SUPPLEMENTAL INFORMATION

Structural and functional insights into methyl fucosidases from glycoside hydrolase family 139

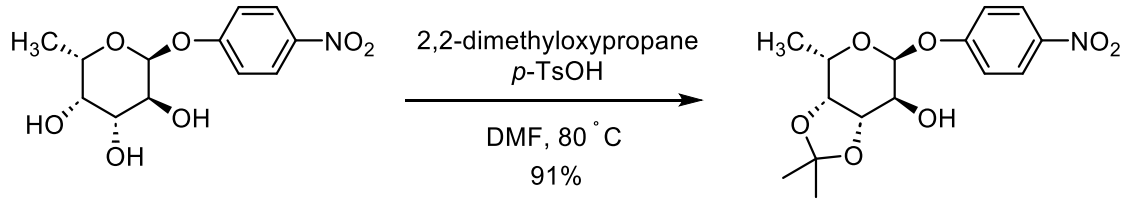
Zak McIver[1,2,3#], Alicia Moraleda-Montoya[4,#], Zongjia Chen[5], Ruwan Epa[5] David Starns[6], Matthew Davy[7], Mikel García-Alija[4], Arnaud Basle[8], Mario Schubert[9], Didier Ndeh[10], Beatriz Trastoy[4,11], Spencer J. Williams[5], Marcelo E. Guerin[12], and Alan Cartmell[1,2,3]*

[1]Department of Biology, University of York, York, YO10 5DD, United Kingdom,
[2]York Structural Biology Laboratory, [3]York Biomedical Research Institute, University of York, York, YO10 5DD, United Kingdom
[3]York Biomedical Research Institute, University of York, York, YO10 5DD, United Kingdom,
[4]Structural Glycoimmunology Laboratory, Biobizkaia Health Research Institute, 48903 Barakaldo, Spain.
[5]School of Chemistry and Bio21, Molecular Science and Biotechnology Institute, University of Melbourne, Parkville, Victoria3010, Australia
[6]School of Molecular and Cellular Biology, Faculty of Biological Sciences, University of Leeds
[7] Department of Chemistry, University of York, York, YO10 5DD, United Kingdom,
[8]Newcastle University Biosciences Institute, Medical School, Newcastle University, Newcastle upon Tyne NE2 4HH, UK
[9] FU Berlin, Department of Biology, Chemistry and Pharmacy, Takustr. 3, 14195 Berlin, Germany
[10] School of Life Sciences, E109, The James Hutton Institute, Errol Road, Invergowrie, Dundee, DD2 5DA, University of Dundee
[11]Ikerbasque, Basque Foundation for Science, 48009 Bilbao, Spain.
[12]Structural Glycobiology Laboratory, Department of Structural and Molecular Biology; Molecular Biology Institute of Barcelona (IBMB), Spanish National Research Council (CSIC), 08028 Barcelona, Catalonia, Spain.

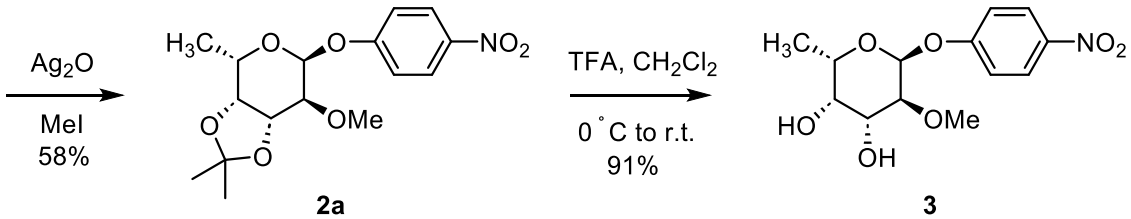
[#]These authors contributed equally

*To whom correspondence should be addressed: alan.cartmell@york.ac.uk

Synthesis of 4-Nitrophenyl-2-*O*-methyl-α-L-fucopyranoside



PNP-a-L-Fuc **1**



2a **3**

4-Nitrophenyl 3,4-*O*-isopropylidene-α-L-fucopyranoside (1)

A mixture of 4-nitrophenyl 3,4-*O*-isopropylidene-α-L-fucoside (320 mg, 1.1 mmol), *p*-TsOH (5 mg, 0.03 mmol) and 2,2-dimethoxypropane (420 mg, 4.0 mmol) in DMF (5 mL) was stirred under a N_2 atmosphere at 80 °C for 1 h. The solvent was removed and the residue was purified by flash chromatography (n-hexane:EtOAc=4:1) to give the product as an oil (332 mg, 91%).

$[\alpha]_D$ = -199.82 (*c* = 1.00, $CHCl_3$)

^{1}H ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.30 (d, *J* = 6.6 Hz, 3H), 1.38 (s, 3H), 1.55 (s, 3H), 4.02 (dd, *J* = 6.6, 3.6 Hz, 1H), 4.14 (dd, *J* = 6.0, 2.3 Hz, 1H), 4.20 (qd, *J* = 6.6, 2.2 Hz, 1H), 4.40 (t, *J* = 6.3 Hz, 1H), 5.61 (d, *J* = 3.6 Hz, 1H), 7.18 (d, *J* = 9.3 Hz, 2H), 8.20 (d, *J* = 9.3 Hz, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 16.4, 26.0, 27.9, 65.8, 69.3, 75.4, 75.8, 96.7, 109.8, 116.6, 126.0, 142.8, 161.6.

HRMS (ESI^+) calcd for $C_{15}H_{19}ClNO_7$ $[M+Cl]^-$ 360.0850. Found 360.0854.

4-Nitrophenyl 3,4-*O*-isopropylidene-2-*O*-methyl-α-L-fucopyranoside (2a)

A mixture of **1** (150 mg, 0.46 mmol) and Ag_2O (871 mg, 3.76 mmol) in CH_3I (1 mL) was stirred at room temperature for 8 h (***). The mixture was filtered through Celite, and the Celite was washed thoroughly with EtOAc. The residue was purified by chromatography (n-hexane:EtOAc = 5:1) to give the product as a white crystalline solid (92 mg, 58%).

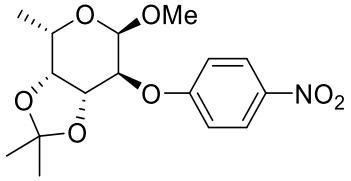
$[\alpha]_D$ = -162.2 (*c* = 1.00, $CHCl_3$)

^{1}H NMR (500 MHz, $CDCl_3$) δ 1.31 (d, *J* = 6.6 Hz, 3H), 1.38 (s, 3H), 1.58 (s, 3H), 3.55 (dd, *J* = 7.6, 3.4 Hz, 1H), 3.57 (s, 3H), 4.05–4.14 (m, 2H), 4.43 (dd, *J* = 7.5, 5.6 Hz, 1H), 5.66 (d, *J* = 3.4 Hz, 1H), 7.19 (d, *J* = 9.2 Hz, 2H), 8.20 (d, *J* = 9.2 Hz, 2H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 16.4, 26.4, 28.4, 59.3, 65.0, 75.5, 75.8, 78.6, 95.3, 109.4, 116.5, 125.9, 142.7, 161.8.

HRMS (ESI^+) calcd for $C_{16}H_{22}NO_7$ $[M+H]^+$ 340.1391. Found 340.1385.

*** Caution! Prolonging the reaction beyond 16 hours will result in rearrangement to the methyl 2-O-nitrophenyl-fucoside **2b** (9% yield by HPLC separation).



