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Supplementary Information for

A SIMPLE AND DIRECT IONIC CHROMATOGRAPHY METHOD TO MONITOR GALACTOSE OXYDASE ACTIVITY

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Table S1. Assignments of ^{1}H and ^{13}C chemical shifts (ppm) of *Fgr*GalOx oxidation of D-galactose as determined by 1D and 2D NMR experiments recorded at 600 MHz and 400 MHz, respectively in D_2O. The peaks are referenced to internal acetone (2.22 ppm for ^{1}H and 30.89 ppm for ^{13}C).

Ring System ^{1}H	δ (ppm)	Multiplicity	Coupling Constant (J) (Hz)	Ring System ^{13}C	δ (ppm)
α-Galactose (Product)				**α-Galactose (Product)**	
H_1	5.26	D	3.70	C_1	92.92
H_2	3.82	M	n.a.	C_2	68.85
H_3	3.82	M	n.a.	C_3	n.a.
H_4	4.12	M	n.a.	C_4	69.44
H_5	3.80	M	n.a.	C_5	72.85
H_6	5.09	D	7.50	C_6	89.07
α-Galactose (Substrate)				**α-Galactose (Substrate)**	
H_1	5.26	D	3.70	C_1	92.92
H_2	3.82	M	n.a.	C_2	68.98
H_3	3.82	M	n.a.	C_3	69.79
H_4	3.98	M	n.a.	C_4	69.94
H_5	4.07	M	n.a.	C_5	71.11
H_6/H'_6	3.74	M	n.a.	C_6	61.81
β-Galactose (Product)				**β-Galactose (Product)**	
H_1	4.57	D	7.98	C_1	97.22
H_2	3.50	M	n.a.	C_2	72.33
H_3	3.63	M	n.a.	C_3	73.37
H_4	4.07	M	n.a.	C_4	68.90
H_5	3.41	M	n.a.	C_5	77.49
H_6	5.12	D	7.18	C_6	88.82
β-Galactose (Substrate)				**β-Galactose (Substrate)**	
H_1	4.57	D	7.98	C_1	97.09
H_2	3.50	M	n.a.	C_2	72.51
H_3	3.63	M	n.a.	C_3	73.43
H_4	3.92	M	n.a.	C_4	69.39
H_5	3.70	M	n.a.	C_5	75.79
H_6/H'_6	3.74	M	n.a.	C_6	61.61

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Table S2. Assignments of ^{1}H and ^{13}C chemical shifts (ppm) of *Fgr*GalOx oxidation of D-lactose as determined by 1D and 2D NMR experiments recorded at 600 MHz and 400 MHz, respectively in D_2O. The peaks are referenced to internal acetone (2.22 ppm for ^{1}H and 30.89 ppm for ^{13}C).

Ring System ^{1}H	δ (ppm)	Multiplicity	Coupling Constant (J) (Hz)	Ring System ^{13}C	δ (ppm)
Galactose (Product)				**Galactose (Product)**	
H_1	4.45	M	n.a.	C_1	103.88 or 103.85
H_2	3.55	M	n.a.	C_2	71.35
H_3	3.66	M	n.a.	C_3	73.10
H_4	4.08	M	n.a.	C_4	68.65
H_5	3.46	D	7.48	C_5	77.59
H_6	5.15	M	n.a.	C_6	88.64
Galactose (Substrate)				**Galactose (Substrate)**	
H_1	4.45	M	n.a.	C_1	103.58
H_2	3.55	M	n.a.	C_2	71.62
H_3	3.66	M	n.a.	C_3	73.17
H_4	3.92	M	n.a.	C_4	69.21
H_5	3.72	M	n.a.	C_5	76.02
H_6/H'_6	3.76	M	n.a.	C_6	61.70
α-Glucose (Product)				**α-Glucose (Product)**	
H_1	5.22	M	n.a.	C_1	92.44
H_2	3.94	M	n.a.	C_2	70.56
H_3	3.55	M	n.a.	C_3	71.74
H_4	3.63	M	n.a.	C_4	80.11
H_5	3.83	M	n.a.	C_5	72.22
H_6/H'_6	3.86	M	n.a.	C_6	60.70
β-Glucose (Product)				**β-Glucose (Product)**	
H_1	5.66	D	7.93	C_1	96.34
H_2	3.28	M	n.a.	C_2	74.40
H_3	3.62	M	n.a.	C_3	75.19
H_4	3.63	M	n.a.	C_4	80.00
H_5	3.62	M	n.a.	C_5	75.31
H_6/H'_6	3.80	M	n.a.	C_6	60.84
α-Glucose (Substrate)				**α-Glucose (Substrate)**	
H_1	5.22	M	n.a.	C_1	92.48
H_2	3.94	M	n.a.	C_2	70.77
H_3	3.55	M	n.a.	C_3	71.81
H_4	3.63	M	n.a.	C_4	79.08
H_5	3.83	M	n.a.	C_5	72.07
H_6/H'_6	3.86	M	n.a.	C_6	60.60
β-Glucose (Substrate)				**β-Glucose (Substrate)**	
H_1	5.66	D	7.93	C_1	96.42
H_2	3.28	M	n.a.	C_2	74.47
H_3	3.62	M	n.a.	C_3	75.02
H_4	3.63	M	n.a.	C_4	78.95
H_5	3.62	M	n.a.	C_5	75.47
H_6/H'_6	3.80	M	n.a.	C_6	60.73

