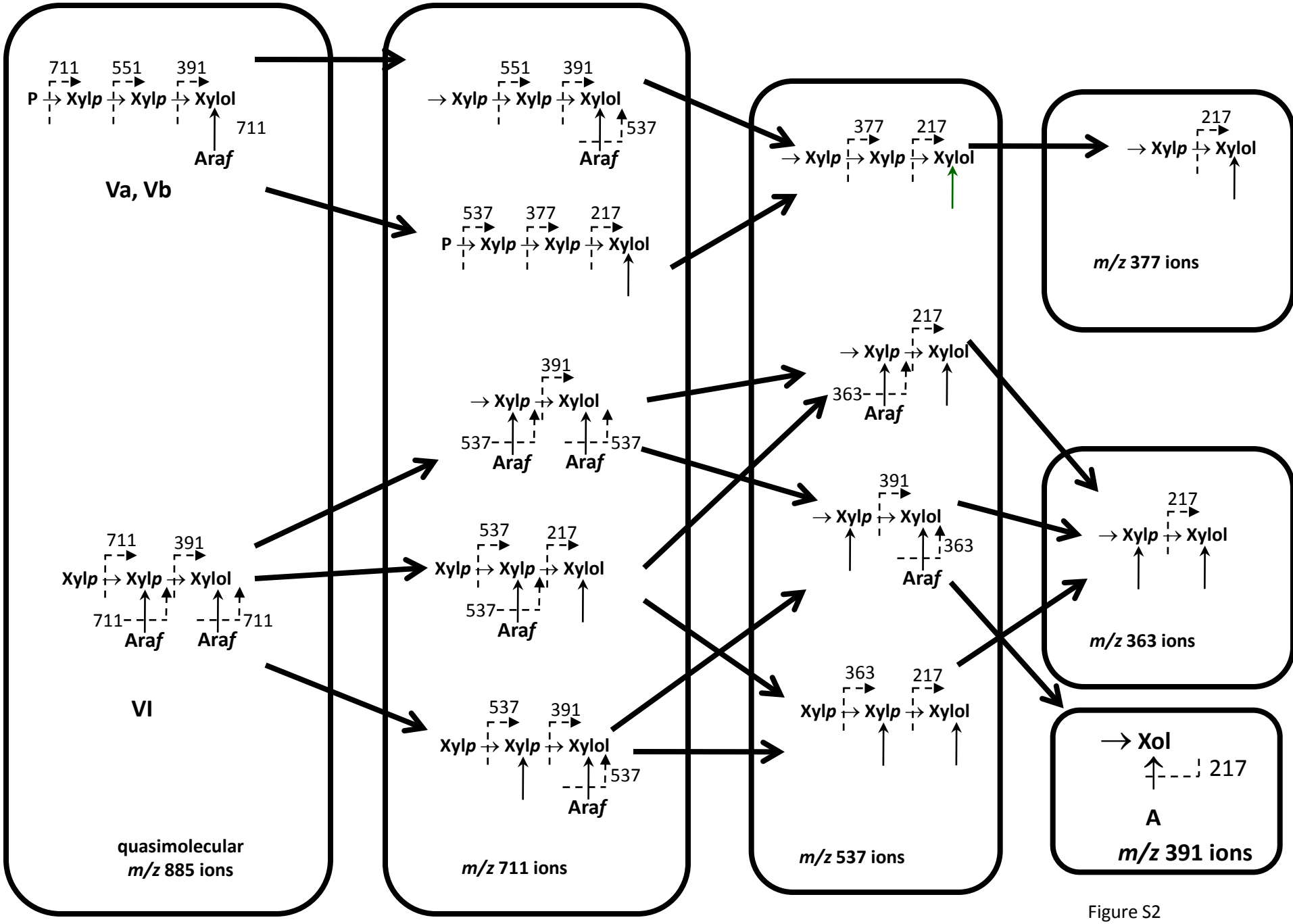
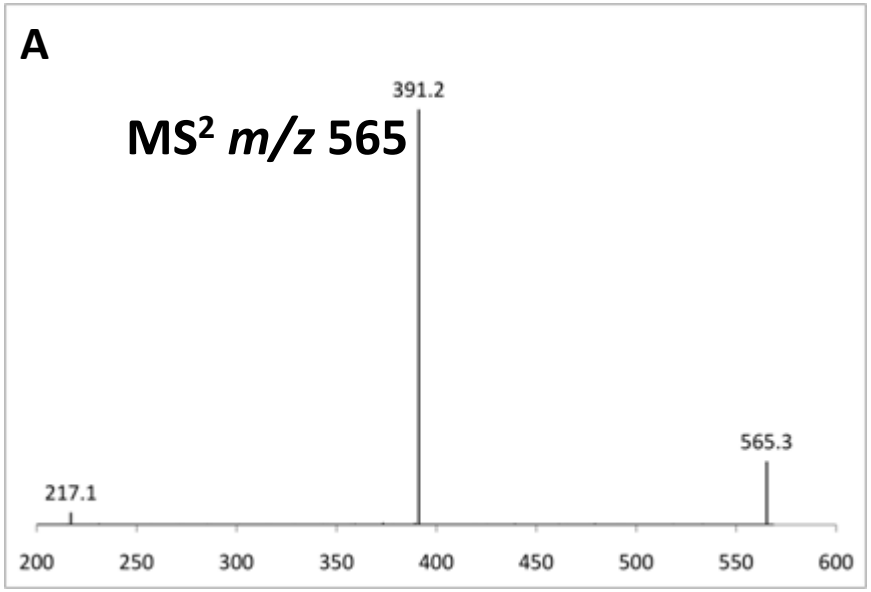

