Supporting information

1) Educts in molar ratio as well as in weight percent

Table S1: Each sample was prepared with a constant amount of ammonium formate and fructose, with a molar ratio of 1.5:1. Water was added in changing amounts as shown below.

Ammonium formate [g]	Fructose [g]	Water [g]	Molar ratio of water	Wt% water
0.47	0.90	0.00	**:0**	0.0
0.47	0.90	0.09	**:1**	6.2
0.47	0.90	0.27	**:3**	16.4
0.47	0.90	0.45	**:5**	24.7
0.47	0.90	0.63	**:7**	31.5
0.47	0.90	0.90	**:10**	39.6
0.47	0.90	1.80	**:20**	56.7
0.47	0.90	4.50	**:50**	76.6
0.47	0.90	9.01	**:100**	86.8

Table S2: For comparison, educts for the synthesis of DOF from previous papers, which were discussed in the introduction.

Reference	Reactants			Molar ratio	Wt% water
Wu[20]	*Ammonium formate [g]*	*Fructose [g]*	*Water [g]*		
	0.39	*0.1*	*2*	***10:1:179***	*95.2*
Shanxi Institute[21]	*Ammonium chloride [g]*	*Fructose [g]*	*Water [g]*		
	15	*5*	*500*	***10:1:1000***	*96.2*
Jia[22]		*Glucosamine [g]*	*Ionic liquid [g]*		
		0.2	*2*	***1:100***	*90.9*

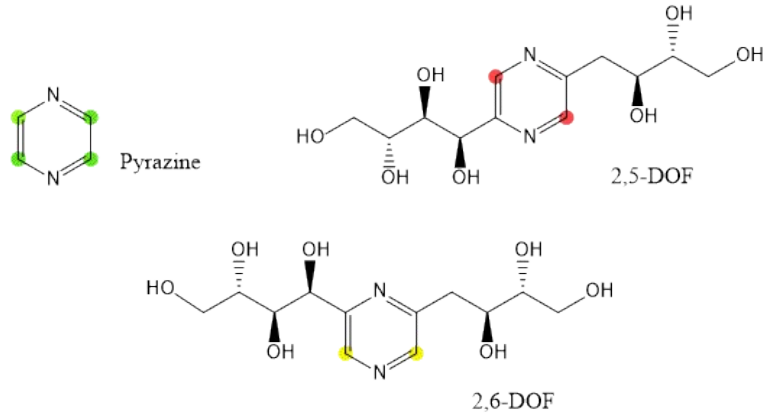
2) Quantitative H-NMR

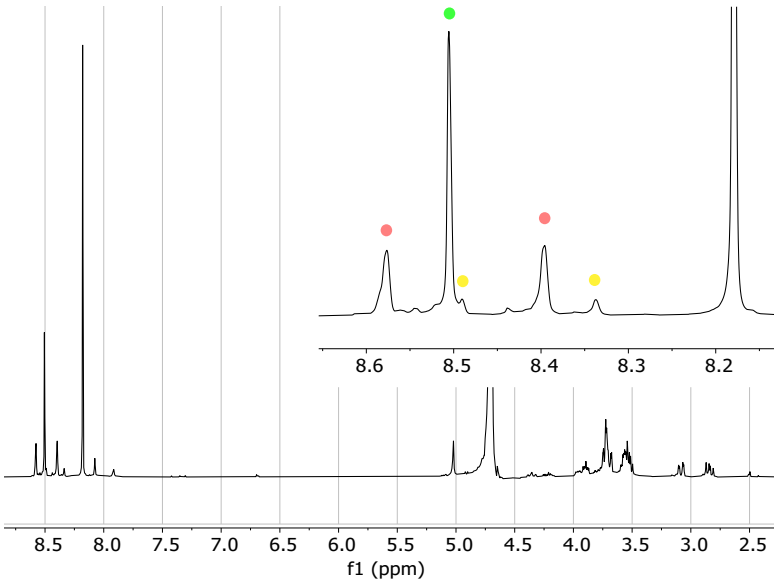
Pyrazine was used as an internal standard and was added to the NMR solvent in the range of 1.5 g/ml. The amount of product was calculated, based on the following equation:

$$m(x) = m(std) \cdot \frac{mw(x)}{mw(std)} \cdot \frac{nH(std)}{nH(x)} \cdot \frac{A(x)}{A(std)}$$

Where m(x) and m(std) are the weights in g, mw(x) and mw(std) are the molecular weights in g/mol, A(x) and A(std) are the integration areas of the selected peaks of the product and the internal standard

DOF content was quantified based on their aromatic proton peaks, shown below for the example of fructose. Fructose and fucose were measured in D_2O, glucose and rhamnose in DMSO- d_6 to avoid overlap between the pyrazine and product peaks.





In the case of water as a third component of the reaction mixture the method was applied in exactly the same way. There was no sign of water evaporation during the synthesis, according to pressure monitoring during the reaction. Also the weight of the reaction mixture remained constant before and after synthesis.

3) Procedure of high throuput screening

Antimicrobial susceptibility tests were performed via the antiBiotic Mode of Action Profile (BioMAP) assay (1, 2). BioMAP utilizes high-throughput screening methodology in order to screen the effect of compounds on a panel of 19 pathogenic bacterial strains, including both Gram negative and Gram positive strains (Table S3).

The assay was performed according to Clinical & Laboratory Standards (CLSI) protocols, and as per Hawkins et al. (2), with the following exceptions: during compound incubation cation-adjusted Muller Hinton Broth (CAMHB; Millipore-Sigma) was used as the growth media for 12 bacterial strains, a 1:1 mixture of CAMHB and brain heart infusion (BHI; Millipore Sigma); (CAMHB:BHI) was used as the growth media for 3 strains, and 4 strains were grown in appropriate complete media (Table S3).

Briefly, each bacterial strain was inoculated in 3 mL of sterile media, as in Hawkins et al. (2), and grown overnight with shaking (200 rpm) at 37°C, with the exception of Streptococcus pneumonia, which was placed in a 5% CO_2 incubator set to 37°C, overnight, without shaking. Saturated overnight cultures were diluted in the appropriate media (Table S3) to achieve approximately 5 x 10^5 CFU of final inoculum density and dispensed via a Matrix dispenser into sterile clear polystyrene 384-well assay microplates (Greiner 781186, Sigma-Aldrich) with a final screening volume of 30 µL. As per Hawkins et al. (2), solutions of test compounds and antibiotic controls were prepared as a 1:1 dilution series in 384-well storage microplates (NUNC 264573, Thermo Fisher Scientific). Two hundred nanoliters of the compound, or antibiotic control, was pinned into each assay plate using a Tecan Freedom EVO 100 equipped with a 384 well pintool. Post-pinning test compounds had a final concentration ranging from 40 to 4 mM per compound, while antibiotic controls had a final concentration ranging from 128 µM to 3.91 nM per compound.

In each 384-well plate, controls were placed in lanes 1, 2, 23 and 24. For the controls, lane 1 contained vehicle (DMSO) and culture medium only; lane 2 contained vehicle (DMSO), culture medium and target bacteria; and lanes 23 and 24 contained vehicle (DMSO), culture medium, target bacteria and antibiotic controls. Ciprofloxacin and gentamicin were used as controls for Gram negative bacteria, while azithromycin and vancomycin were used as controls for Gram positive bacteria.

After pinning and dispensing, absorbance values were obtained at OD600 for timepoint T_0 using an automated plate reader (Synergy Neo2, BioTek). Plates were then sealed with a lid and placed in a 37°C incubator. S. pneumoniae was incubated in a separate incubator (37°C; 5% CO2). After an incubation period of 18-20 hours absorbance measurements were obtained for timepoint T_{20}.

MIC90 values were calculated using GRAPHPAD PRISM (version 8). Percent growth (PG) was calculated via the following equation

$$PG = [(Treat T_{20} - Treat T_0)/(Cpos - Cneg)] \times 100$$

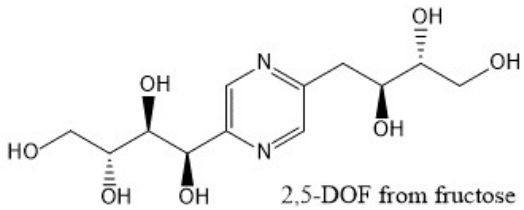
Where, Treat represents absorbance values at T_0 and T_{20}; Cneg and Cpos are the averaged absorbance values of the controls in Lane 1 (DMSO + culture media) and lane 2 (DMSO + culture media + bacteria), respectively. Percent inhibition was calculated as 100 – PG.

Table S3. Information related to bacterial strains used in BioMAP assay.

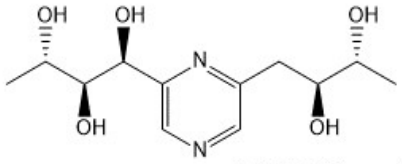
Strain Name	Strain Designation	Growth Media
Gram-Positive		
Bacillus subtilis	ATCC 6051	CAMHB
Enterococcus faecalis	ATCC 29212	CAMHB:BHI
Enterococcus faecium	ATCC 6569	CAMHB:BHI
Listeria ivanovii	BAA-139	TSB
Staphylococcus aureus (Methicillin-Resistant)	BAA-44	CAMHB
Staphylococcus aureus (Methicillin-Sensitive)	ATCC 29213	CAMHB
Staphylococcus epidermidis	ATCC 14990	TSB
Streptococcus pneumoniae	ATCC 49619	CAMHB:BHI
Gram-Negative		
Acinetobacter baumanii	ATCC 19606	CAMHB
Escherichia coli	K-12 MG1655	CAMHB
Klebsiella aerogenes	ATCC 35029	CAMHB
Klebsiella pneumoniae	ATCC 700603	CAMHB
Ochrobactrum anthropi	ATCC 49687	TSB
Providencia alcalifaciens	ATCC 9886	CAMHB
Pseudomonas aeruginosa	ATCC 27853	CAMHB
Salmonella enterica	ATCC 13311	CAMHB
Shigella sonnei	ATCC 25931	CAMHB
Vibrio cholera	A1552 El Tor	CAMHB
Yersinia pseudotuberculosis	ATCC 6904	BHI

4) Characterization of products

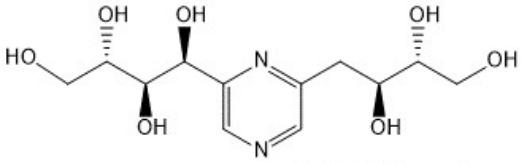
Major products are depicted below. The respective isomers (2,5 vs. 2,6) were also detected in smaller amounts. We did not aim to identify any byproducts but the Maillard reaction is also known as the browning reaction and produces a range of heterogeneous polymeric material for prolonged reaction times. The typical products were recognizable by the dark brown color as well as by the pleasant smell of the crude samples.