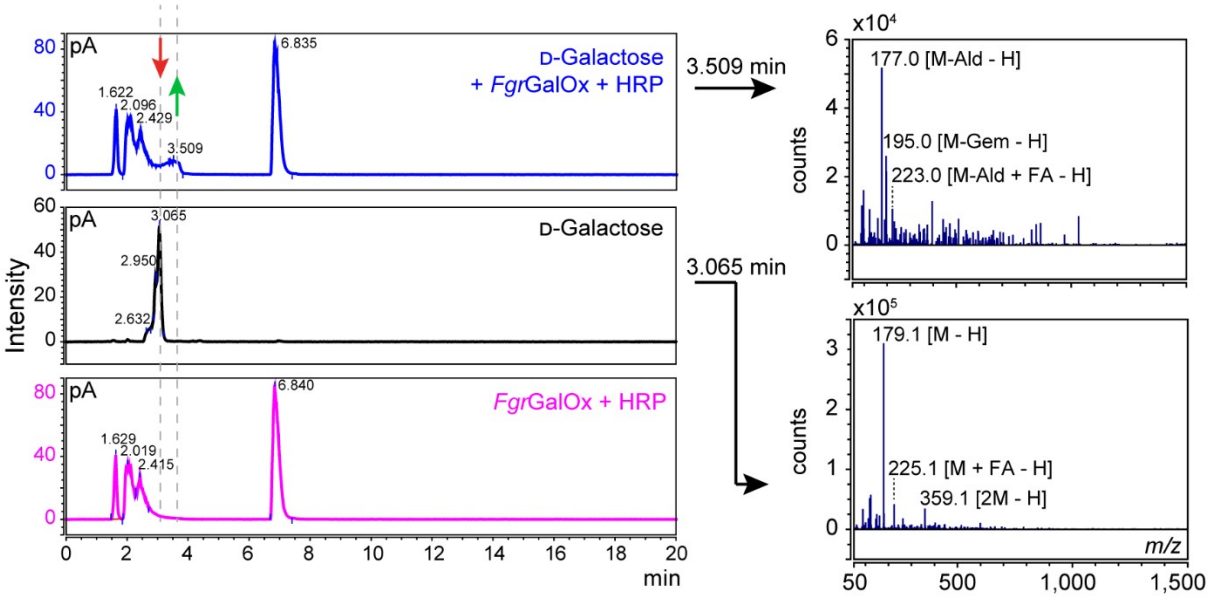

Figure S1. LC-MS analysis of *Fgr*GalOx reaction products with D-galactose as substrate. **(A)** LC-MS analysis showing the detection of adducts (in negative mode; FA, formate) of D-galactose, *m/z* (g.mol^{-1}) = 179.1 [M-H], 225.1 [M + FA - H] and 359.1 [2M-H]; and derived oxidized forms : aldehyde, *m/z* = 177.0 [M-Ald -H], 223.0 [M-Ald + FA -H]; and *geminal*-diol, *m/z* = 195.0 [M-Gem + FA -H]. Reaction mixtures contained D-galactose (1 mM final concentration), *Fgr*GalOx (50 nM) and HRP (0.05 mg.mL^{-1}) in water and were incubated in a Thermomixer (23 °C, 1,000 rpm, 24 h).