2b

Methyl 3,4-*O*-isopropylidene-2-(4-nitrophenyl)-α-L-fucopyranoside (2b)

$[\alpha]_D$ = -122.5 (*c* = 0.50, $CHCl_3$)

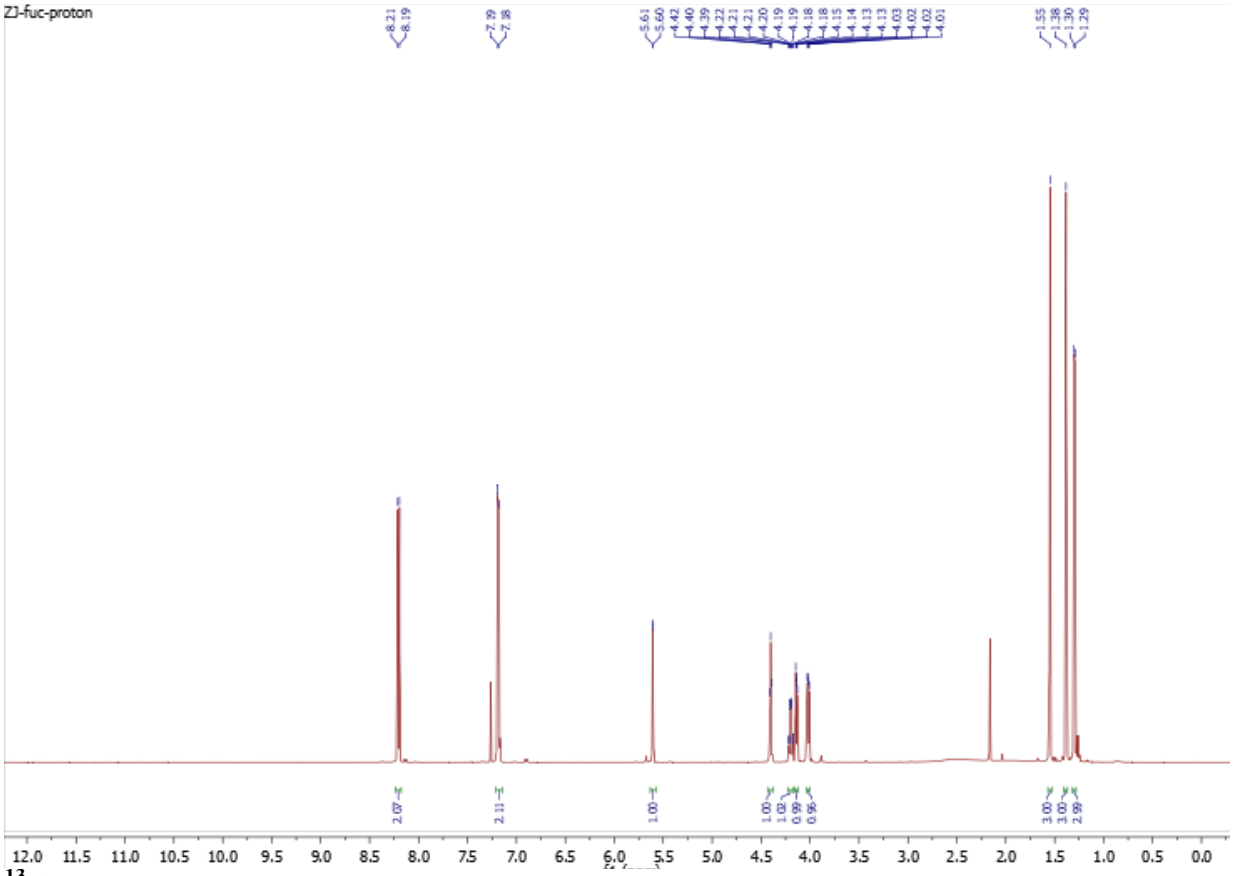
^{1}H NMR (600 MHz, $CDCl_3$) δ 1.34 (s, 3H), 1.45 (d, *J* = 6.6 Hz, 3H), 1.53 (s, 3H), 3.46 (s, 3H), 3.93 (qd, *J* = 6.5, 2.2 Hz, 1H), 4.10 (dd, *J* = 5.6, 2.2 Hz, 1H), 4.25–4.21 (m, 1H), 4.30–4.27 (m, 1H), 4.31 (d, *J* = 7.9 Hz, 1H), 7.11 (d, *J* = 9.3 Hz, 2H), 8.14 (d, *J* = 9.3 Hz, 2H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 16.5, 26.1, 28.1, 57.0, 69.1, 76.5, 78.2, 80.7, 102.4, 110.2, 116.5, 125.7, 141.9, 164.3.
HRMS (ESI^+) calcd for $C_{16}H_{22}NO_7$ $[M+H]^+$ 340.1391. Found 340.1390.

4-Nitrophenyl 2-*O*-methyl-α-L-fucopyranoside (3)

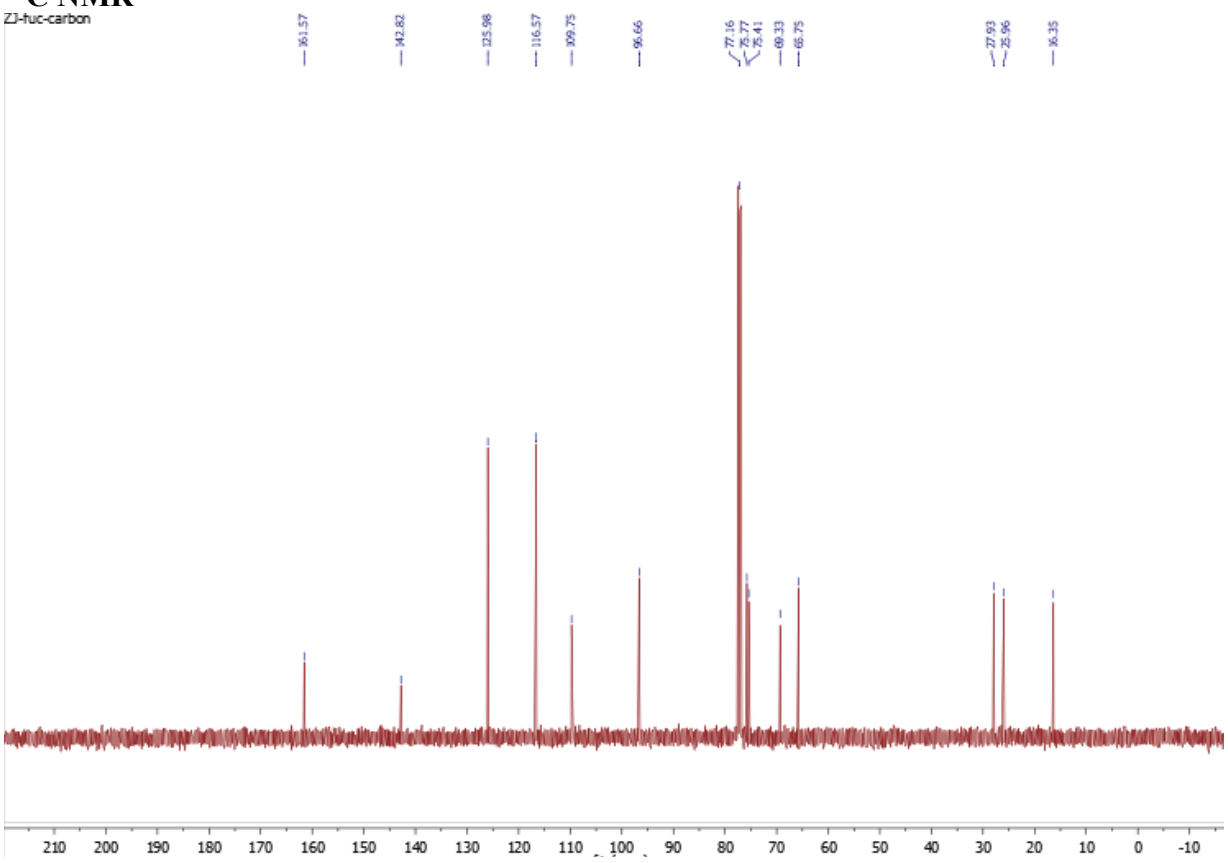
A solution of **2a** (25 mg, 0.074 mmol) in CH_2Cl_2 (2 mL) was cooled in an ice bath, and TFA (19 μL, 0.26 mmol) was added under a N_2 atmosphere. The mixture was warmed to room temperature and stirred for 4 h. The reaction was neutralized with NEt_3, and the solvent was removed under reduced pressure. The residue was purified by chromatography (n-hexane:EtOAc = 1:4) to give the product as a white foam (20 mg, 91%).
$[\alpha]_D$ = -98.9 (*c* = 0.88, $CHCl_3$)
^{1}H NMR (500 MHz, $CDCl_3$) δ 1.27 (d, *J* = 6.6 Hz, 3H), 2.49 (s, 1H), 2.71 (d, *J* = 3.0 Hz, 1H), 3.50 (s, 3H), 3.72 (dd, *J* = 9.8, 3.4 Hz, 1H), 3.90 (s, 1H), 3.97 (q, *J* = 6.6 Hz, 1H), 4.16 (dt, *J* = 9.8, 3.0 Hz, 1H), 5.78 (d, *J* = 3.4 Hz, 1H), 7.21 (d, *J* = 9.2 Hz, 2H), 8.22 (d, *J* = 9.2 Hz, 2H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 16.2, 58.5, 67.4, 69.5, 71.2, 77.6, 94.6, 116.6, 126.0, 142.8, 161.9.
HRMS (ESI^+) calcd for $C_{13}H_{18}NO_7$ $[M+H]^+$ 300.1078. Found 300.1073.