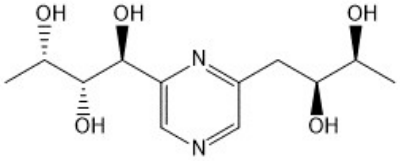
2,5-DOF from fructose



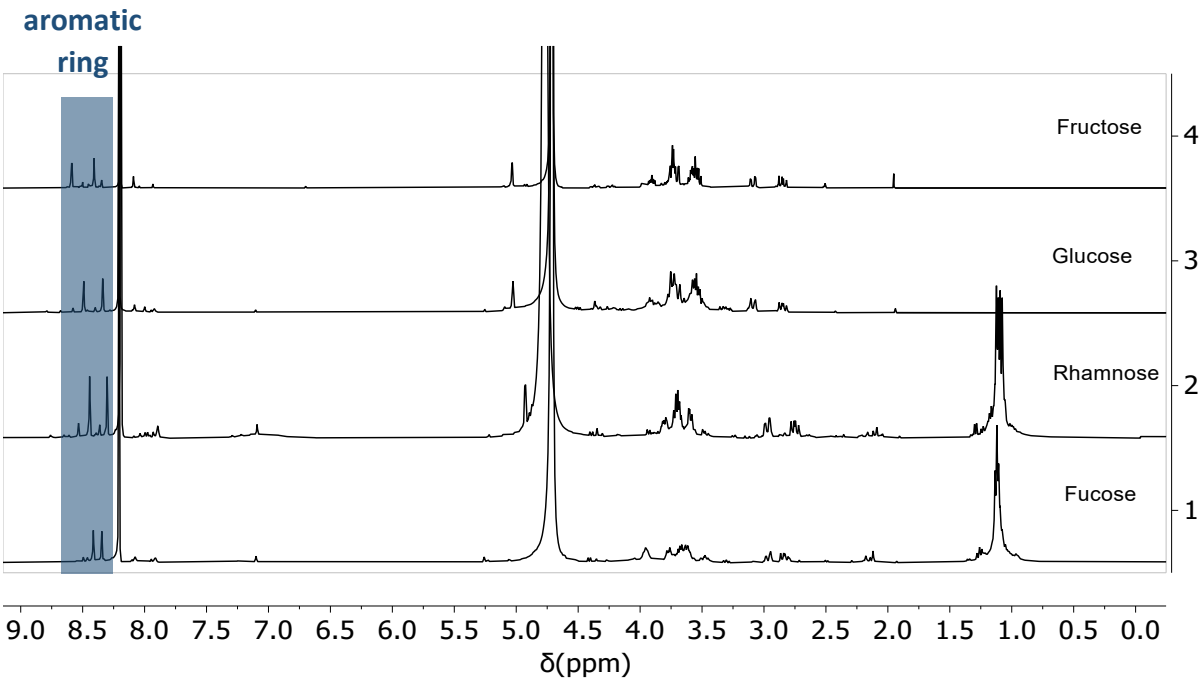
2,6-DOR from rhamnose



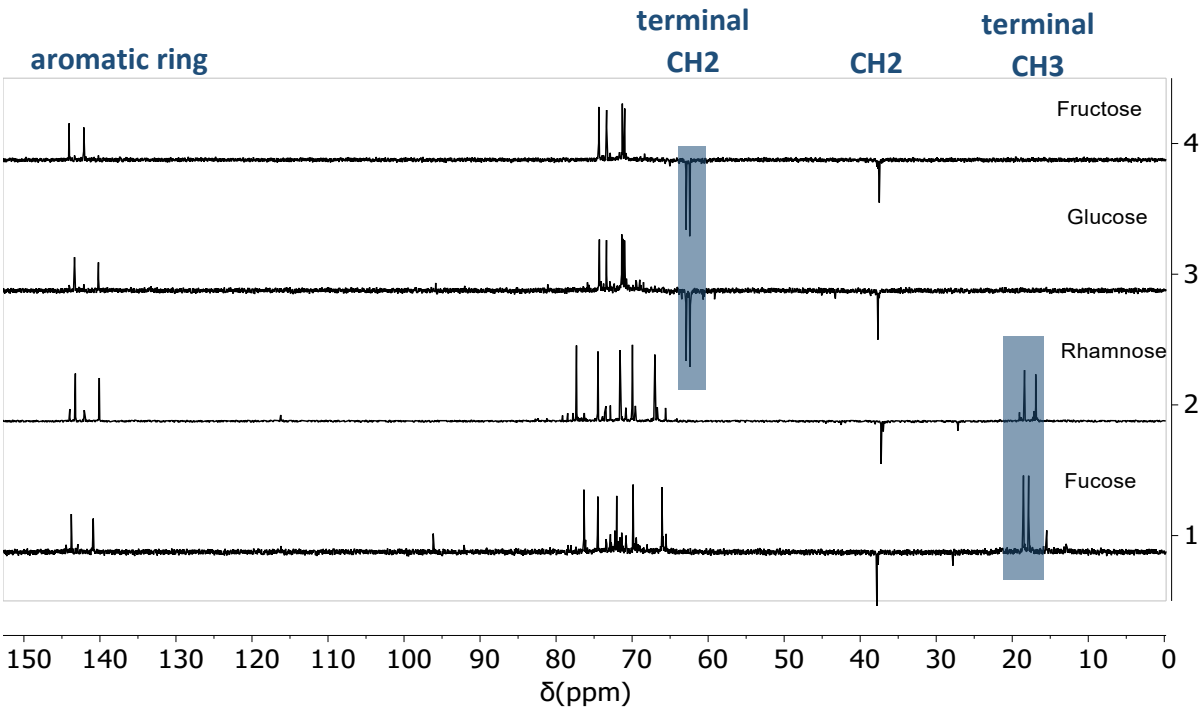
2,6-DOF from glucose



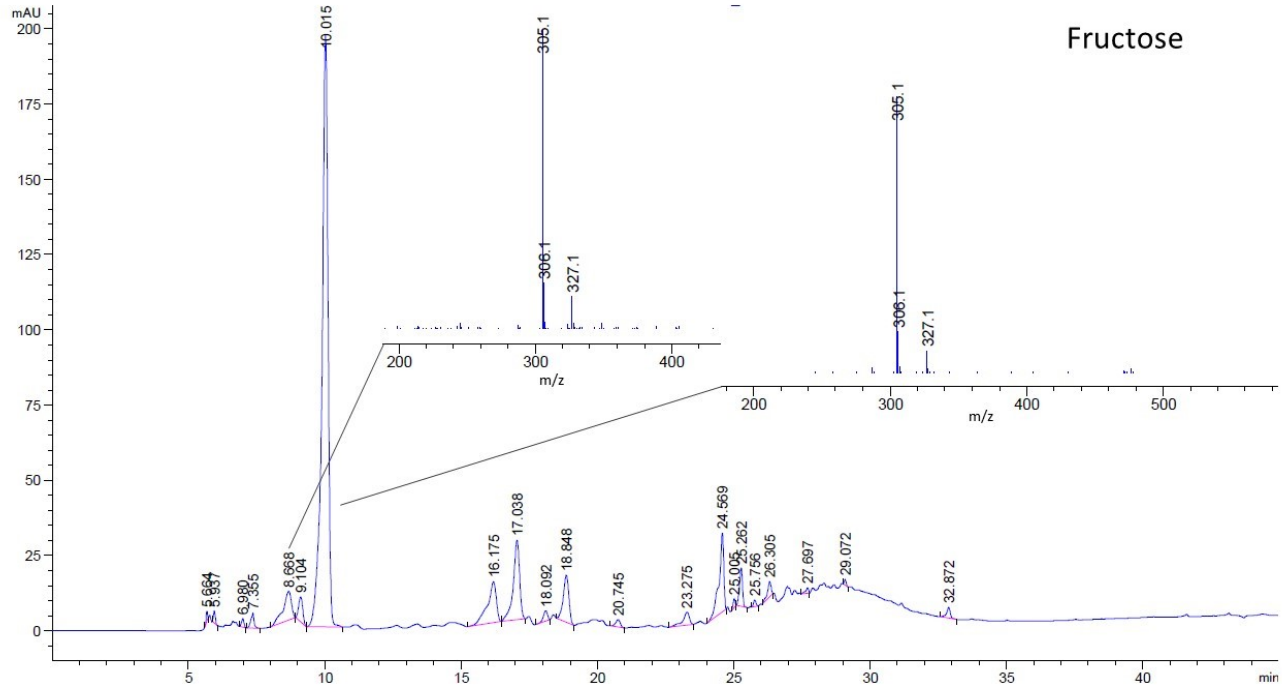
2,6-DOFu from fucose

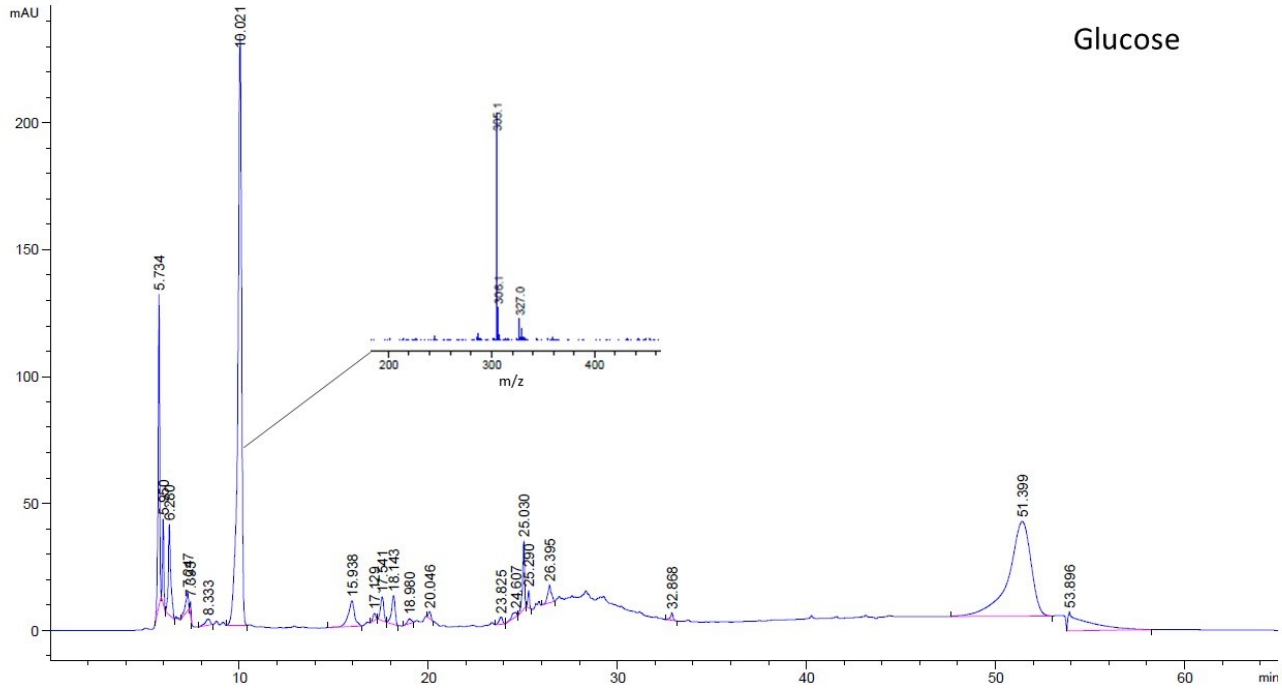


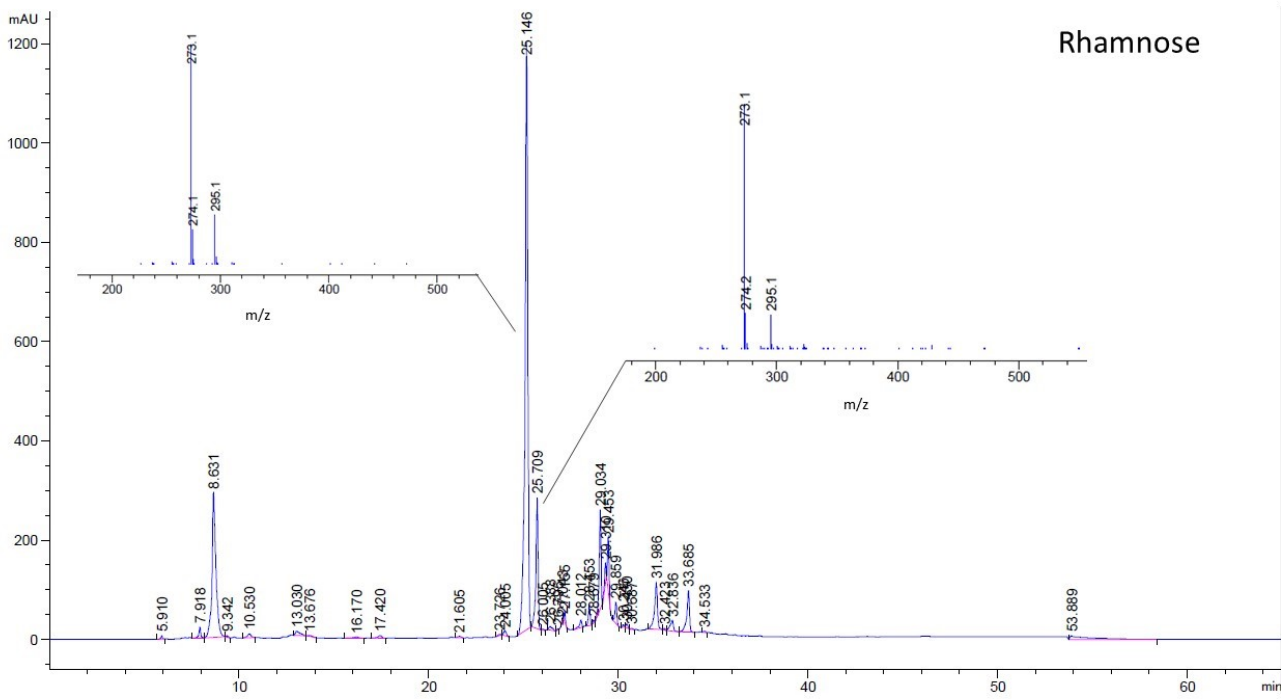
^{1}H-NMR in D_2O of the crude reaction mixture from different monosaccharides