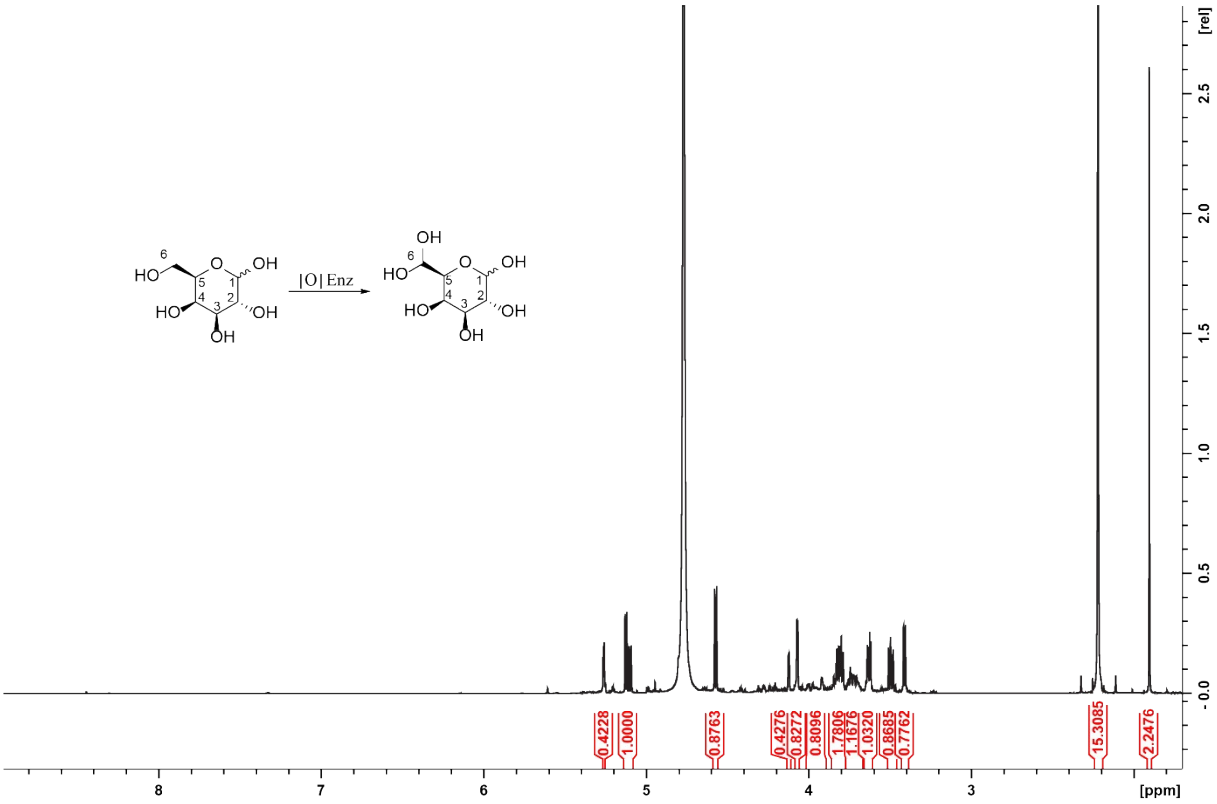

Figure S2. Full ^{1}H-NMR spectrum of the oxidation of D-galactose by *Fgr*GalOx. ^{1}H NMR spectra (600 MHz) of reaction product profiles after 24 h incubation with enzyme in the presence of catalase and HRP with 50 mM of substrate. The peak at 2.22 pm corresponds to acetone that was used as an internal standard. See **Table S1** for peak assignment.