4-Nitrophenyl 3,4-*O*-isopropylidene-α-L-fucopyranoside (1)

^{1}H NMR

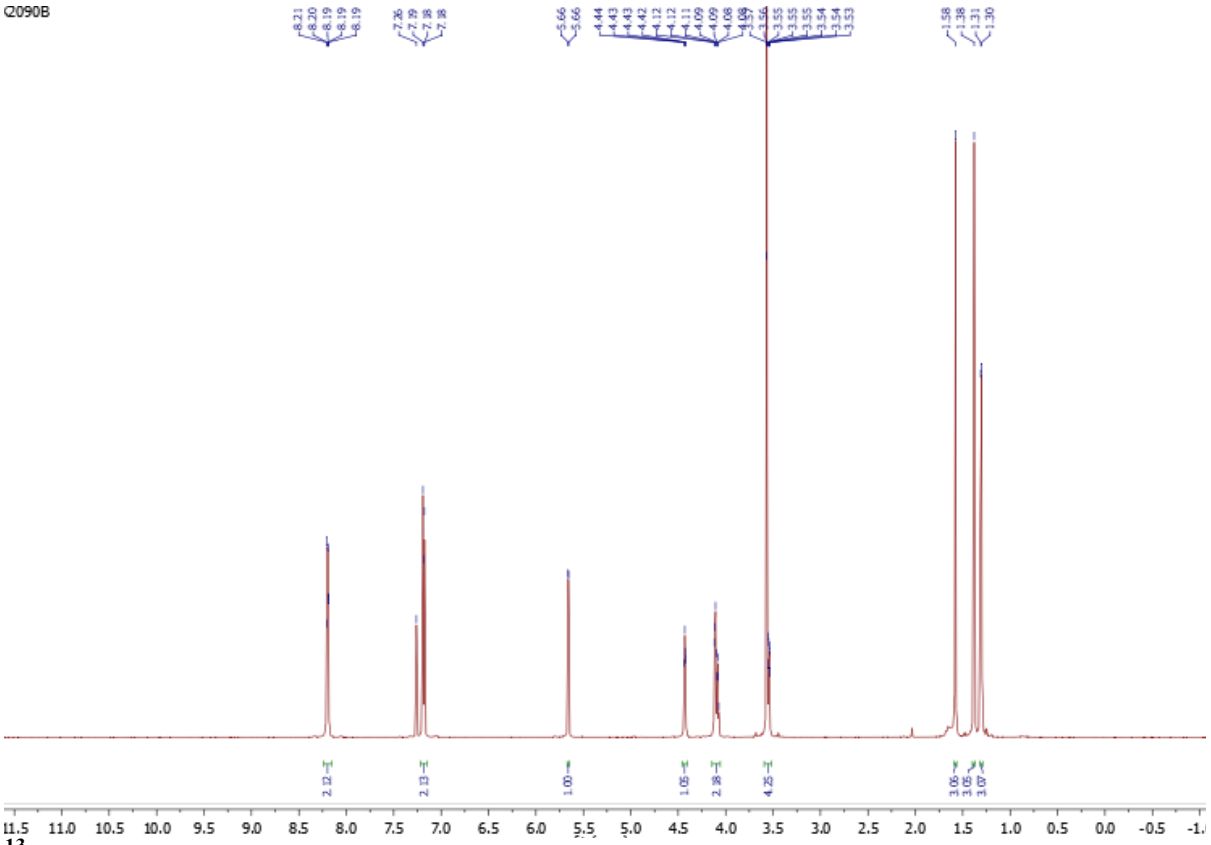


^{13}C NMR

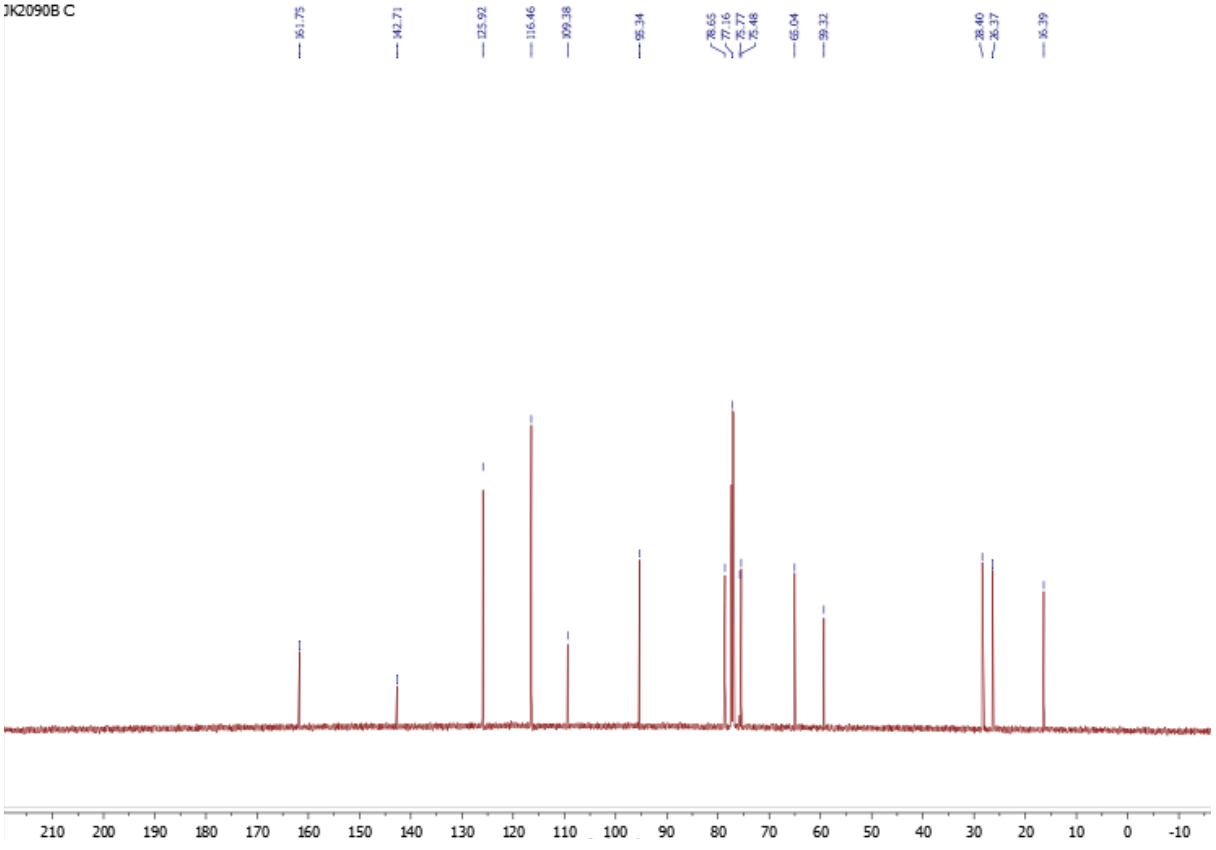


4-Nitrophenyl 3,4-*O*-isopropylidene-2-*O*-methyl-α-L-fucopyranoside (2a)