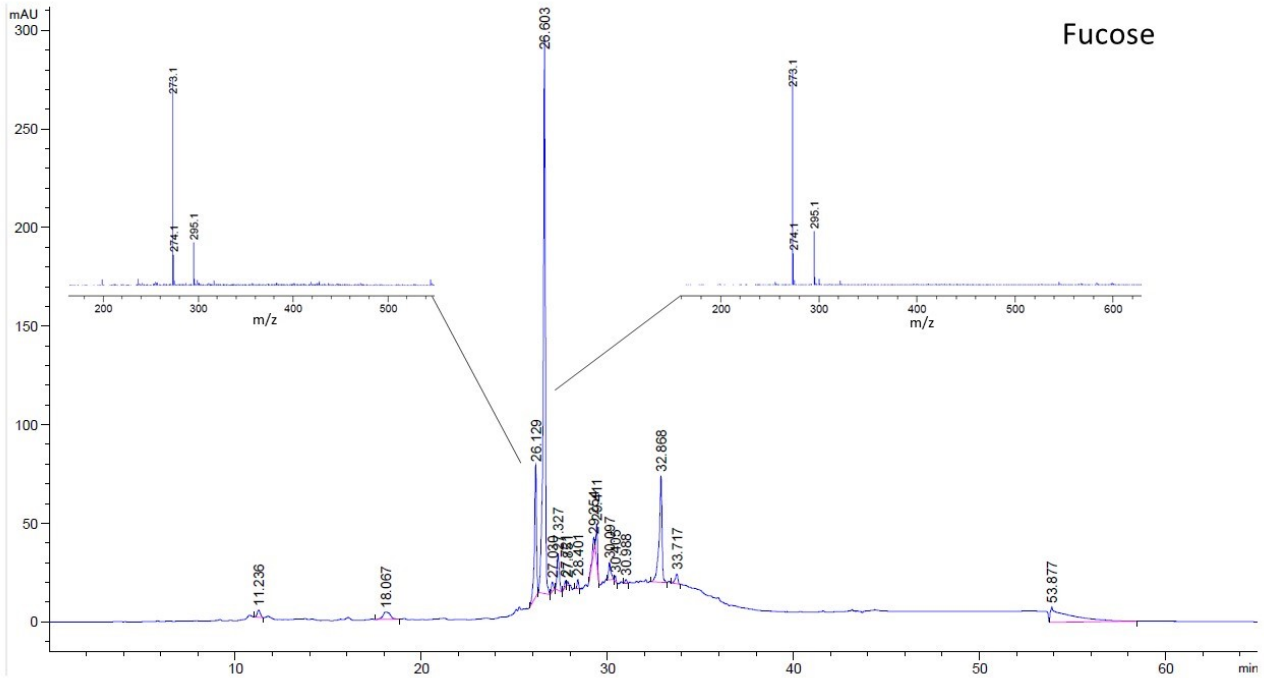


DEPT ^{13}C NMR in D_2O of the crude reaction mixture from different monosaccharides


Fructose
mAU
min
m/z
10.015
305.1
306.1
327.1
16.175
17.038
18.848
24.569
29.072
32.872


Glucose
mAU
min
m/z
10.021
5.734
15.938
25.030
26.395
32.868
51.399
53.896





HPLC-MS of the crude reaction mixture from different monosaccharides

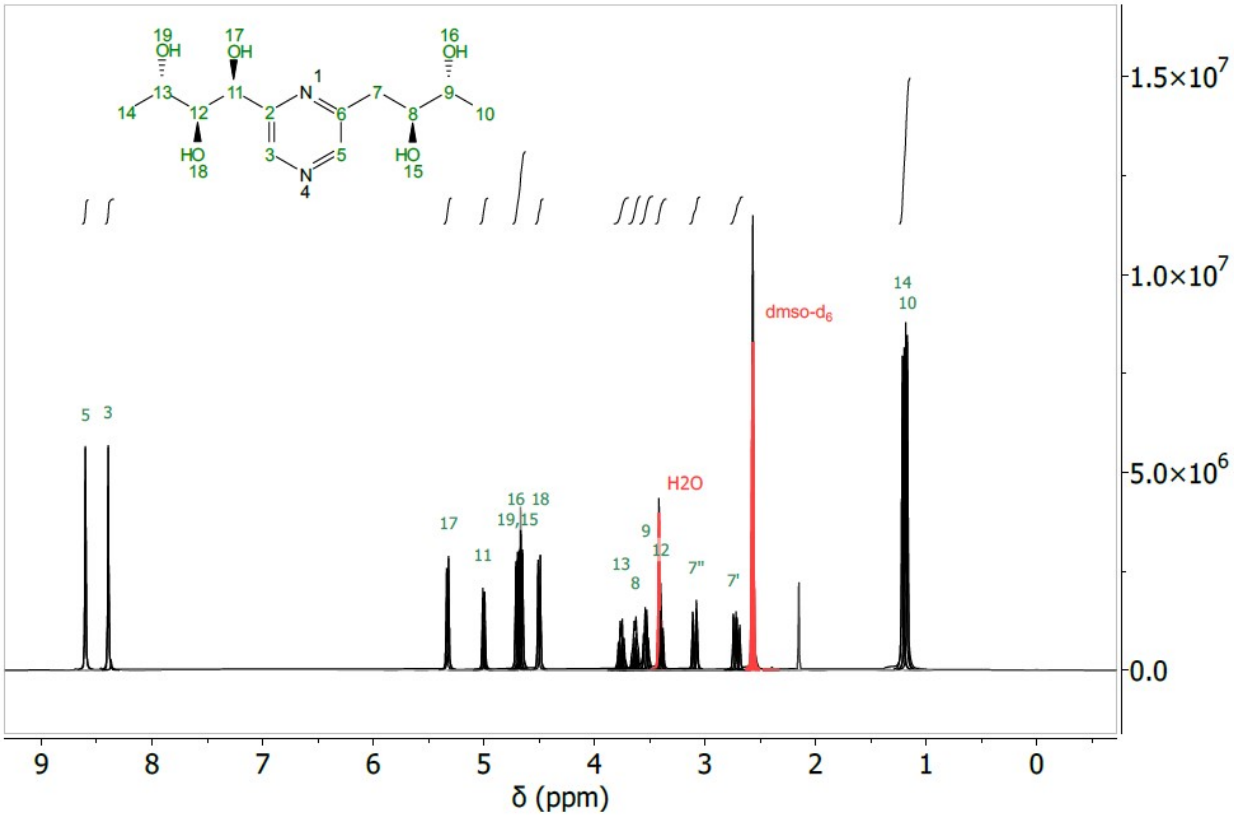
5) Characterization of separated DOR derivatives

Analytical data for 2,6-DOR from Rhamnose

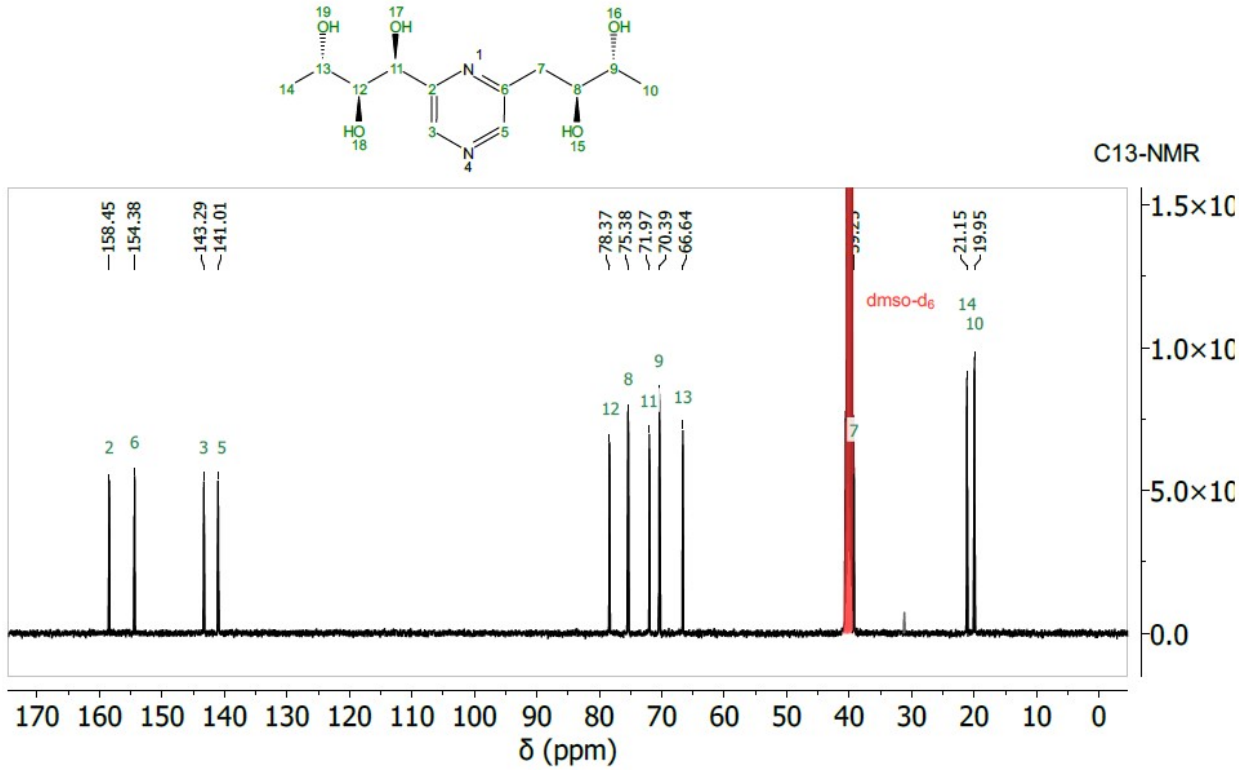
^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.60 (s, 1H), 8.40 (s, 1H), 5.33 (d, *J* = 6.5 Hz, 1H), 5.06 – 4.97 (d looking m, 1H), 4.73 – 4.62 (m, 3H), 4.50 (d, *J* = 8.1 Hz, 1H), □3.77 – 3.61 (m, 1H), □3.62 – 3.52 (m, 1H), 3□.47 (q, J = 6.0 Hz, 1H), □3.38 – 3.23 (m, 1H), □3.02 (dd, J = 13.8, 2.8 Hz, 1H), 2.65 □2.68 – 2.60 (dd, J = 13.8, 9.7 Hz, 1H), □1.14 (d, J = 6.2 Hz, 3H), 1.11 (d, J = 6.1 Hz, 3H) ppm.

^{13}C NMR (101 MHz, DMSO-d_6) δ = 19.95, 21.15, 39.25, 66.64, 70.39, 71.97, 75.38, 78.37, 141.01, 143.29, 154.38, 158.45 ppm.