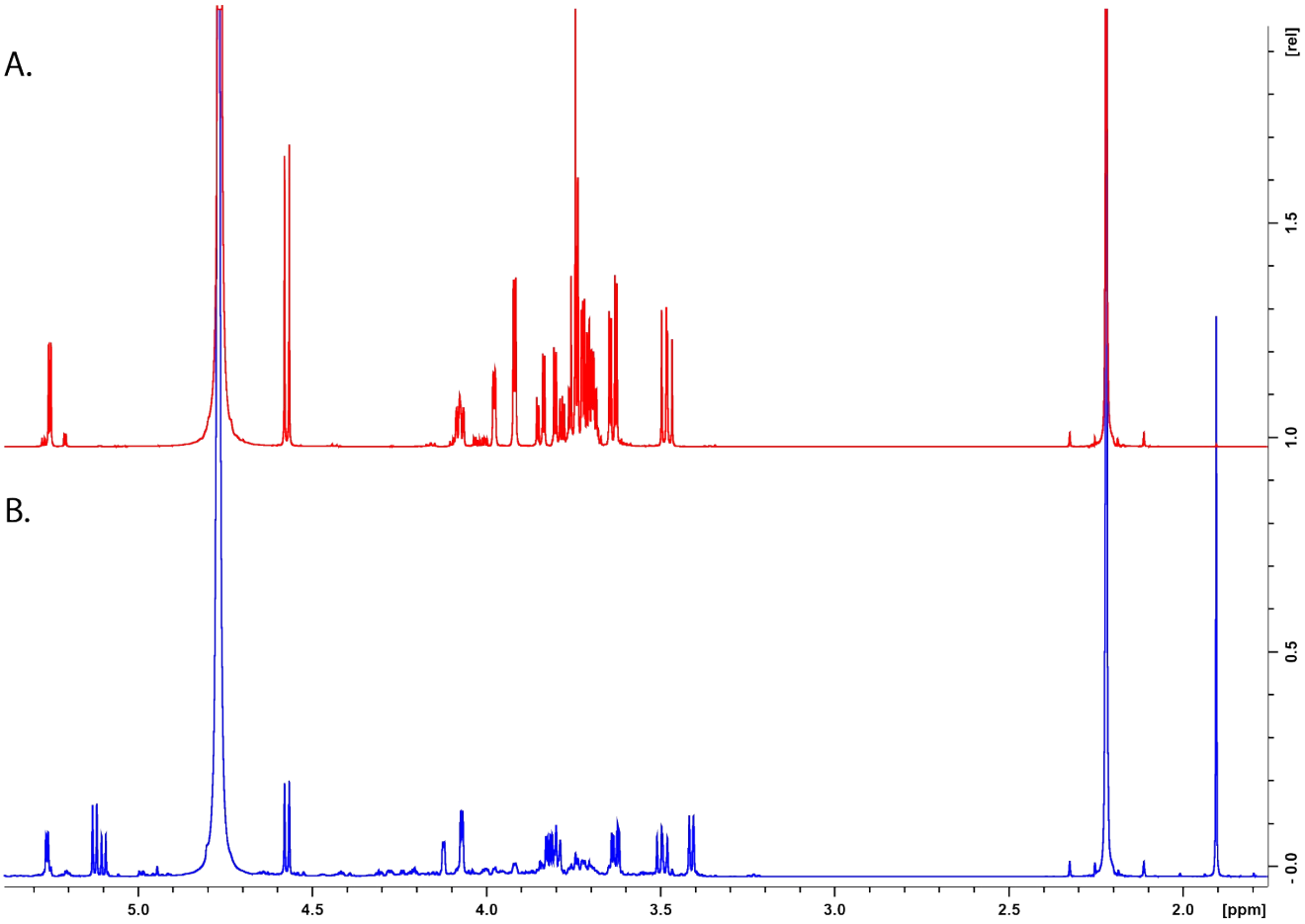

Figure S3. ^{1}H-NMR analysis of the control reaction (A) and the oxidative reaction with *Fgr*GalOx (B) of D-galactose. Panel B shows ^{1}H NMR spectra (600 MHz) of reaction product profiles after 24 h incubation with enzyme in the presence of catalase and HRP with 50 mM of substrate, and panel A shows the control without enzymes. The peak at 2.22 pm corresponds to acetone that was used as an internal standard. See **Table S1** for peak assignment.