^{1}H NMR

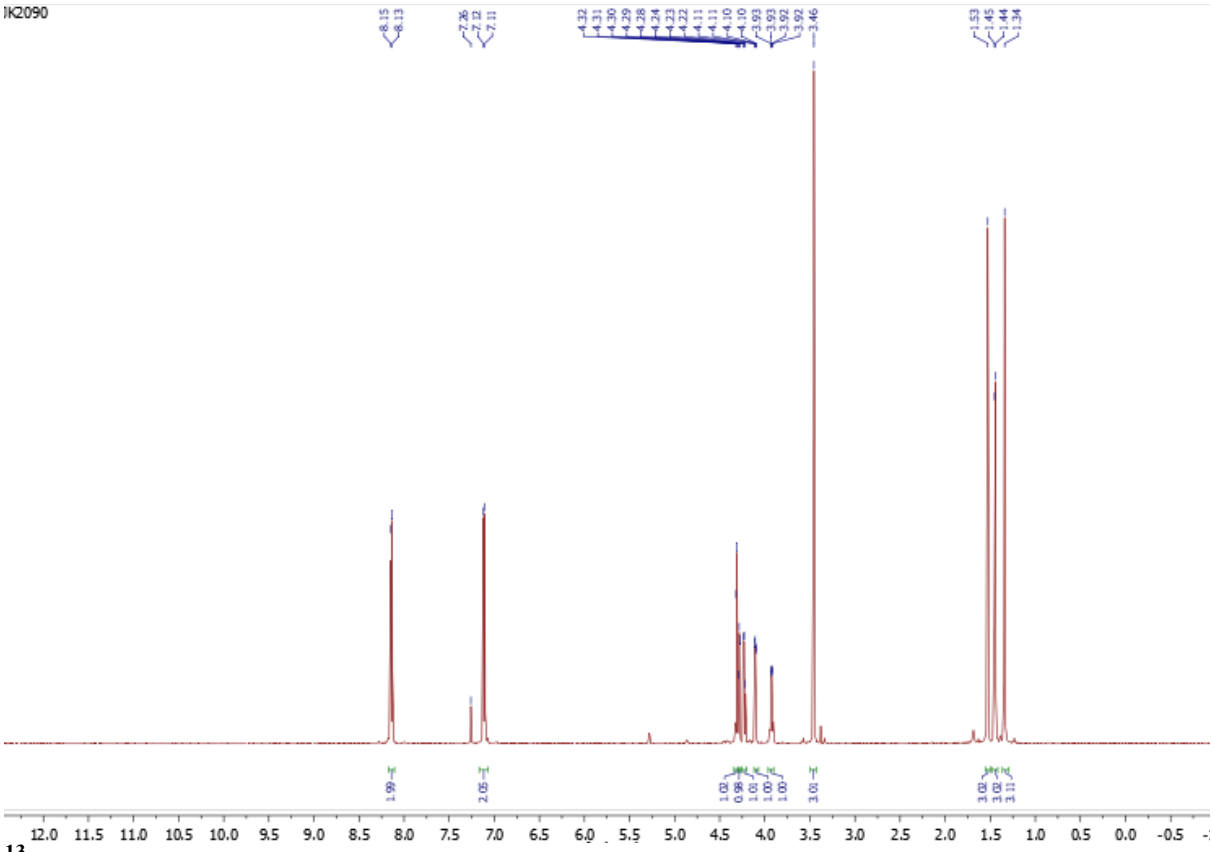


^{13}C NMR

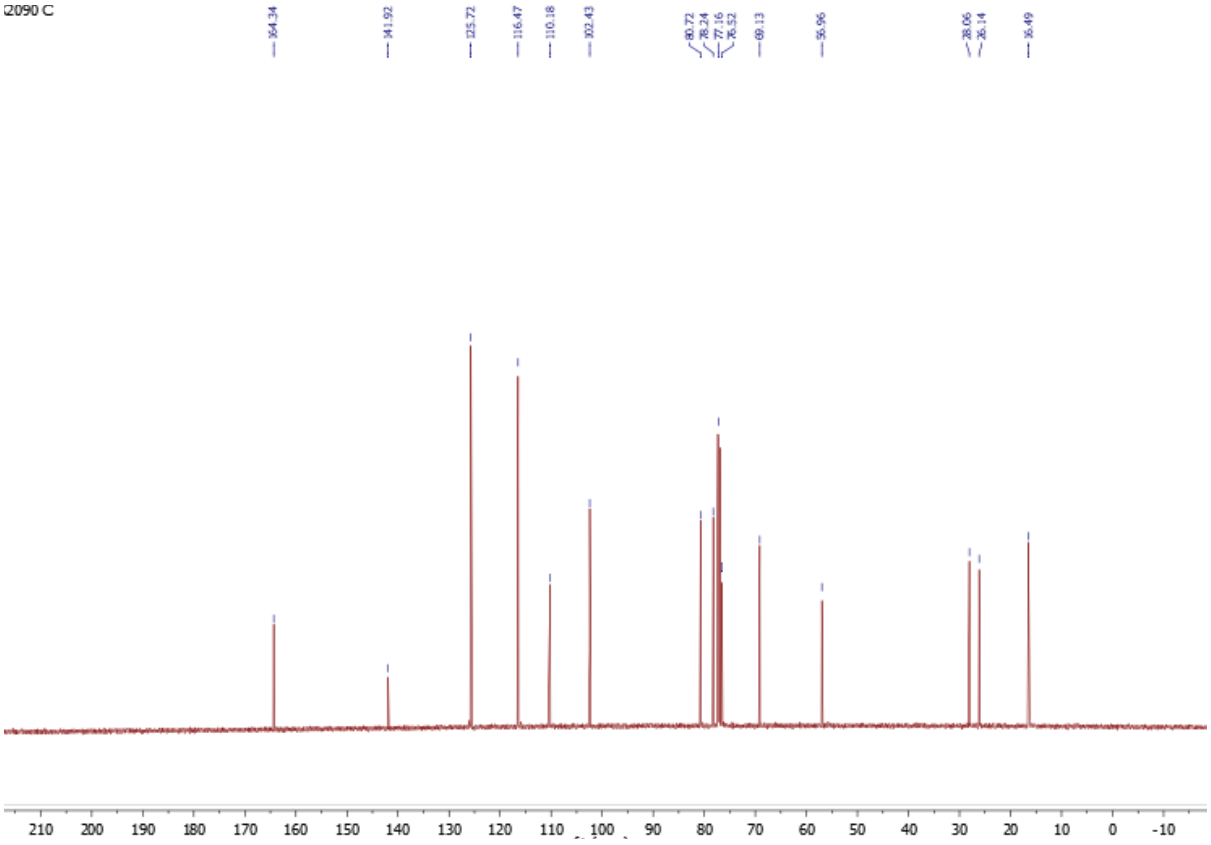


Methyl 3,4-*O*-isopropylidene-2-(4-nitrophenyl)-α-L-fucopyranoside (2b)