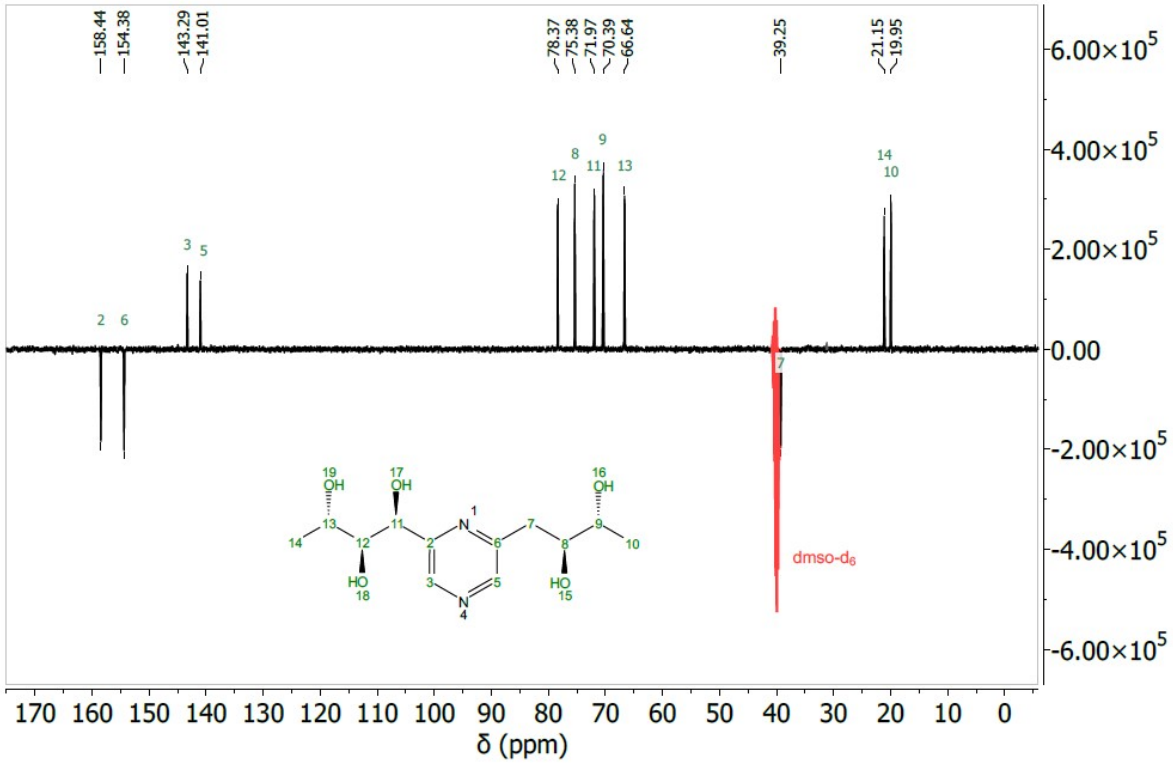
HRMS (ESI^{+}): Calculated for $C_{12}H_{21}N_2O_5$: 273.1445, Found: 273.1453



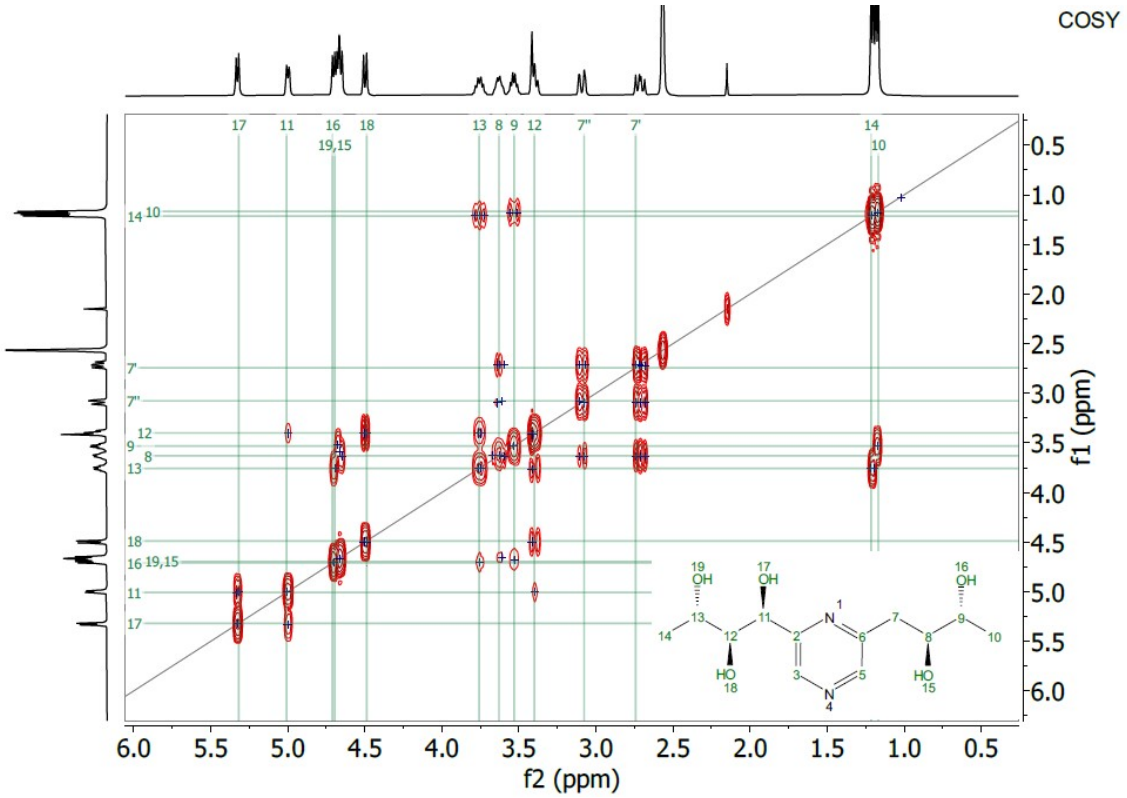
^{1}H -NMR (400MHz, DMSO-d_6) for 2,6-DOR from rhamnose



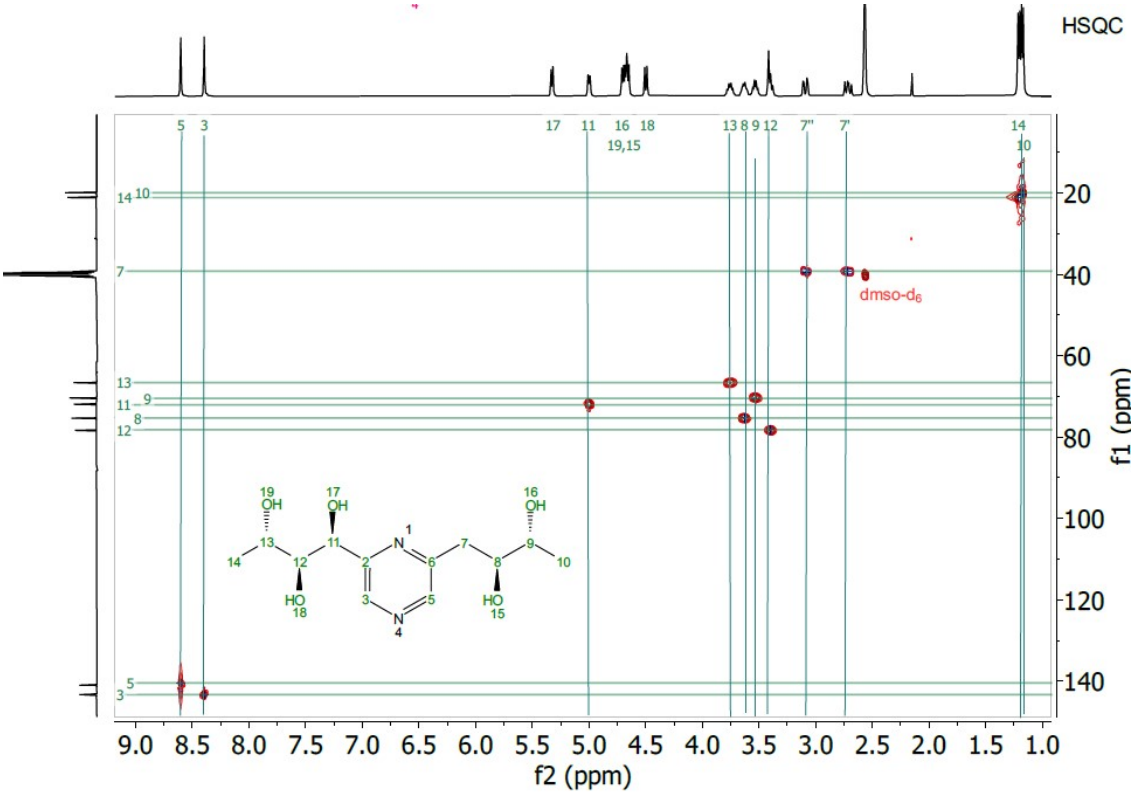
^{13}C -NMR (400MHz, DMSO-d_6) for 2,6-DOR from rhamnose



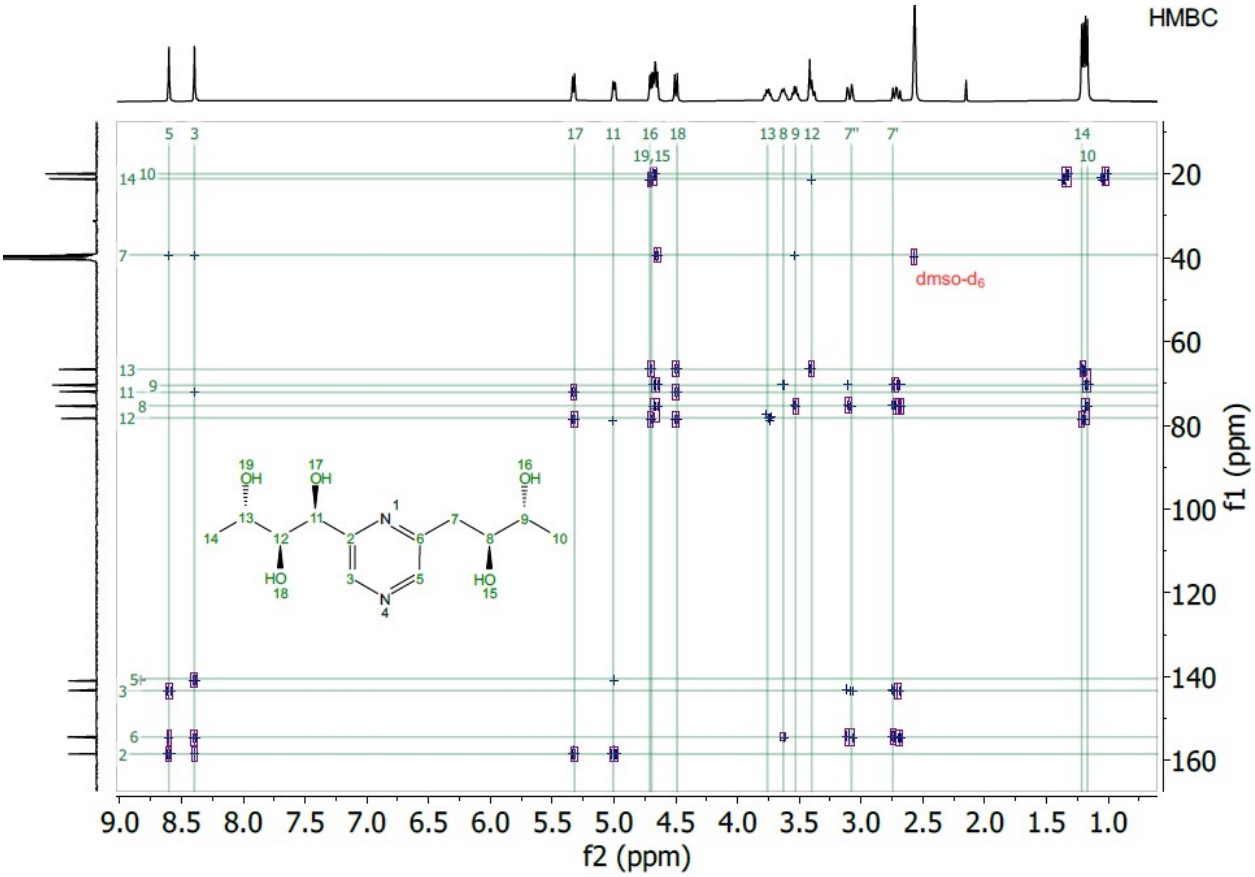
^{13}C-APT-NMR (400MHz, DMSO-d_6) for 2,6-DOR from rhamnose



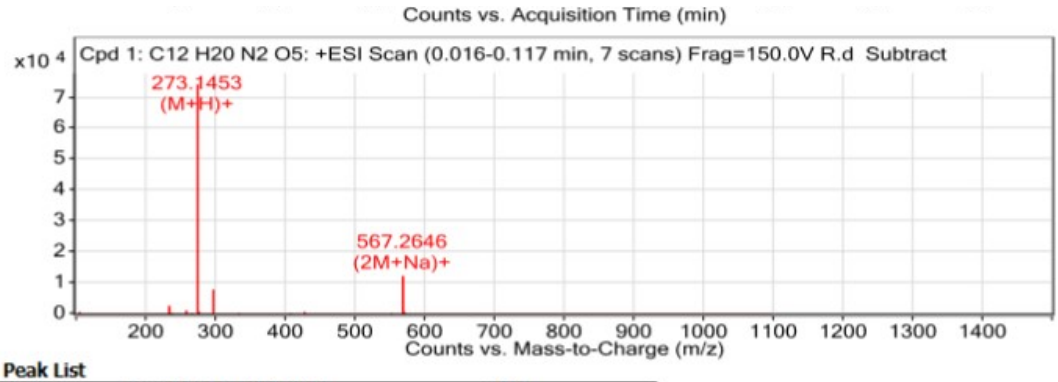
COSY -NMR (400MHz, DMSO-d_6) for 2,6-DOR from rhamnose