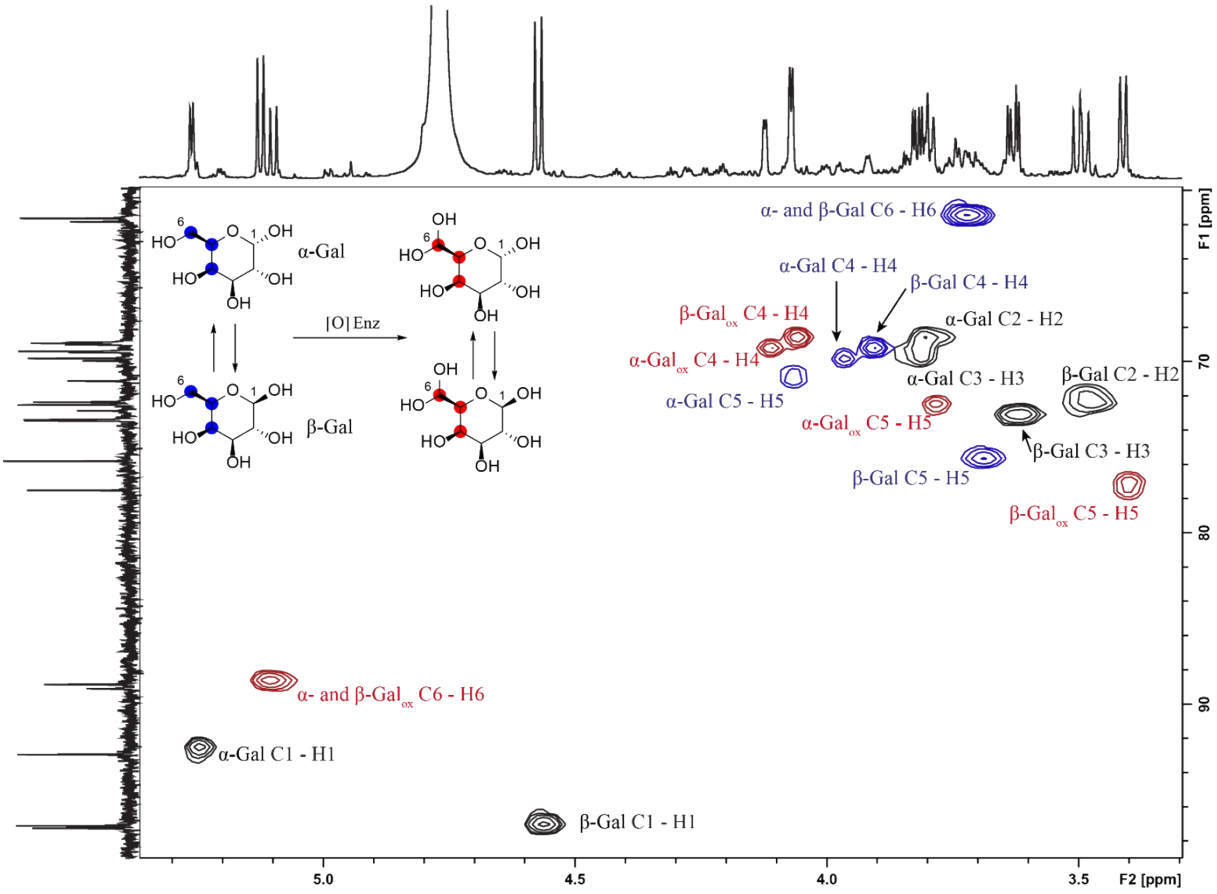

Figure S4. Complete assignment of proton and carbon nuclei from oxidized D-galactose by *Fgr*GalOx using $^{1}H,^{13}C$ HSQC experiments. Each crosspeak corresponds to a $^{1}J_{C,H}$-coupling interaction from the HSQC experiment. Red crosspeaks correspond to peaks unique to the oxidized product, blue crosspeaks correspond to unique peaks found in the unoxidized substrate and black crosspeaks correspond to signals overlapping from substrate and product. The notation states the sugar ring and its corresponding atoms(s) below it. See **Table S1** for peak assignment. Abbreviations used: Gal, galactose; ox, Oxidized.