^{1}H NMR

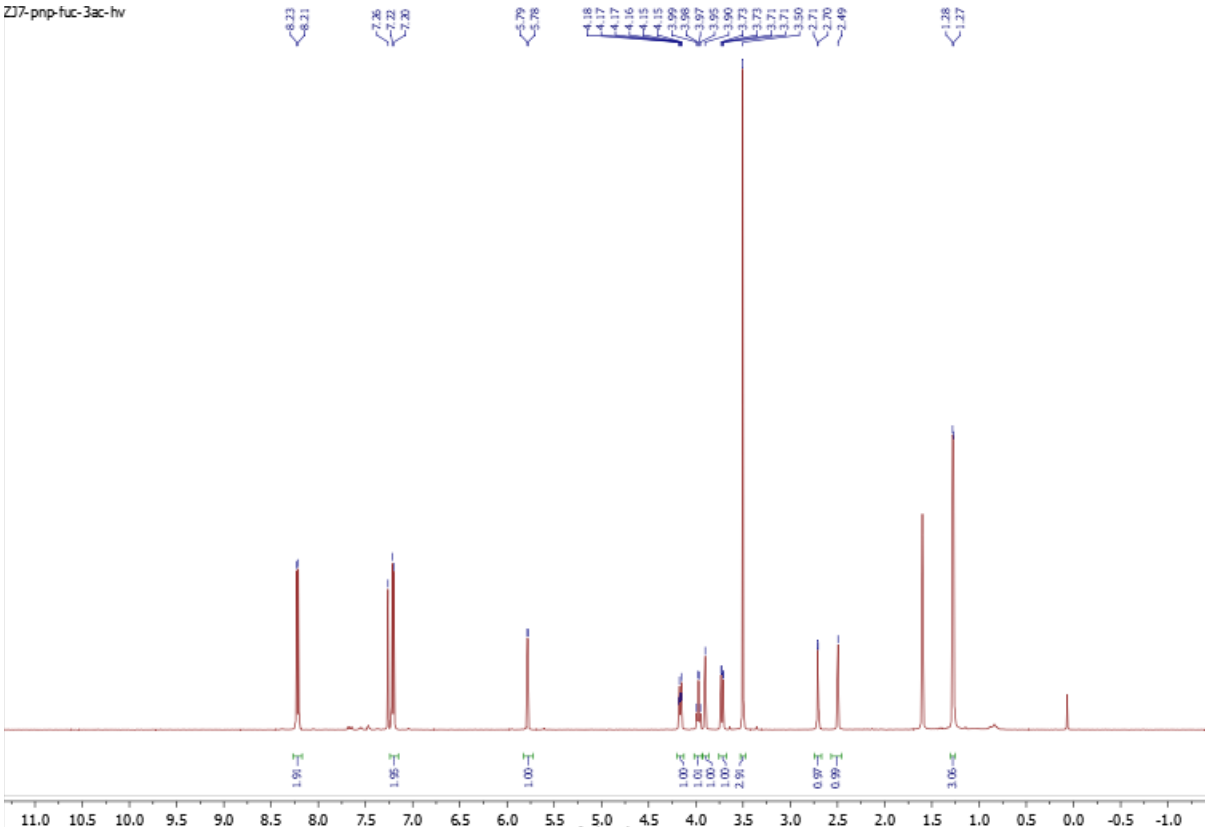


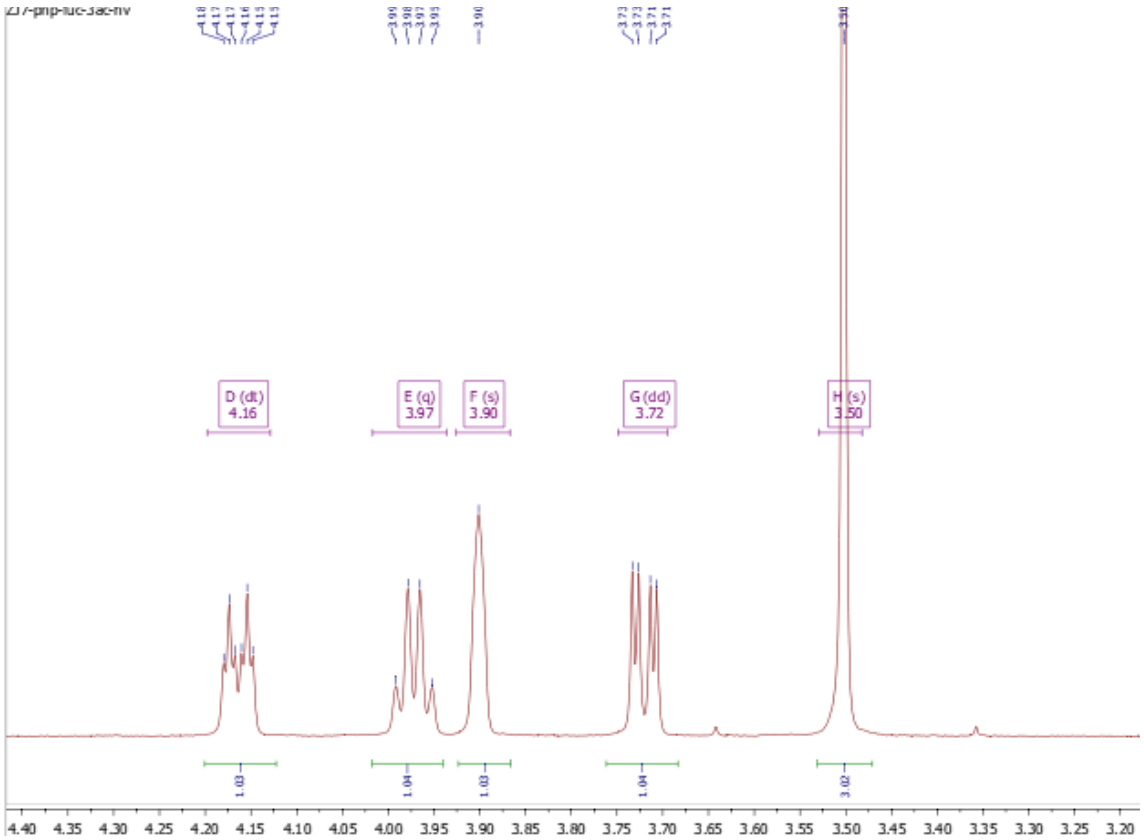
^{13}C NMR



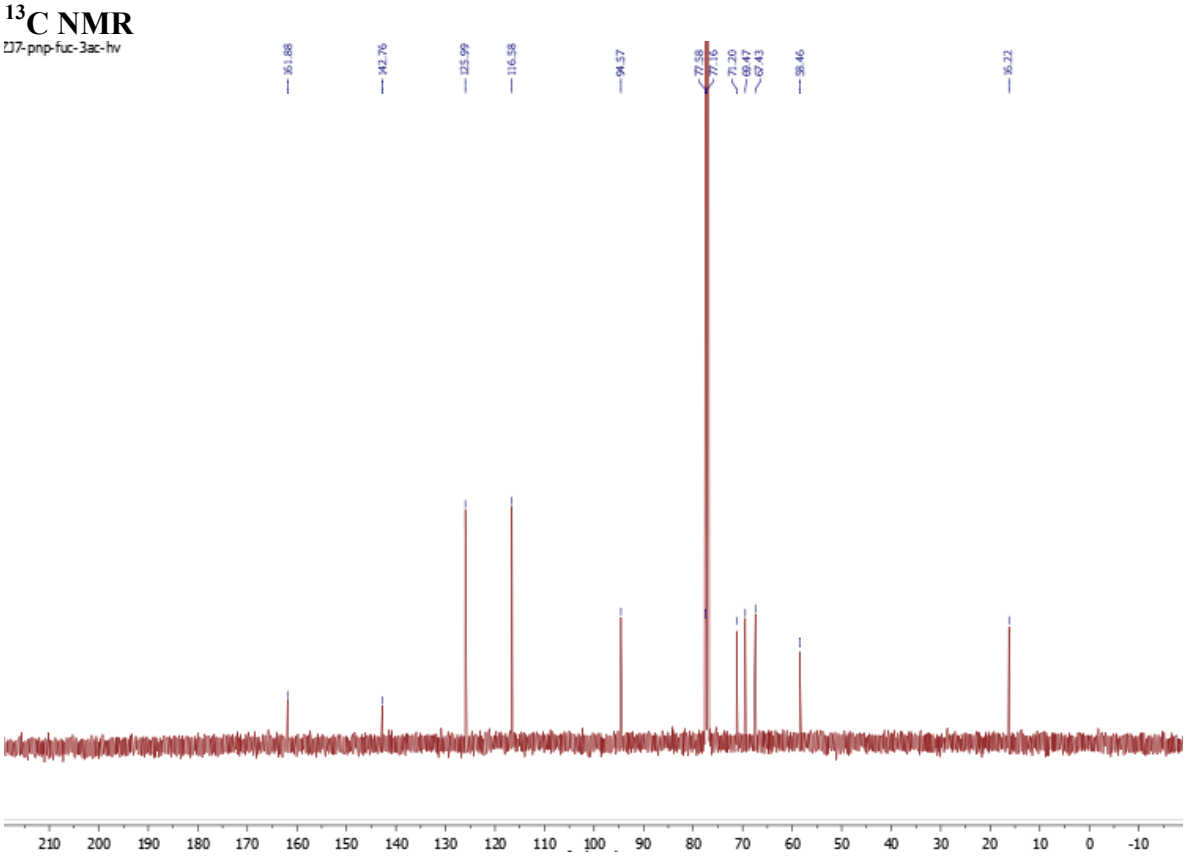
4-Nitrophenyl 2-*O*-methyl-α-L-fucopyranoside (3)