711
551
391
P → Xylp → Xylp → Xylol
711
Araf
Va, Vb
711
391
Xylp → Xylp → Xylol
711
Araf
Araf
711
VI
quasimolecular
m/z 885 ions
551
391
→ Xylp → Xylp → Xylol
537
Araf
537
377
217
P → Xylp → Xylp → Xylol
391
→ Xylp → Xylol
537
Araf
Araf
537
537
217
Xylp → Xylp → Xylol
537
Araf
537
391
Xylp → Xylp → Xylol
537
Araf
m/z 711 ions
377
217
→ Xylp → Xylp → Xylol
217
363
→ Xylp → Xylol
Araf
391
→ Xylp → Xylol
363
Araf
363
217
Xylp → Xylp → Xylol
m/z 537 ions
217
→ Xylp → Xylol
m/z 377 ions
217
→ Xylp → Xylol
m/z 363 ions
→ Xol
217
A
m/z 391 ions

Figure S2


A
391.2
MS² m/z 565
565.3
217.1
200
250
300
350
400
450
500
550
600

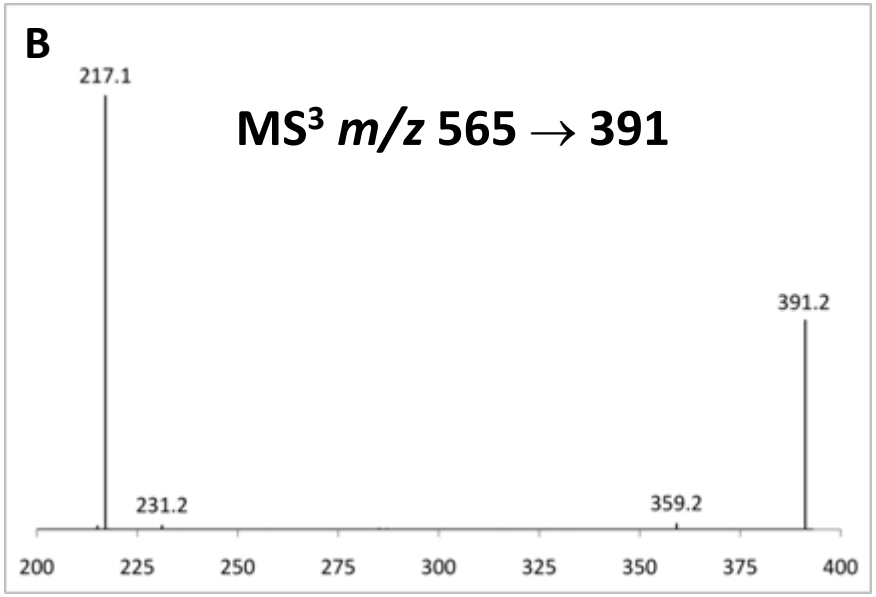

B
217.1
MS³ m/z 565 → 391
391.2
231.2
359.2
200
225
250
275
300
325
350
375
400

Figure S3

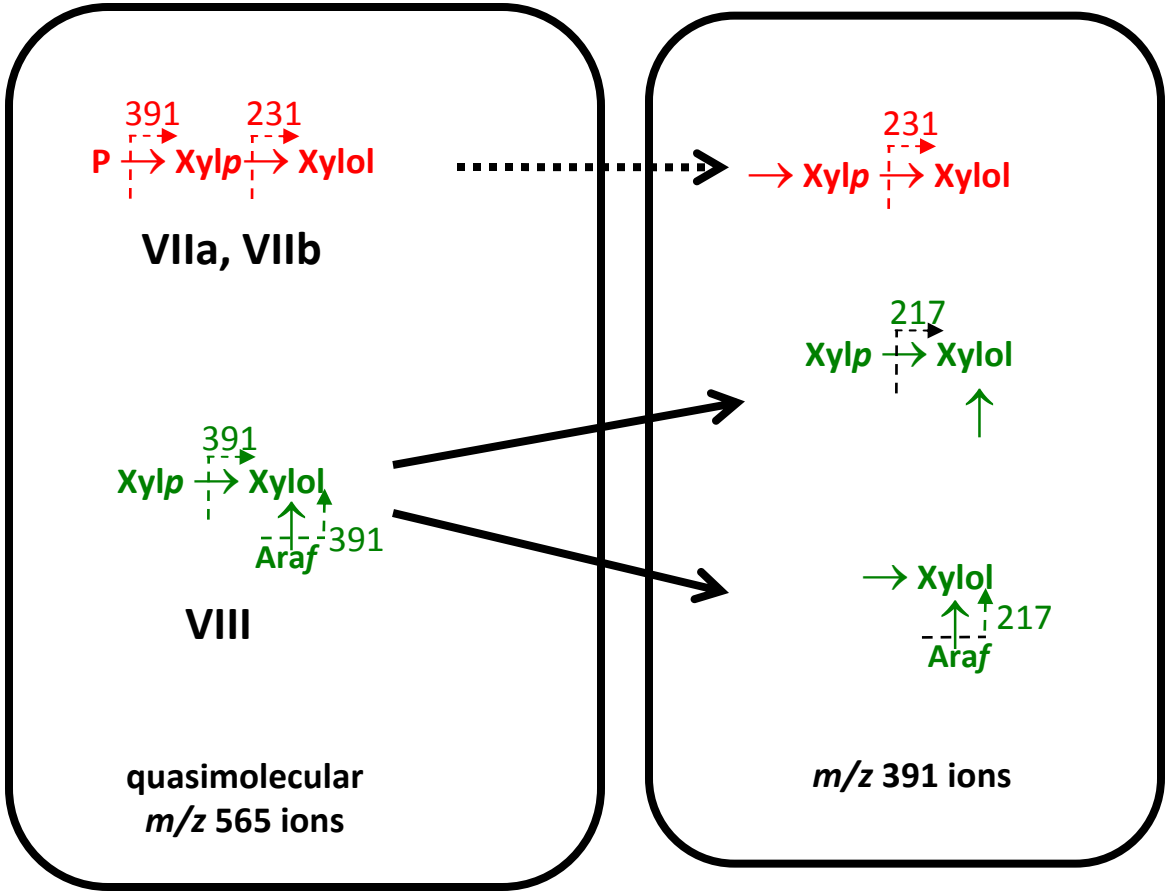

391
231
P
Xylp
Xylol
VIIa, VIIb
231
Xylp
Xylol
217
Xylp
Xylol
391
Xylp
Xylol
391
Araf
VIII
Xylol
217
Araf
quasimolecular
m/z 565 ions
m/z 391 ions

Figure S4

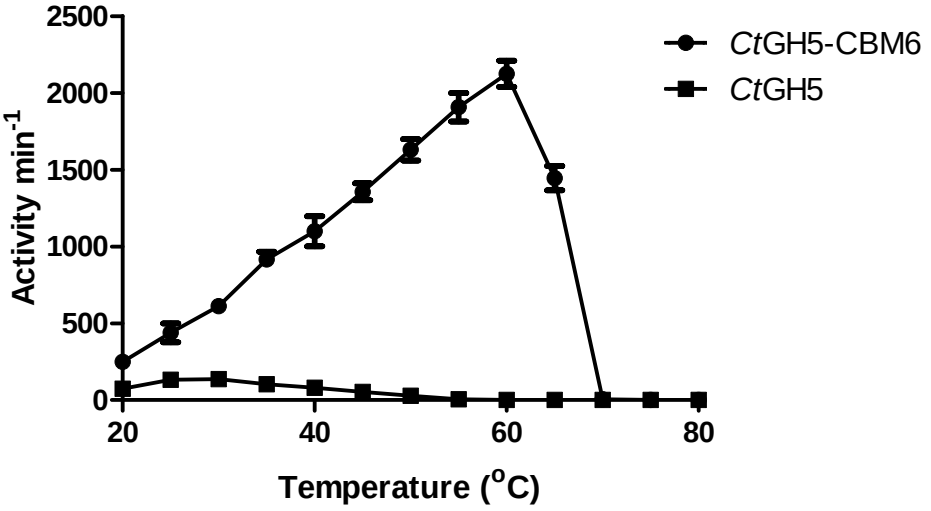

2500
2000
1500
1000
500
0
Activity min-1
20
40
60
80
Temperature (oC)
CtGH5-CBM6
CtGH5

Figure S5