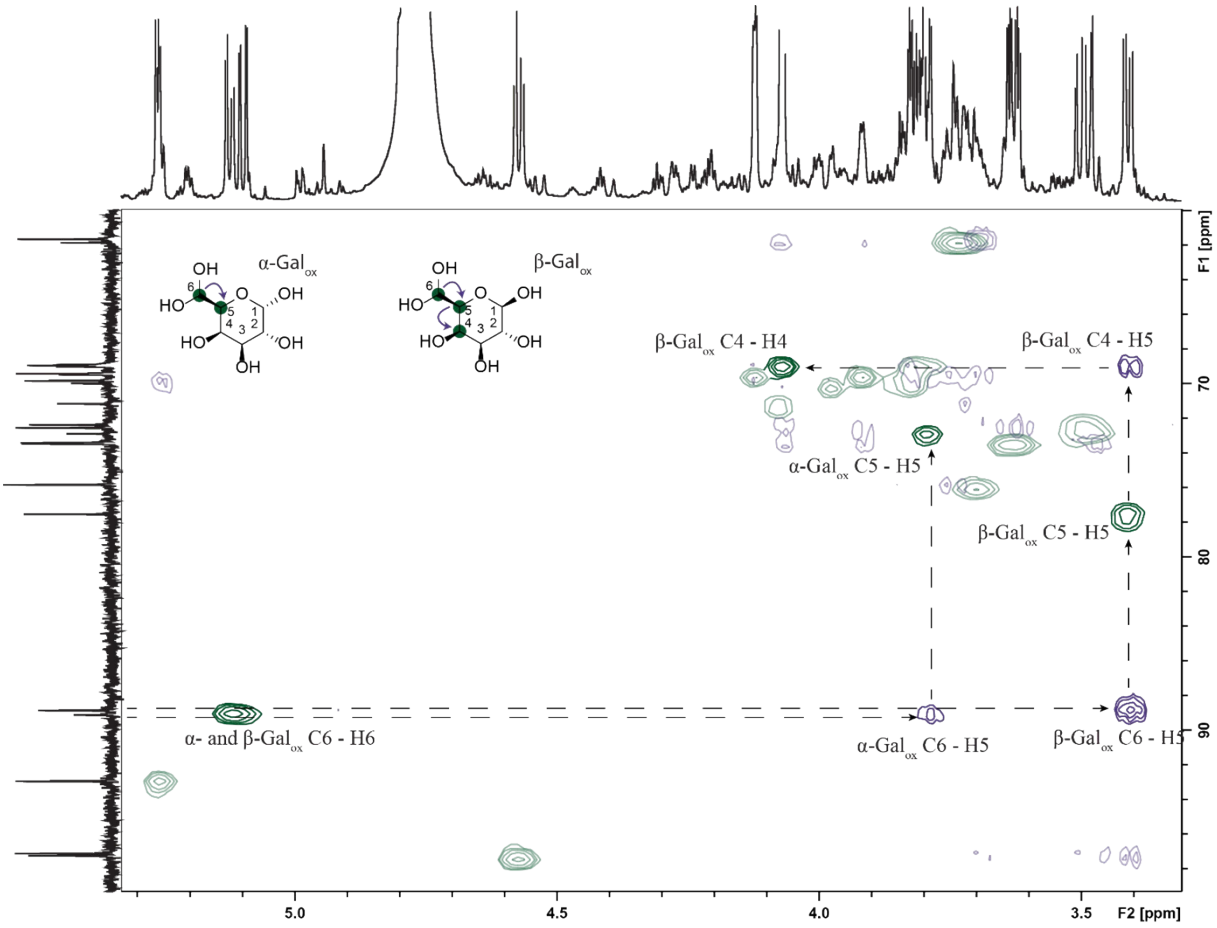

Figure S5. Selective assignment of proton and carbon nuclei from ^{1}H,^{13}C HSQC and HMBC of oxidation product *Fgr*GalOx with D-galactose. Green crosspeaks correspond to a $^{1}J_{C,H}$-coupling interaction from the HSQC experiment while purple crosspeaks correspond to a $^{2}J_{C,H}$- or $^{3}J_{C,H}$-coupling interaction from the HMBC experiment. The dashed arrows showcase the path between the green HSQC crosspeaks and purple HMBC crosspeaks of the oxidized galactose used to confirm the site of oxidation. The annotation of each crosspeak states the sugar residue and the corresponding carbon and hydrogen atom(s). See **Table S1** for peak assignment. Abbreviations used: Gal, galactose; ox, Oxidized.