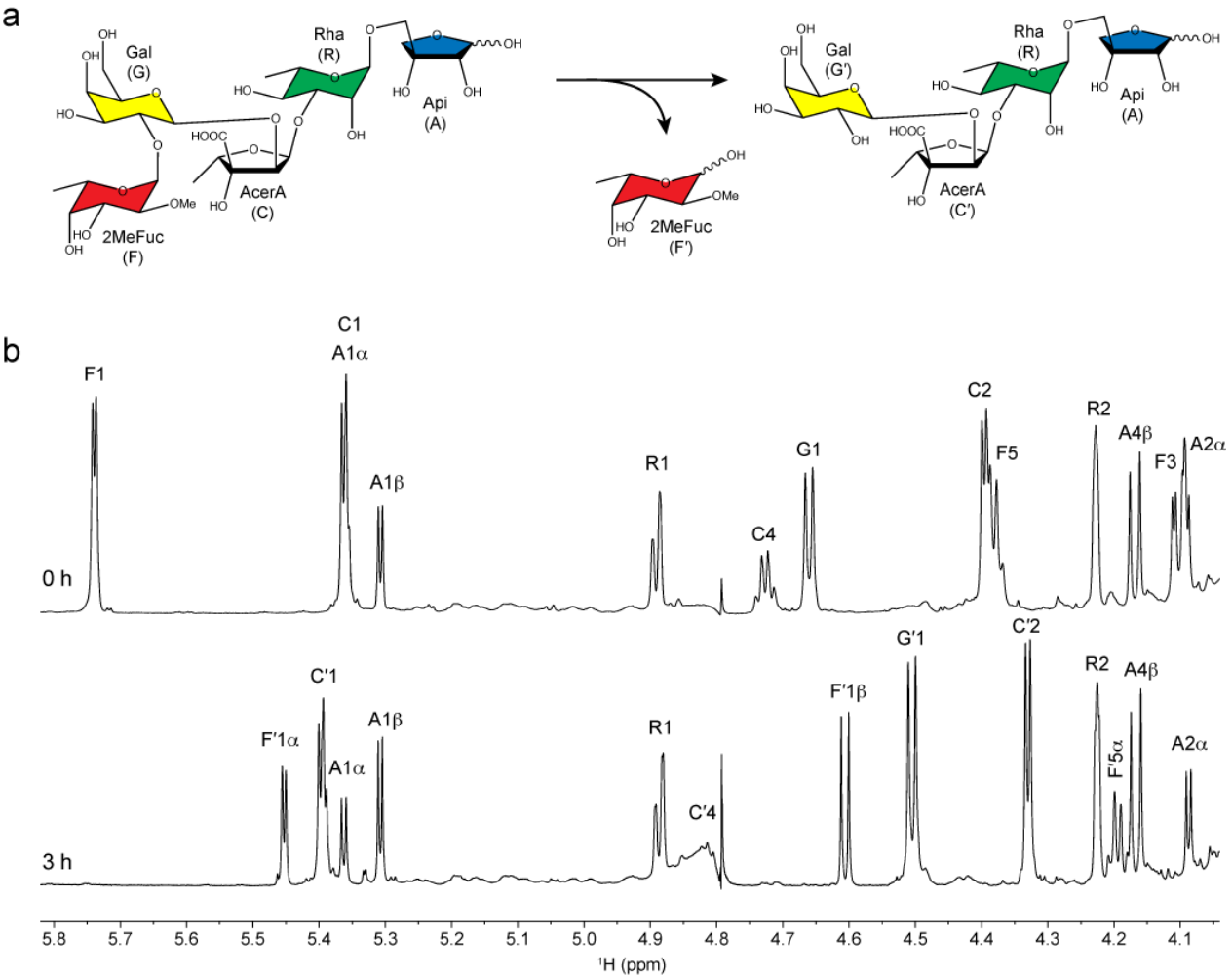
^{1}H NMR



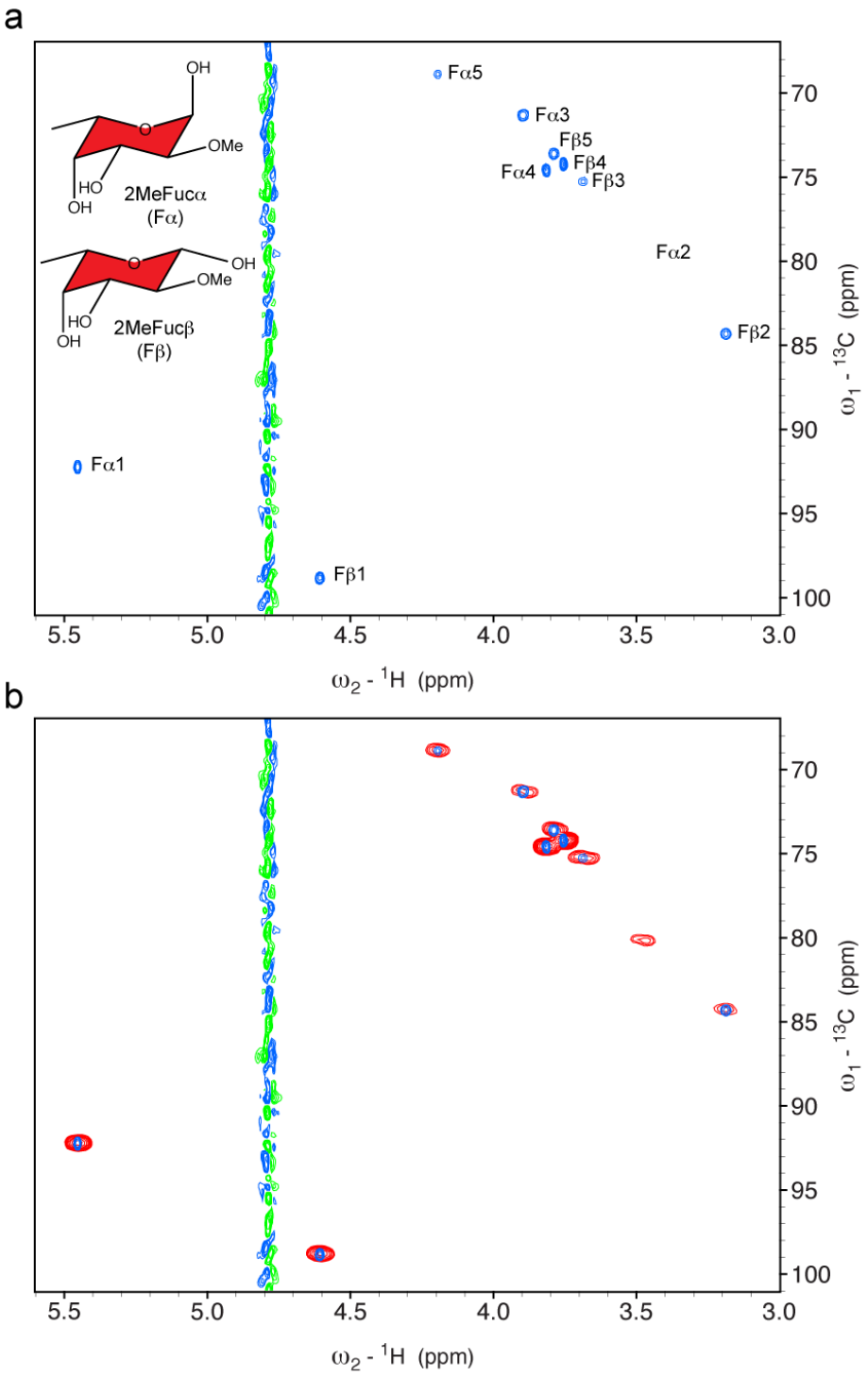


^{13}C NMR

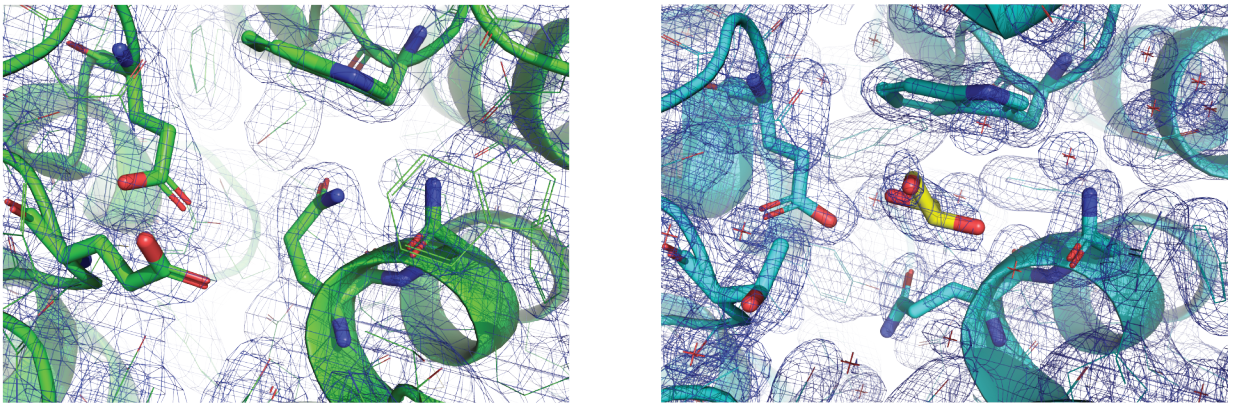

DJ7-pnp-fuc-3ac-hv
161.88
142.76
125.99
116.58
94.57
77.58
77.16
71.20
69.47
62.43
58.46
16.22
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10



Supplementary Figure S1. Chemical shift assignment of the ^{1}H 1D spectra of Δ0984 oligo before and after enzymatic treatment. (a) Chemical structure of Δ0984 oligo and the products of the enzymatic cleavage with used nomenclature. (b) ^{1}H 1D spectra of Δ0984 oligo before (top) and after enzymatic treatment (bottom) with assignment. Capital letters denote the building block, numbers proton number and in case of a free reducing end α and β denotes the anomer, e.g. F'1α stands for H1 of the free fucose α anomer.



Supplementary Figure S2. NMR spectra confirm the released carbohydrate is free 2MeFuc. (a) ^{1}H-^{13}C HSQC of the released carbohydrate with assignments. (b) Same spectrum as before (blue) overlayed on a ^{1}H-^{13}C HMQC spectrum of a reference sample of 2MeFuc (red) showing identical peak positions.



Supplementary Figure S3. Electron density maps of the GH139 active sites.
Weighted 2Fobs-Fc electron density maps of the active site residues of BT0984, left (2.7 Å), and SDT91673.1. right (2.05 Å). Both maps are contoured at 1σ.

Table S1 Observed chemical shifts of the Δ0984 oligo measured at 298 K in 10 mM HEPES, with 150 mM NaCl, buffer referenced to DSS in comparison with related structures reported earlier (1-4).