HSQC -NMR (400MHz, DMSO-d_6) for 2,6-DOR from rhamnose



HMBC -NMR (400MHz, DMSO-d_6)



Peak List

m/z	z	Abund	Formula	Ion
255.1336	1	134	C12 H19 N2 O4	(M+H)+[-H2O]
273.1453		75954	C12 H21 N2 O5	(M+H)+
273.3493		993		
274.1483		7596	C12 H21 N2 O5	(M+H)+
275.15		925	C12 H21 N2 O5	(M+H)+
295.1273	1	8129	C12 H20 N2 Na O5	(M+Na)+
296.1307	1	1059	C12 H20 N2 Na O5	(M+Na)+
567.2646	1	13067	C24 H40 N4 Na O10	(2M+Na)+
568.2678	1	3006	C24 H40 N4 Na O10	(2M+Na)+
569.2701	1	621	C24 H40 N4 Na O10	(2M+Na)+

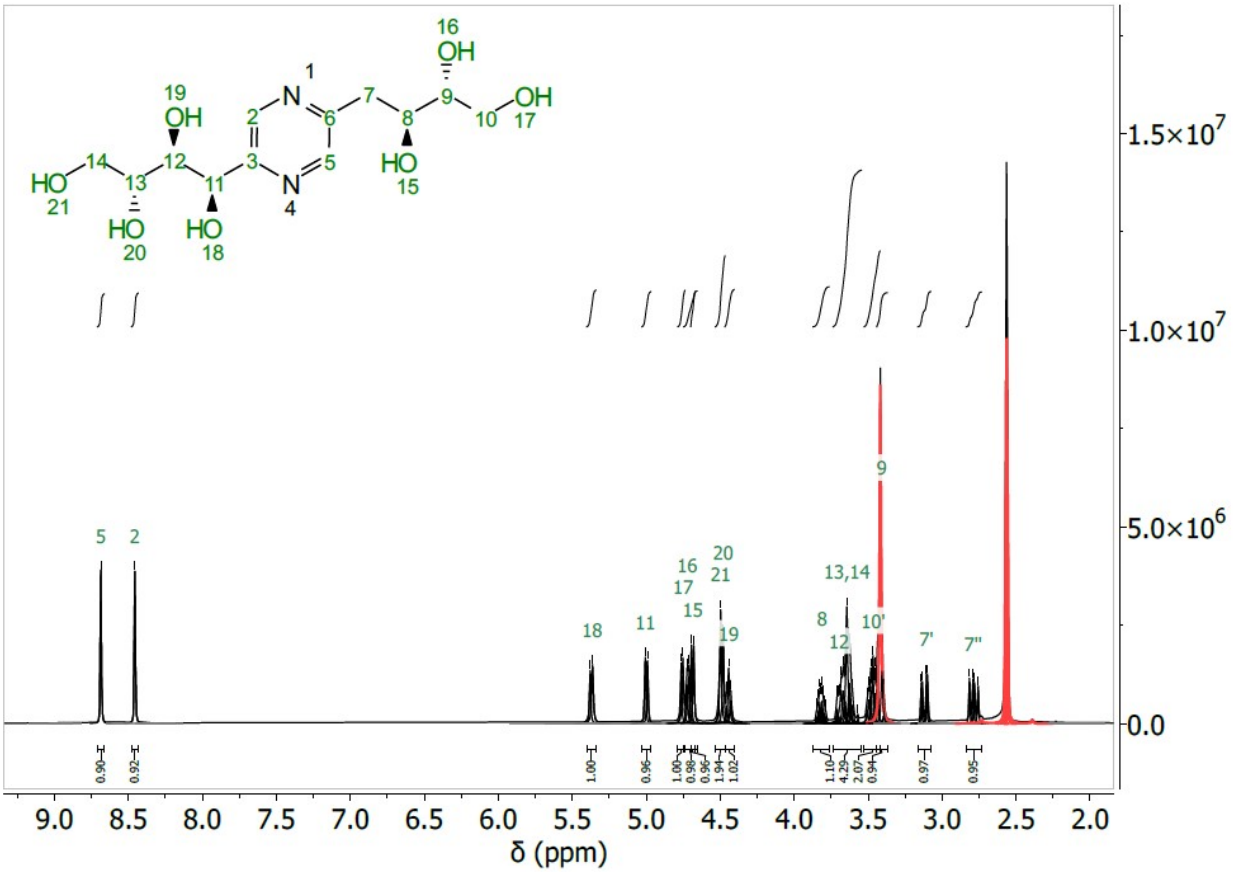
HR-MS of for 2,6-DOR from rhamnose

Analytical data for 2,5-DOF from fructose.

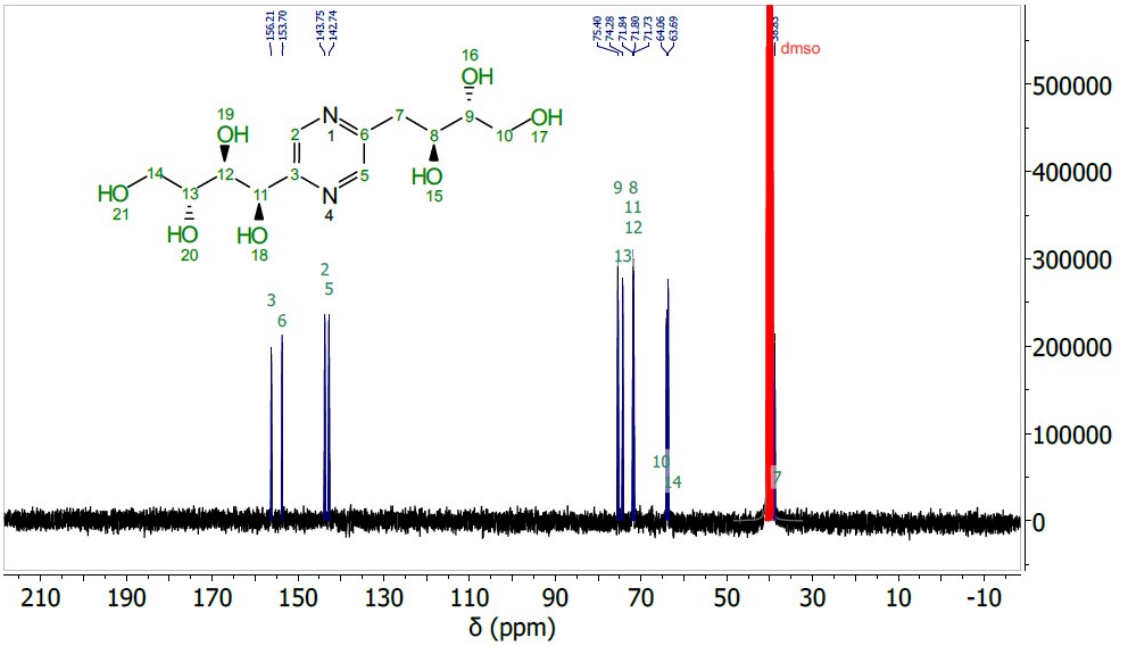
^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.62 (s, 1H), 8.39 (s, 1H), 5.31 (d, *J* = 6.4 Hz, 1H), 4.93 (d, *J* = 5.9 Hz, 1H), 4.67 (dd, *J* = 15.9, 4.7 Hz, 2H), 4.62 (d, *J* = 6.6 Hz, 1H), 4.42 (d, *J* = 7.0 Hz, 2H), 4.37 (d, *J* = 5.9 Hz, 1H), 3.75 (dtd, *J* = 9.5, 6.4, 6.3, 2.9 Hz, 1H), 3.69 – 3.52 (m, 5H), 3.44-3.37 (m, 2H, overlapping with H_2O peak in DMSO-d_6), 3.06 (dd, *J* = 14.0, 3.0 Hz, 1H), 2.72 (dd, *J* = 13.9, 9.5 Hz, 1H) ppm.

^{13}C NMR (101 MHz, DMSO-d_6) δ = 38.39, 63.25, 63.62, 71.29, 71.36, 71.40, 73.84, 74.96, 142.30, 143.31, 153.26, 155.77 ppm.

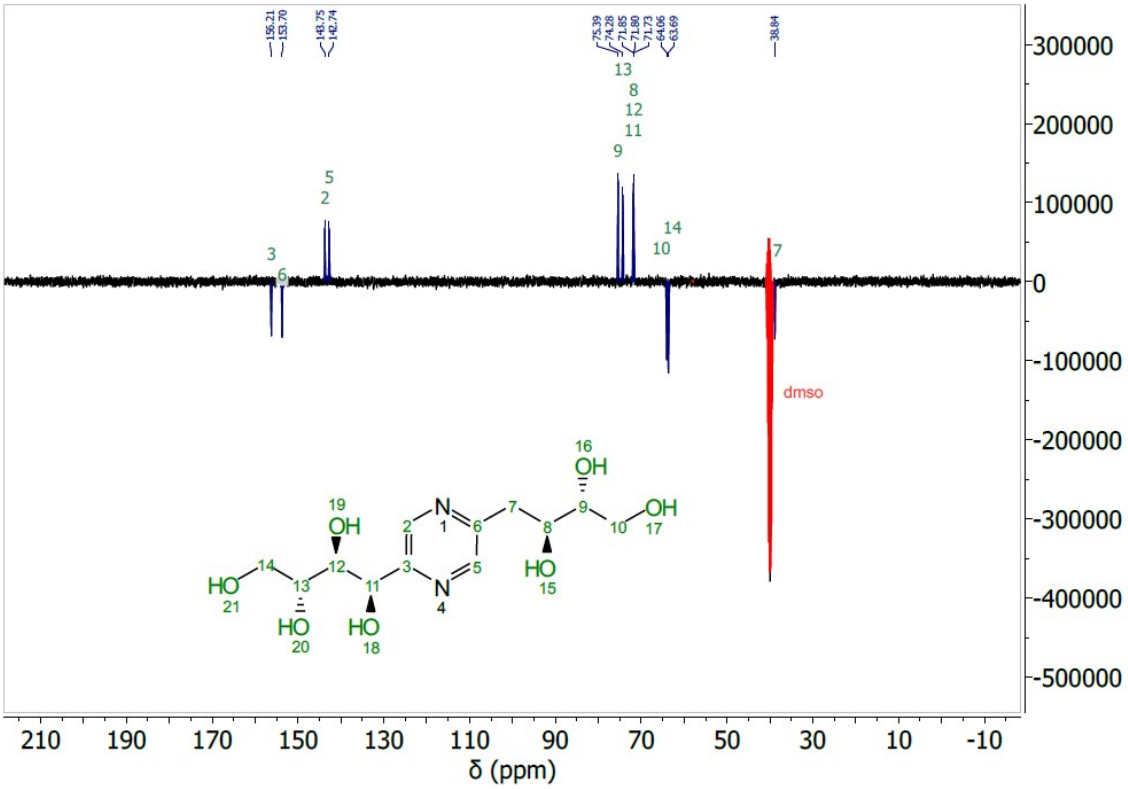
HRMS (ESI$^+$): Calculated for $C_{12}H_{21}N_2O_7$: 305.1343, Found: 305.1356.