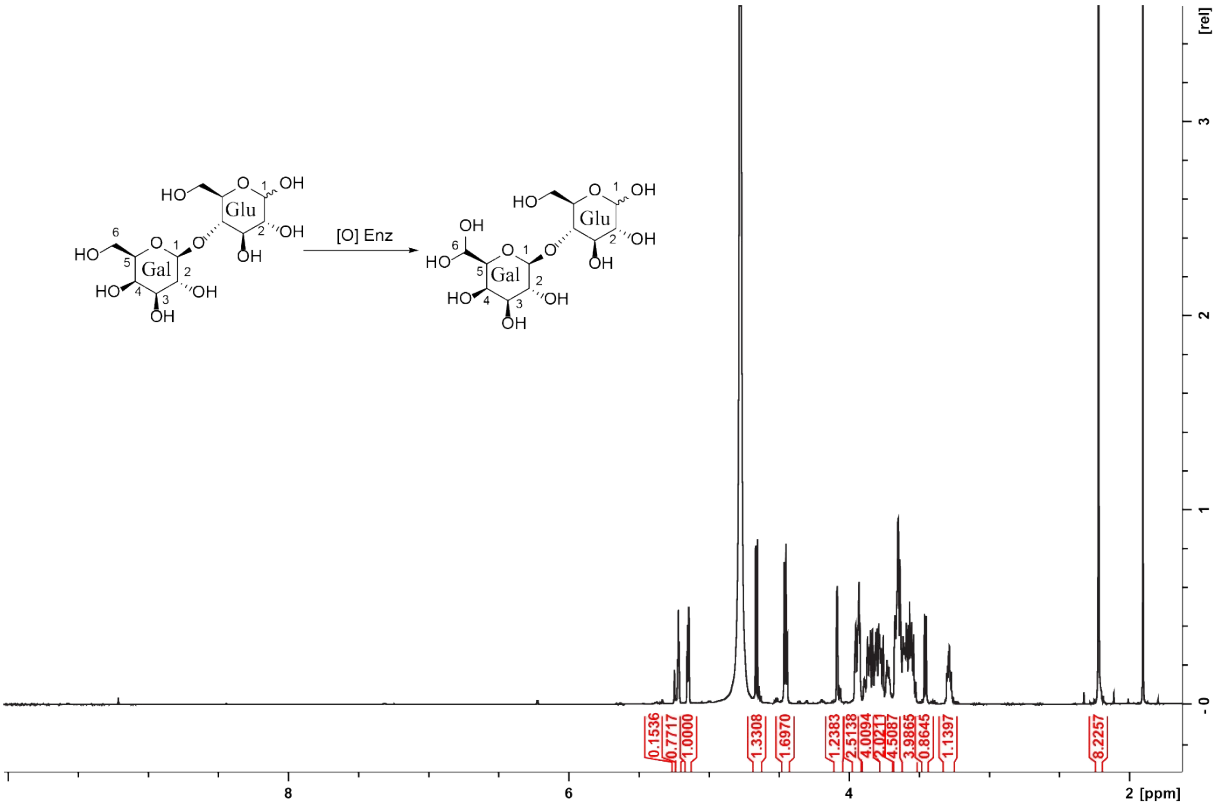

Figure S6. Full ^{1}H-NMR spectrum of the oxidation of D-lactose by *Fgr*GalOx. ^{1}H NMR spectra (600 MHz) of reaction product profiles after 24 h incubation with enzyme in the presence of catalase and HRP with 50 mM of substrate. The peak at 2.22 pm corresponds to acetone that was used as an internal standard. See **Table S2** for peak assignment.

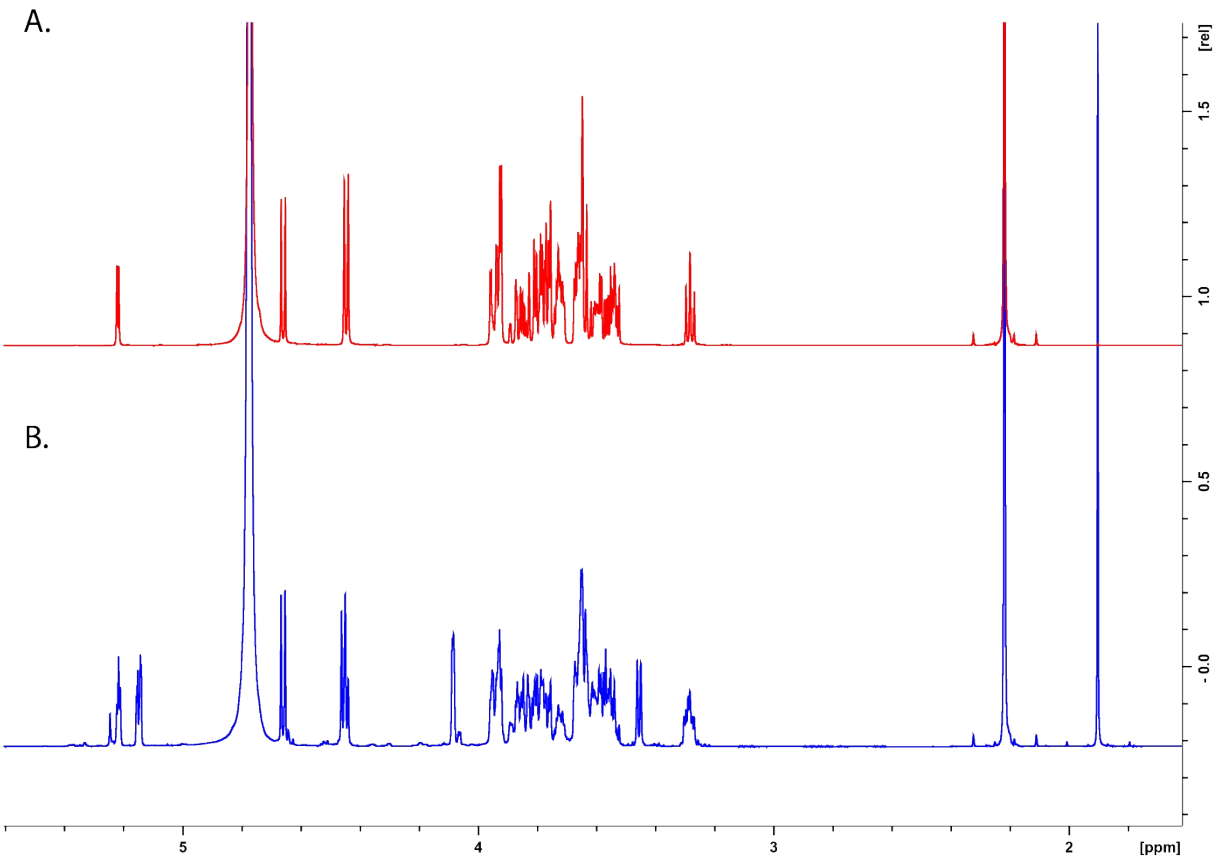

Figure S7. ^{1}H-NMR analysis of the control reaction (A) and the oxidative reaction with *Fgr*GalOx (B) of D-lactose. Panel B shows ^{1}H NMR spectra (600 MHz) of reaction product profiles after 24 h incubation with enzyme in the presence of catalase and HRP with 50 mM of substrate, and panel A shows the control without enzymes. The peak at 2.22 pm corresponds to acetone that was used as an internal standard. See **Table S2** for peak assignment.