Moiety	H1	H2	H3	H4	H4'	H5	H6/H5'	H7	C1	C2	C3	C4	C5	C6	C7	
β-Api [a]	5.294	3.954	—	4.155	3,876	3.828	3.558	—	104.4	80.1	80.8	75.7	71.9	—	—	this work
	5.233	4.158	—	4.348	3.935	3.986	3.655	—	112.5	n.d.	n.d.	77.3	73.1	—	—	Vidal [b]
	5.12	n.d.	—	4.23	3.81	4.08	3.53	—	112.5	n.d.	n.d.	76.9	73.3	—	—	Rodriguez[c]
	5.09	4.12	—	4.16	4.01	3.72	3.72	—	110.9	78.0	80.8	75.0	65.0	—	—	Ishi [d]
α-Api [a]	5.342	4.089	—	3.792	3.527	3.986	3.989	—	99.0	76.0	79.2	75.6	72.5	—	—	this work
	5.11	4.12	—	4.16	4.12	3.72	3.72	—	104.7	73.2	78.4	75.1	65.9	—	—	Ishi [d]
Rha	4.878	4.222	3.938	3.626	—	3.783	1.325	—	102.5	69.3	78.1	72.9	71.3	19.5	—	this work
	4.87	4.19	3.89	3.57	—	3.75	1.31	—	102.5	69.4	78.8	72.7	71.2	19.5	—	Glushka [e]
	4.92	4.23	3.50	3.64	—	3.95	1.34	—	102.7	69.3	77.8	72.7	74.3	19.4		Rodriguez[c]
Aceric acid	5.373	4.424	—	4.730	—	1.190	—	—	100.0	90.2	85.6	81.4	16.2	177.0	—	this work
	5.38	4.39	—	4.84	—	1.19	—	—	100.4	90.4	n.d.	80.5	15.3	n.d.	—	Glushka [e]
	5.41	4.38	—	4.74	—	1.17	—	—	98.9	89.7	85.2	81.2	16.1	n.d.		Rodriguez[c]
Gal	4.649	3.778	3.872	3.867	—	3.717	3.794	—	105.0	75.5	72.0	77.3	78.0	63.5	—	this work
2MFuc	5.738	3.480	4.049	3.842	—	4.318	1.229	3.496	97.4	80.1	70.8	74.7	69.1	18.2	59.9	this work
	5.65	3.45	4.00	3.81	—	4.37	1.17	3.47	97.8	80.1	70.9	74.6	69.0	18.1	60.0	Glushka [f]
	5.732	3.48	4.10	n.d.	—	4.395	1.24	3.47	97.5	80.0	70.3	n.d.	68.8	18.1		Rodriguez[c]

[a] nomenclature of apiose: C3' is named in our table C5 and the corresponding protons H5 and H5'

[b] Vidal et al. reported the chemical shifts of a 12-mer including a Gal-β1,2-Ace*f* (OAc)-β1,3-Rha-β1,3'-Api*f*-β1,2-Gal side chain; for better comparison 1.2 ppm was added to the reported ^{13}C values and 0.023 ppm to the ^{1}H chemical shifts

[c] for better comparison reported ^{13}C chemical shifts were corrected by + 2.3 ppm and ^{1}H chemical shifts by + 0.12

[d] Ishii et al. reported chemical shifts of free methyl apiofuranosides, to the reported ^{13}C values 0.7 ppm was added, to ^{1}H chemical shifts 0.1 ppm was added.

[e] for better comparison 2 ppm was added to the reported ^{13}C values.

[f] for better comparison 0.2 ppm was added to the reported ^{13}C values.

Table S2 Observed chemical shifts of oligo after enzyme treatment, plus released monosaccharide referenced to DSS in comparison with related structures reported earlier (1-4).

Moiety	H1	H2	H3	H4	H4'	H5	H6/ H5'	H7	C1	C2	C3	C4	C5	C6	
β-Api [a]	5.308	3.974	—	4.166	3.893	3.844	n.d.	—	104.6	80.1	80.4	75.8	72.0	—	this work
	5.233	4.158	—	4.348	3.935	3.986	3.655	—	112.5	n.d.	n.d.	77.3	73.1	—	Vidal [b]
	5.12	n.d.	—	4.23	3.81	4.08	3.53	—	112.5	n.d.	n.d.	76.9	73.3	—	Rodriguez[c]
	5.09	4.12	—	4.16	4.01	3.72	3.72	—	110.9	78.0	80.8	75.0	65.0	—	Ishi [d]
α-Api [a]	5.364	4.089	—	4.002	4.002	n.d.	n.d.	—	99.0	74.4	79.2	75.7	n.d.	—	this work
	5.11	4.12	—	4.16	4.12	3.72	3.72	—	104.7	73.2	78.4	75.1	65.9	—	Ishi [d]
Rha	4.886	4.228	3.935	3.642	—	3.793	1.349	—	102.6	69.4	78.5	72.8	71.3	19.6	this work
	4.87	4.19	3.89	3.57	—	3.75	1.31	—	102.5	69.4	78.8	72.7	71.2	19.5	Glushka [e]
	4.92	4.23	3.50	3.64	—	3.95	1.34	—	102.7	69.3	77.8	72.7	74.3	19.4	Rodriguez[c]
	4.913	4.246	3.96	3.629	—	3.829	1.356	—	102.7	69.8	n.d.	n.d.	n.d.	19.7	Vidal [b]
Aceric acid	5.396	4.331	—	4.809	—	1.190	—	—	100.3	91.0	86.2	81.2	15.8	—	this work
	5.38	4.39	—	4.84	—	1.19	—	—	100.4	90.4	n.d.	80.5	15.3	n.d.	Glushka [e]
	5.41	4.38	—	4.74	—	1.17	—	—	98.9	89.7	85.2	81.2	16.1	n.d.	Rodriguez[c]
	5.412	4.329	—	4.809	—	1.193	—	—	100.9	91.1	n.d.	n.d.	15.6	n.d.	Vidal [b]
Gal (terminal)	4.506	3.636	3.712	3.932	—	3.797	3.793	—	106.9	73.3	78.2	71.2	74.8	63.6	this work
	4.503	3.633	3.669	3.934	—	n.d.	3.793	—	107.1	73.2	75.3	71.2	n.d.	63.7	Vidal [b]
free β-2MeFuc	4.608	3.187	3.687	3.754	—	3.790	1.254	n.d.	98.8	84.2	75.2	74.2	73.6	18.2	this work
free α-2MeFuc	5.454	3.479	3.896	3.812	—	4.193	1.220	n.d.	92.2	80.1	71.3	74.6	68.8	18.2	this work

[a] nomenclature of apiose: C3' is named in our table C5 and the corresponding protons H5 and H5'

[b] Vidal et al. reported the chemical shifts of a 12-mer including a Gal-β1,2-Ace*f* (OAc)-β1,3-Rha-β1,3'-Api*f*-β1,2-Gal side chain; for better comparison 1.2 ppm was added to the reported ^{13}C values and 0.023 ppm to the ^{1}H chemical shifts

[c] for better comparison reported ^{13}C chemical shifts were corrected by + 2.3 ppm and ^{1}H chemical shifts by + 0.12

[d] Ishii et al. reported chemical shifts of free methyl apiofuranosides, to the reported ^{13}C values 0.7 ppm was added, to ^{1}H chemical shifts 0.1 ppm was added.

Table S3. X-ray data collection and refinement statistics
Statistics for the highest-resolution shell are shown in parentheses

	BT0984^{GH139}	**SDT091673 ^{GH139}**
Data collection		
Beamline	iO4-1 (DLS)	i24 (DLS)
Wavelength (Å)	0.98	0.999
Resolution range (Å)	47.43 – 2.70 (2.76 – 2.70)	76.74 - 2.05 (2.123 - 2.05)
Space group	$P2_12_12_1$	$P\ 6_522$
Unit cell		
a, b, c (Å)	101.20, 138.22, 195.53	209.45 209.45 143.96
a, b, g (°)	90, 90, 90	90.00 90.00 120.00
Total reflections	251340 (15118)	4209044 (681959)
Unique reflections	75053 (4450)	5422 (4518)
Multiplicity	3.3 (3.4)	39.5 (24.4)
Completeness (%)	98.9 (99.5)	99.96 (99.92)
Mean I/sigma(I)	10.4 (1.3)	71.7 (4)
Wilson B-factor (Å^2)	59.22	26.62
Rmerge	0.089 (1.050)	0.050 (0.773)
Rmeas	0.105 (1.246)	0.051 (0.789)
CC1/2	0.997 (0.502)	1 (0.887)
Refinement		
R-work/ R-free	0.22/0.26	0.18/0.19
RMS(bonds) (Å)	0.005	0.01
RMS(angles) (º)	1.40	1.03
Ramachandran favored (%)	94.33	97.37
Ramachandran allowed (%)	99.93	99.87
Ramachandran outliers (%)	0.07	0.13
Rotamer outliers (%)	2.85	0.31
Clashscore	4.72	1.96
Average B-factor (Å^2)	75.46	33.45
macromolecules	70.6/ 80.31	32.74
ligands	0	0
solvent	42.80	39.54
PDB code	9HYQ	9HMB

Table S4 Melting temperatures of GH139 enzymes and their mutants.