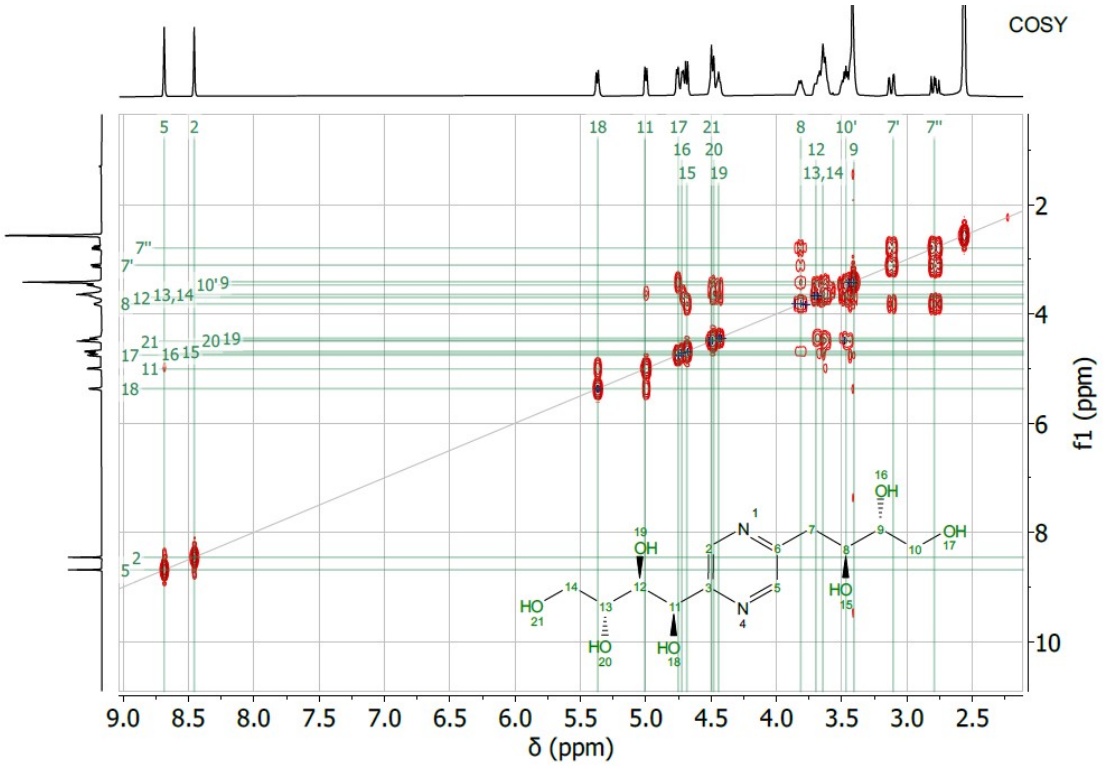
^{1}H -NMR (400MHz, DMSO-d_6) for 2,5-DOF from fructose



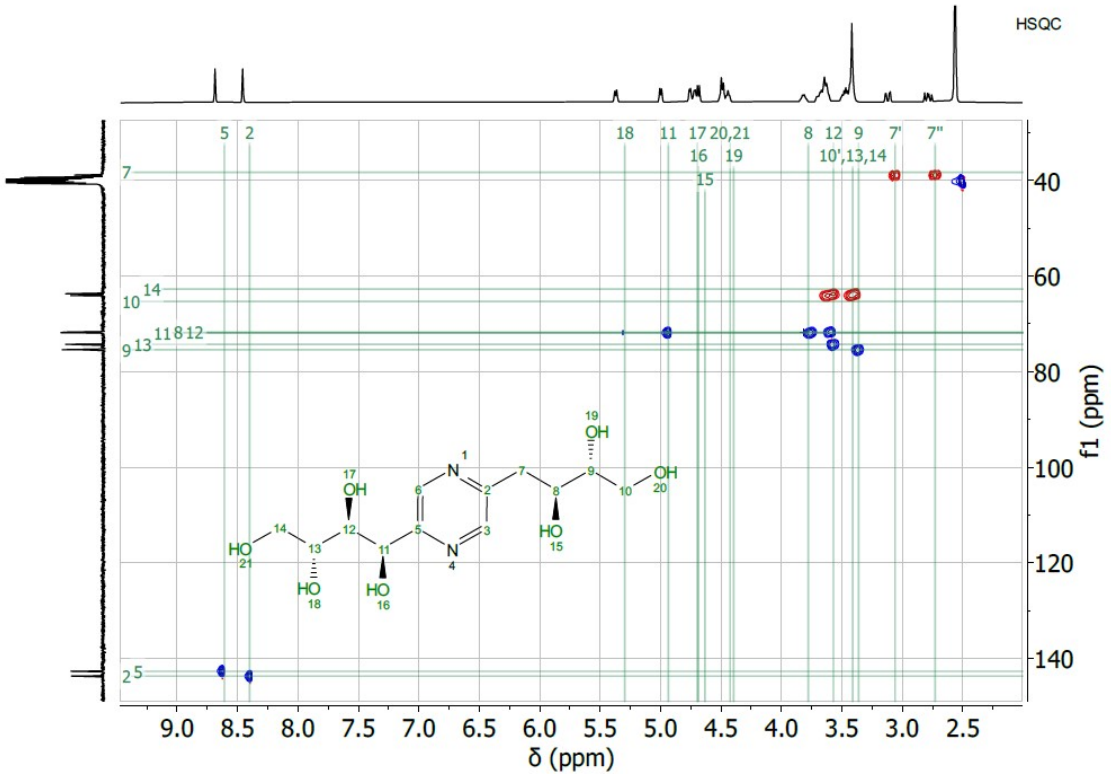
^{13}C -NMR (400MHz, DMSO-d_6) for 2,5-DOF from fructose



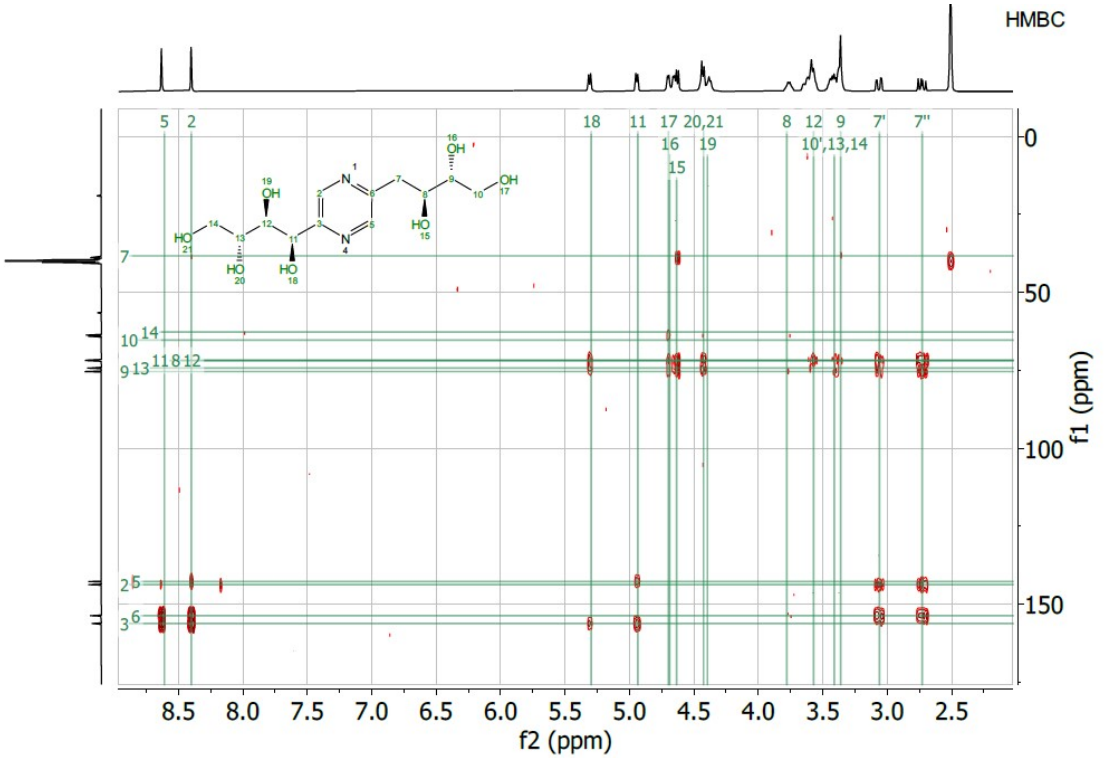
^{13}C-APT -NMR (400MHz, DMSO-d_6) for 2,5-DOF from fructose



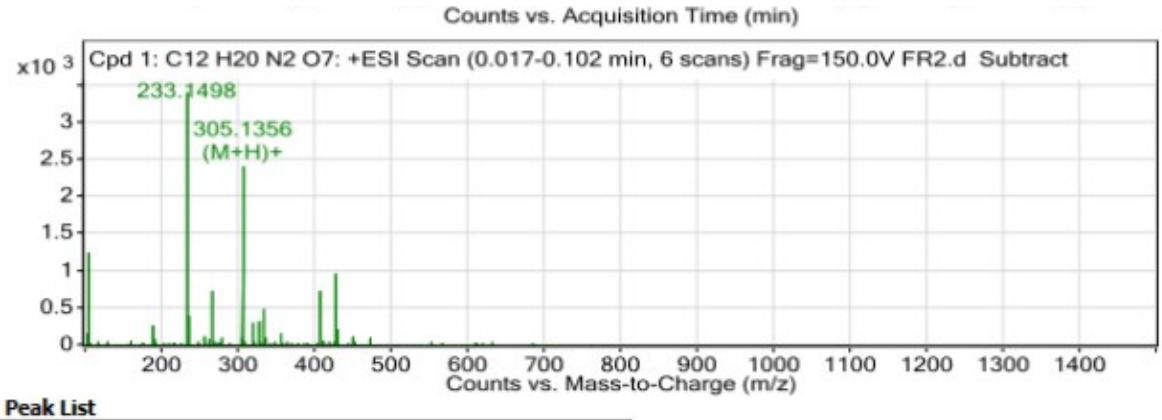
COSY -NMR (400MHz, DMSO-d_6) for 2,5-DOF from fructose



HSQC -NMR (400MHz, DMSO-d_6) for 2,5-DOF from fructose



HMBC-NMR (400MHz, DMSO-d_6) for 2,5-DOF from fructose



Peak List

m/z	z	Abund	Formula	Ion
233.1498		3426		
233.3431		14		
233.635		12		
233.7796		8		
234.1526		417		
235.1552		54		
305.1356	1	2455	C12 H21 N2 O7	(M+H)+
306.1377	1	381	C12 H21 N2 O7	(M+H)+
307.1458	1	57	C12 H21 N2 O7	(M+H)+

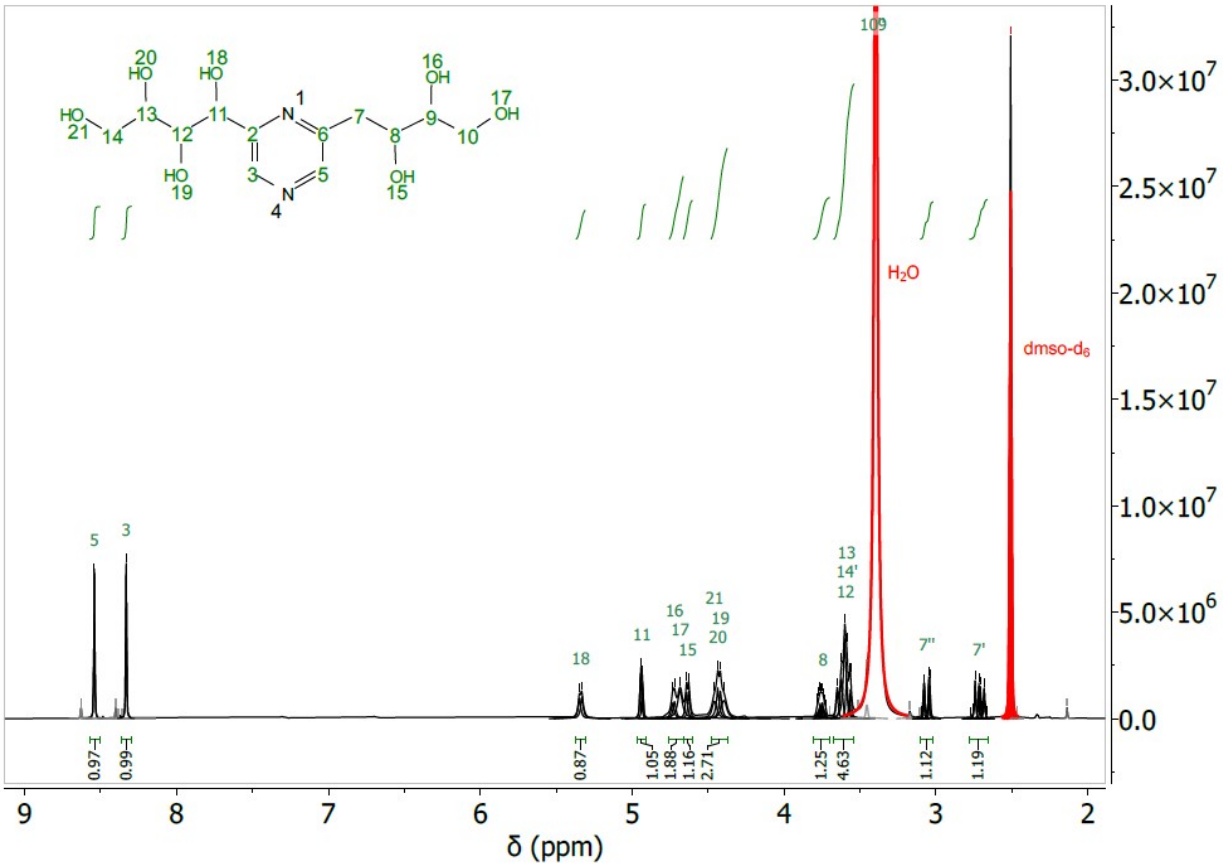
HR-MS for 2,5-DOF from fructose

Analytical data for 2,6-DOF from glucose

^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.54 (s, 1H), 8.33 (s, 1H), 5.34 (d, *J* = 6.4 Hz, 1H), 4.94 (d, *J* = 4.3 Hz, 1H), 4.78 – 4.55 (m, 3H), 4.53 – 4.32 (m, 3H), 3.75 (dt, *J* = 6.5, 3.3, 3.3 Hz, 1H), 3.68 – 3.54 (m, 5H), 3.06 (dd, *J* = 13.8, 2.7 Hz, 1H), 2.71 (dd, *J* = 13.9, 9.9 Hz, 1H) ppm.