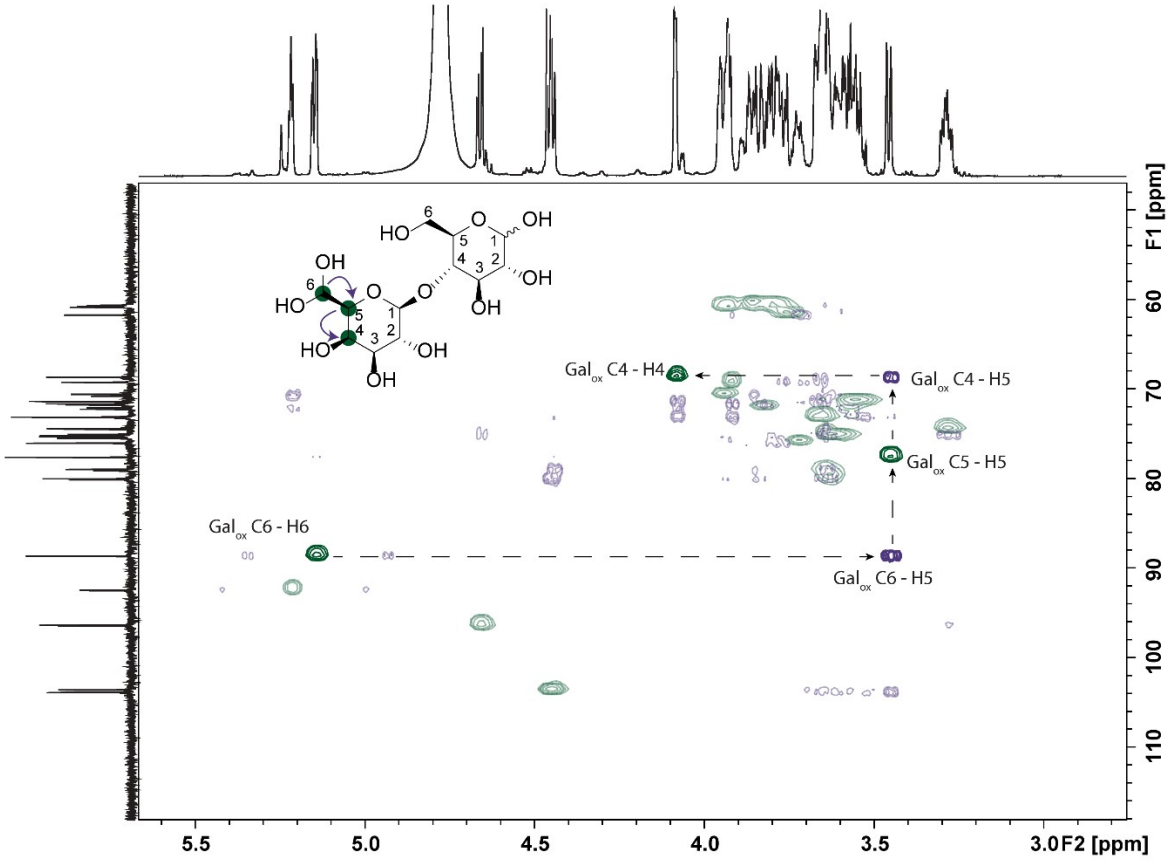

Figure S8. Selective assignment of proton and carbon nuclei from ^{1}H,^{13}C HSQC and HMBC of oxidation product *Fgr*GalOx with D-lactose. Green crosspeaks correspond to a $^{1}J_{C,H}$-coupling interaction from the HSQC experiment while purple crosspeaks correspond to a $^{2}J_{C,H}$- or $^{3}J_{C,H}$-coupling interaction from the HMBC experiment. The dashed arrows showcase the path between the red HSQC crosspeaks and blue HMBC crosspeaks of the oxidized galactose ring in lactose used to confirm the site of oxidation. The annotation of each crosspeak states the sugar residue and the corresponding carbon and hydrogen atom(s). Abbreviations used: Gal, galactose; ox, Oxidized. See **Table S2** for peak assignment.