All melting temperatures were performed in 10 mM MOPS pH 7.0 with 150 mM NaCl deploying 5 μM protein unless stated.

Enzyme	Melting point	Confidence interval
	Run 1	
BT_0984_WT	54.38	44.24 to 50.71
W162A	54.78	54.59 to 55.55
W403A	51.48	51.02 to 52.39
Q411A	55.01	54.03 to 56.05
N412A	54.22	53.61 to 55.23
E472A	56.56	55.78 to 57.29
E472Q	55.37	55.07 to 55.83
W490A	56.64	56.13 to 57.11
E561A	58.62	57.18 to 59.25
E561Q	57.21	55.22 to 60.26
N639A	56.07	55.54 to 56.99
E641A	54.33	53.10 to 55.66
E641Q	56.00	54.58 to 56.95
W683A	47.48	45.79 to 49.92
	Run2	
SDT	59.29	59.18 to 59.40
SDT T115Q	41.1	40.83 to 41.36
Q411A + N412A	53.96	53.45 to 54.81
Q411A + E562Q	58.91	59.17 to 59.74
N412A + E562Q	56.55	56.71 to 58.48
Q411A + N412A + E562Q	56.89	57.06 to 58.16

Table S5. Total hydrogen bond interactions between 2-methoxy-α-fucose and BT0984 during molecular dynamics simulations.

		% of the simulation time	
Ligand Atom	**AA residue**	**Simulation 1**	**Simulation 1**
Hemi acetal O	T409	11.30	3.00
Hemi acetal O	Q411	-	3.30
Hemi acetal O	N412	72.00	67.00
Hemi acetal O	Q461	4.90	2.20
Hemi acetal O	H2O	74.80	96.10
C1-O	Q461	1.60	1.90
C1-O	Q461	25.80	-
C1-O	H2O	99.20	99.80
C1-OH	E561	86.60	76.80
C1-OH	E561	36.80	73.80
C1-OH	H2O	8.70	16.90
C2-O	N639	1.40	14.40
C2-O	H2O	95.40	83.20
C3-O	W683	93.30	90.20
C3-O	Y722	6.00	5.60
C3-O	H2O	72.30	82.50
C3-OH	H2O	97.10	96.00
C4-O	N412	99.90	98.30
C4-O	W683	94.30	93.40
C4-O	H2O	13.60	11.30
C4-OH	D723	83.40	100.00
C4-OH	D723	94.80	77.30

Table S6. Hydrogen bond occupancy at subsite −1 residues of BT0984 in complex with 2-methoxy-α-fucose.

		% of the simulation time	
AA residue	**Ligand atom**	**Simulation 1**	**Simulation 2**
D723	C4-OH	83.40%	100.00%
D723	C4-OH	94.80%	77.30%
E561	C1-OH	86.60%	76.81%
E561	C1-OH	36.80%	73.80%
N412	Hemi acetal O	72.00%	67.00%
N412	C4-O	99.90%	98.30%
N729	__	0.00%	0.00%
Q411	C2-O	0%	3.30%
W683	C3-O	93.30%	90.20%
W683	C4-O	94.30%	93.40%

Table S7. Binding free energy (ΔG_{bind}) of 2-methoxy-α-fucose to BT0984 calculated by MM-GBSA (values in kcal/mol).

	Binding free energy	Van der Waal contribution	Electrostatic contribution
Simulation 1	-18.05	-19.13	-43.80
Simulation 2	-16.17	-20.34	-40.81

Table S8. Mutant primer table

Mutant primer	Primer sequence 5' – 3'
BT0984_W162A_F	TCC TAT AAG GCC GCTCCTCCAAAAGGAACGATGACG
BT0984_W162A_R	TGG AGG AGC GGC CTTATAGGAACATTGCTGCCCCTC
BT0984_W404A_F	TAC CGA AAG GCC GGAGGAGGAACGATGACTGCACAG
BT0984_W403A_R	GCA TTC ACT GGC GACTACCGAAAGTGGGGAGGAGGA
BT0984_Q461A_F	TTC TGT GAG GCC ATAGAAAACTTCGGCTTACCCAAT
BT0984_Q461A_R	GTT TTC TAT GGC CTCACAGAAGCAGGCTCCCTCGTG
BT0984_E472A_F	AAT CCC GCA GCC TATGGTTTCAAACGTCCGGCTTGG
BT0984_E472A_R	GAA ACC ATA GGC TGCGGGATTGGGTAAGCCGAAGTT
BT0984_E472Q_F	AAT CCC GCA CAG TATGGTTTCAAACGTCCGGCTTGG
BT0984_E472Q_R	GAA ACC ATA CTG TGCGGGATTGGGTAAGCCGAAGTT
BT0984_W490A_F	TAC AAT GCG GCC CTGGAATATGAATGGGATACTATT
BT0984_W490A_R	ATA TTC CAG GGC CGCATTGTATTCCAGTCCTTTGTC
BT0984_E561A_F	TCT GCC TGC GCC ACTTACAAGATGACCAACAACGCC
BT0984_E561A_R	CTT GTA AGT GGC GCAGGCAGAGCCGGGGAACAGTAT
BT0984_E561Q_F	TCT GCC TGC CAG ACTTACAAGATGACCAACAACGCC
BT0984_E561Q_R	CTT GTA AGT CTG GCAGGCAGAGCCGGGGAACAGTAT
BT0984_N639A_F	CGA ATC AAC GCC ATAGAGACACCACAACTCTACCCG
BT0984_N639A_R	TGT CTC TAT GGC GTTGATTCGTTCCCAGCTTTTGGC
BT0984_E641A_F	AAC AAT ATA GCC ACACCACAACTCTACCCGGTTTTT
BT0984_E641A_R	TTG TGG TGT GGC TATATTGTTGATTCGTTCCCAGCT
BT0984_E641Q_F	AAC AAT ATA CAG ACACCACAACTCTACCCGGTTTTT
BT0984_E641Q_R	TTG TGG TGT CTG TATATTGTTGATTCGTTCCCAGCT
BT0984_W683A_F	CAT ACC GGA GCC AAGCAAGACAACATCTGGGCAGCC
BT0984_W683A	GTC TTG CTT GGC TCCGGTATGGGAGCGGAATTTGAG
BT0984_N412A_F	ACT GCA CAG GCC CAGCGTCTTGTCTACTGGCCG
BT0984_N412A	AAG ACG CTG GGC

	CTGTGCAGTCATCGTTCCTCC
BT0984_Q411A_F	ATG ACT GCA GCC AATCAGCGTCTTGTCTACTGG
BT0984_Q411A_R	ACG CTG ATT GGC TGCAGTCATCGTTCCTCCTCC
BT0984_Q411A/N412A_F	ATG ACT GCA GCC GCC CAG CGT CTT GTC TAC TGG
BT0984_Q411A/N412A_R	ACG CTG GGC GGC TGC AGT CAT CGT TCC TCC TCC
SDT_T551E_F	CTGCTCAGAGCCTGGAGACCTACCAGTCCC
SDT_T551E_R	GGGACTGGTAGGTCTCCAGGCTCTGAGCAG

1. Glushka, J. N., Terrell, M., York, W. S., O'Neill, M. A., Gucwa, A., Darvill, A. G. *et al.* (2003) Primary structure of the 2-O-methyl-alpha-L-fucose-containing side chain of the pectic polysaccharide, rhamnogalacturonan II Carbohydr Res **338**, 341-352 10.1016/s0008-6215(02)00461-5
2. Ishii, T., Yanagisawa, M (1998) Synthesis, separation and NMR spectral analysis of methyl apiofuranosides Carbohydrate Research **313**, 189-192 https://doi.org/10.1016/S0008-6215(98)00262-6
3. Rodriguez-Carvajal, M. A., Herve du Penhoat, C., Mazeau, K., Doco, T., andPerez, S. (2003) The three-dimensional structure of the mega-oligosaccharide rhamnogalacturonan II monomer: a combined molecular modeling and NMR investigation Carbohydr Res **338**, 651-671 10.1016/s0008-6215(03)00003-x
4. Vidal, S., Doco, T., Williams, P., Pellerin, P., York, W. S., O'Neill, M. A. *et al.* (2000) Structural characterization of the pectic polysaccharide rhamnogalacturonan II: evidence for the backbone location of the aceric acid-containing oligoglycosyl side chain Carbohydr Res **326**, 277-294 10.1016/s0008-6215(00)00036-7