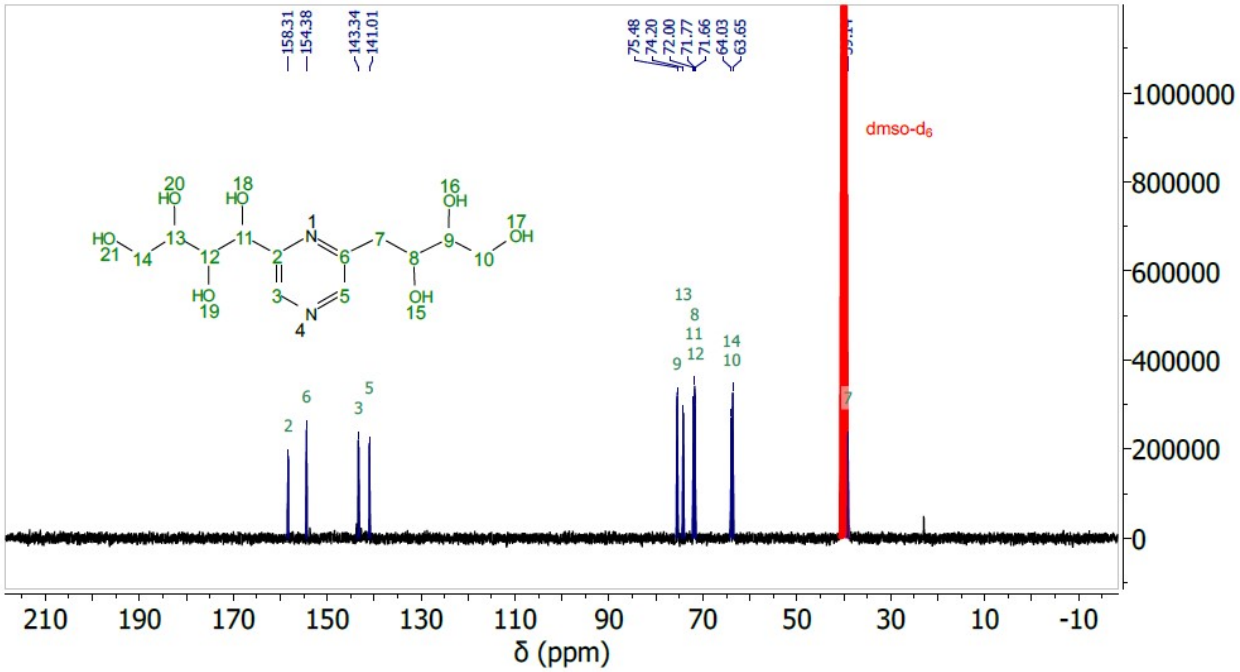
^{13}C NMR (101 MHz, DMSO-d_6) δ = 22.56, 63.24, 63.62, 71.25, 71.36, 71.59, 73.79, 75.07, 140.59, 142.93, 153.97, 157.90 ppm.

HRMS (ESI^{+}): Calculated for $C_{12}H_{21}N_2O_7$: 305.1343, Found: 305.1350

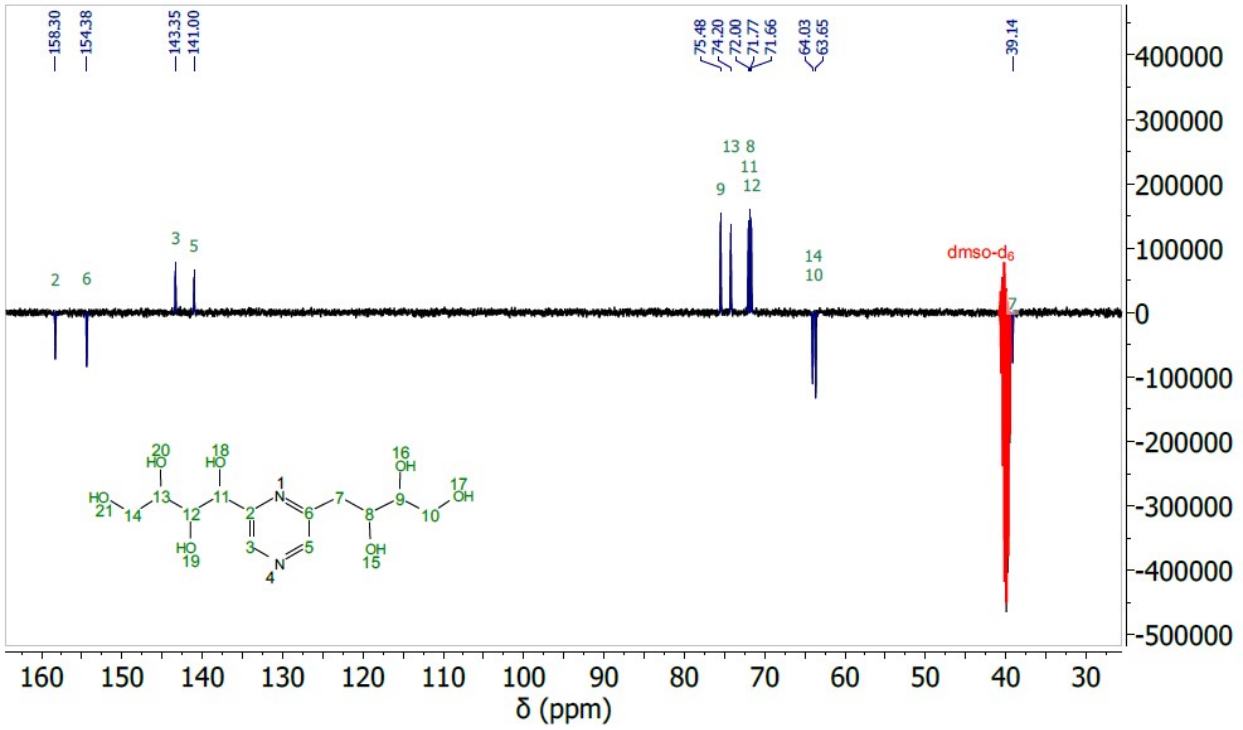


^{1}H -NMR (400MHz, DMSO-d_6) for 2,6-DOF from glucose. The integration misses the proton peaks from 9 and 10. These are hidden behind the water peak as shown by HSQC spectra.

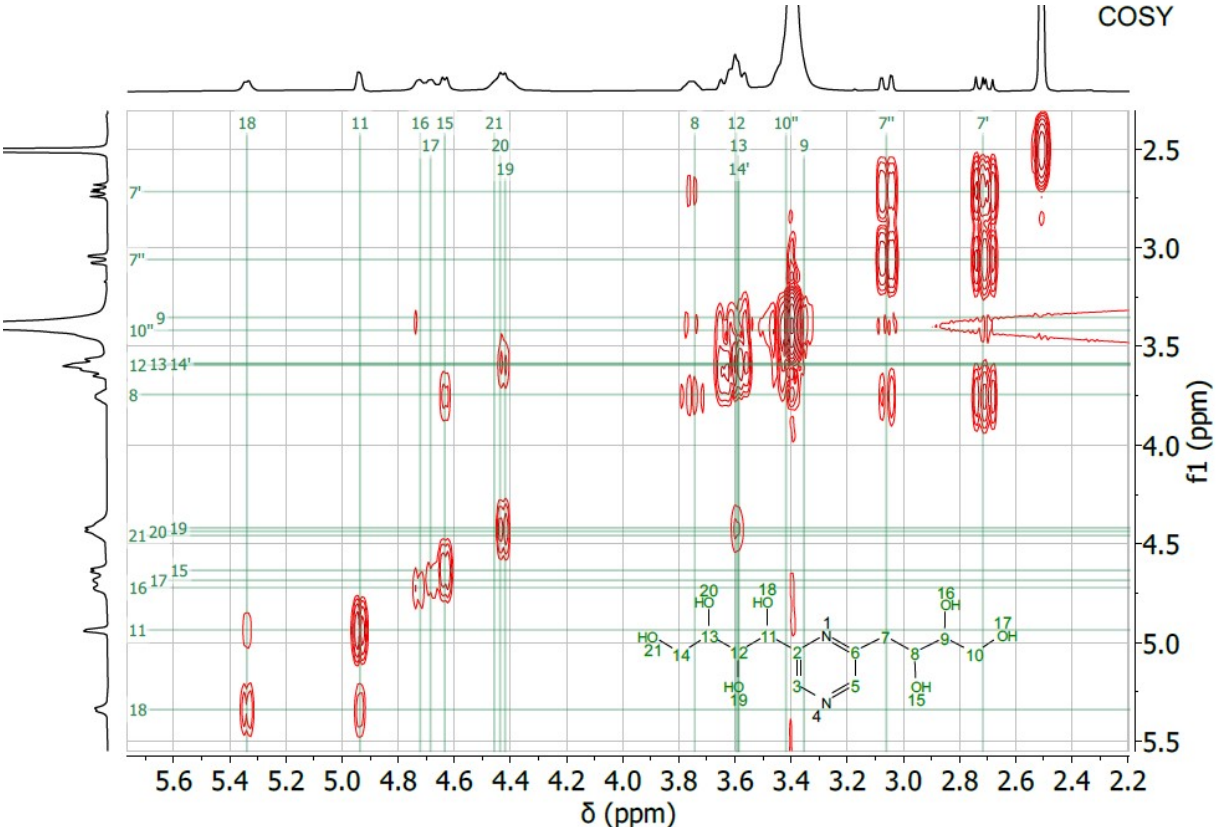
13



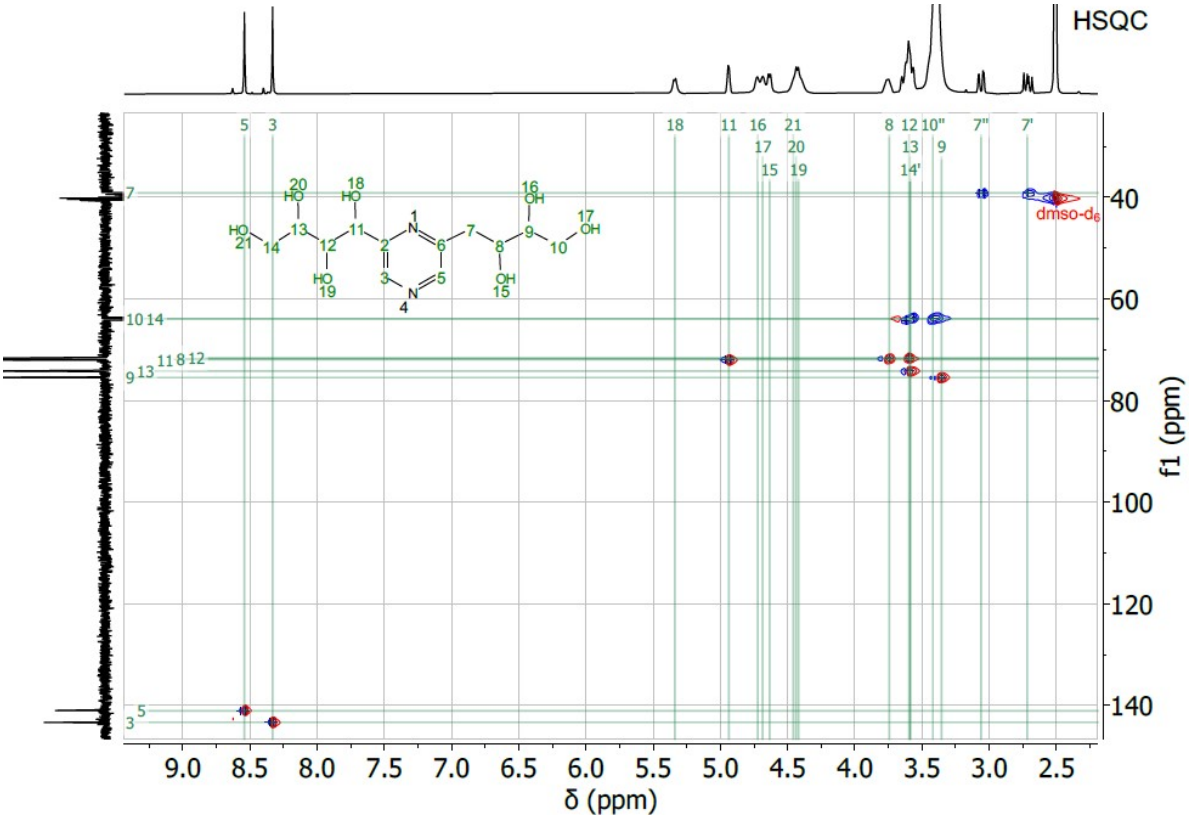
C -NMR (400MHz, DMSO-d_6) for 2,6-DOF from glucose



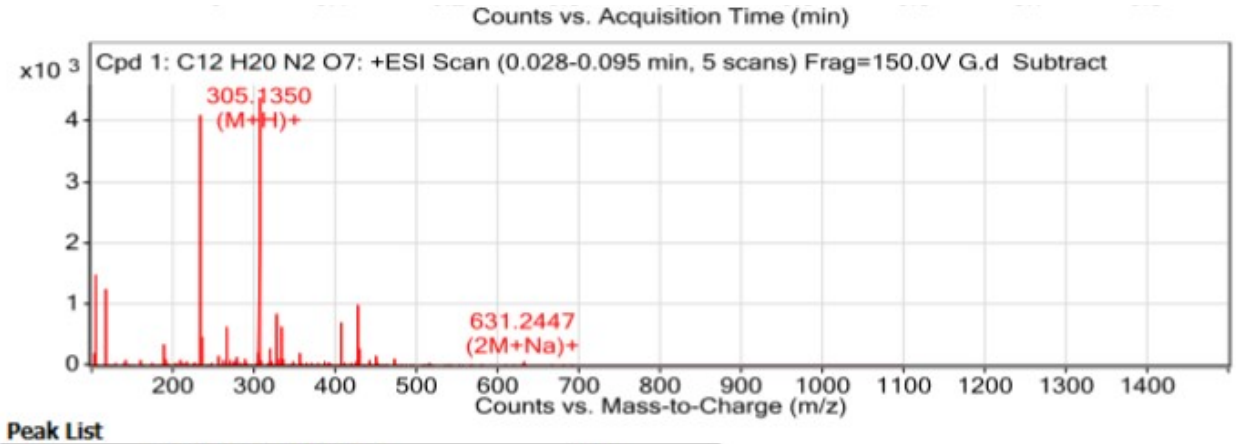
^{13}C-APT-NMR (400MHz, DMSO-d_6) for 2,6-DOF from glucose



COSY-NMR (400MHz, DMSO-d_6) for 2,6-DOF from glucose



HSQC-NMR (400MHz, DMSO-d_6) for 2,6-DOF from glucose



Peak List

m/z	z	Abund	Formula	Ion
302.2089		39		
304.1678		25		
305.135		4450	C12 H21 N2 O7	(M+H)+
306.1388		591	C12 H21 N2 O7	(M+H)+
307.1347		104	C12 H21 N2 O7	(M+H)+
327.1176	1	869	C12 H20 N2 Na O7	(M+Na)+
328.1261	1	121	C12 H20 N2 Na O7	(M+Na)+
329.1256	1	27	C12 H20 N2 Na O7	(M+Na)+
631.2447	1	75	C24 H40 N4 Na O14	(2M+Na)+
632.2437	1	23	C24 H40 N4 Na O14	(2M+Na)+

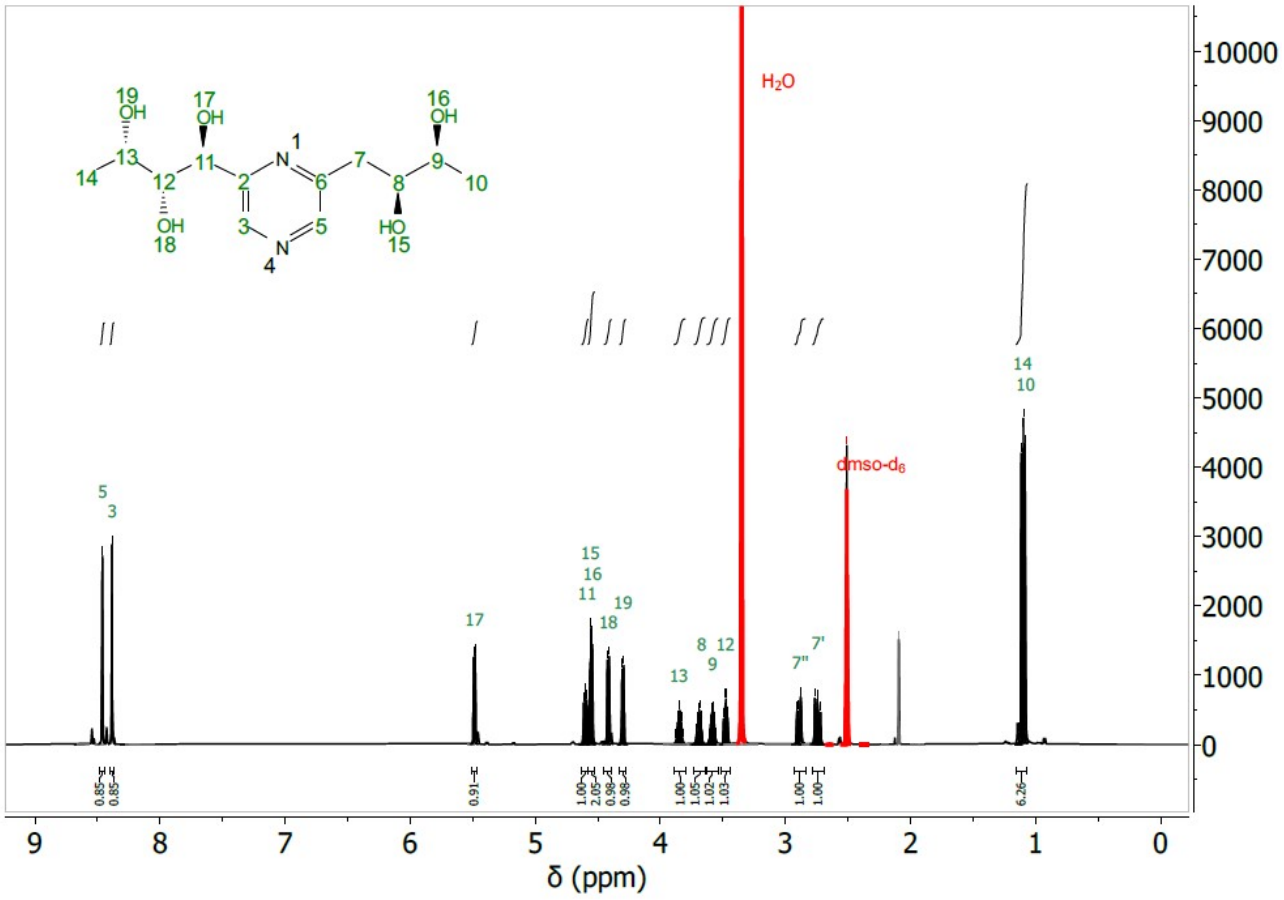
HR-MS for 2,6-DOF from glucose

Analytical data for 2,6-DOFu from fucose

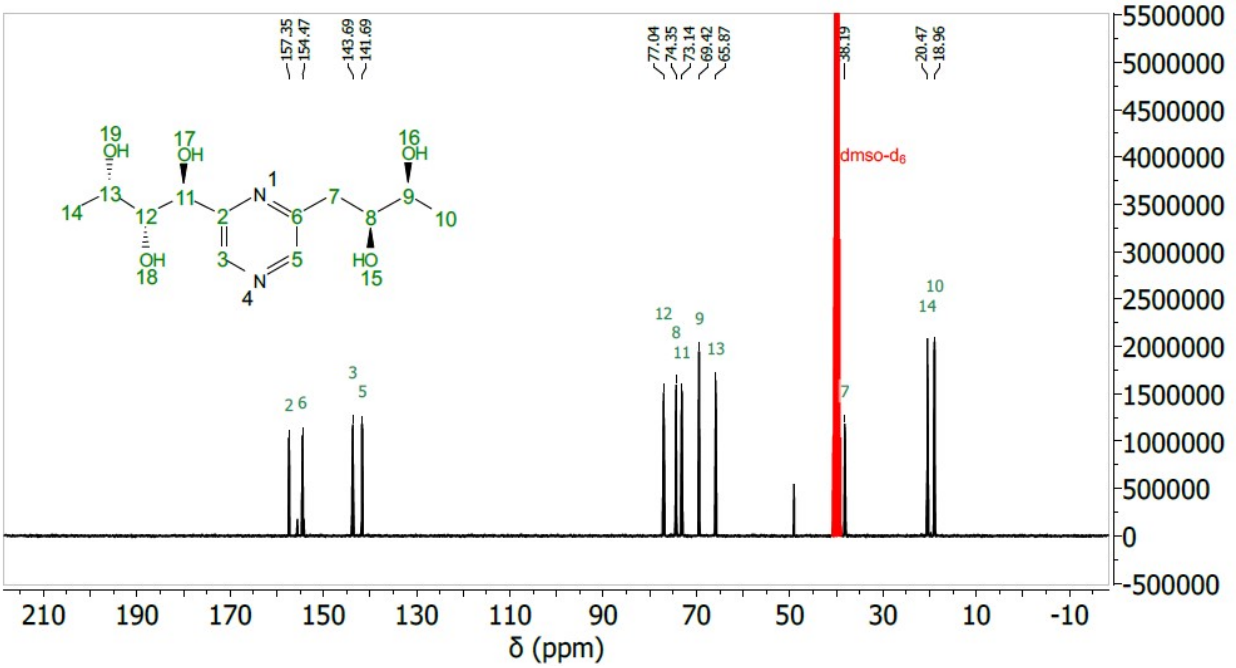
^{1}H NMR (500 MHz, DMSO-d_6) δ = 8.45 (s, 1H), 8.38 (s, 1H), 5.48 (d, *J* = 5.7 Hz, 1H), 4.59 (dd, *J* = 7.5, 5.6 Hz, 1H), 4.54 (dd, *J* = 5.5, 3.5 Hz, 2H), 4.41 (d, *J* = 6.9 Hz, 1H), 4.29 (d, *J* = 6.1 Hz, 1H), 3.87-3.81 (m, 1H), 3.73 – 3.64 (m, 1H), 3.59-3.56 (m, 1H), 3.47 (td, *J* = 7.2, 3.0 Hz, 1H), 2.88 (dd, *J* = 13.8, 3.3 Hz, 1H), 2.73 (dd, *J* = 13.8, 9.5 Hz, 1H), 1.10 (d, J = 6.5 Hz, 3H) 1.08 (d, J = 6.3 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ = 18.58, 20.09, 37.81, 65.49, 69.04, 72.76, 73.97, 76.66, 141.31, 143.31, 154.09, 156.97ppm.

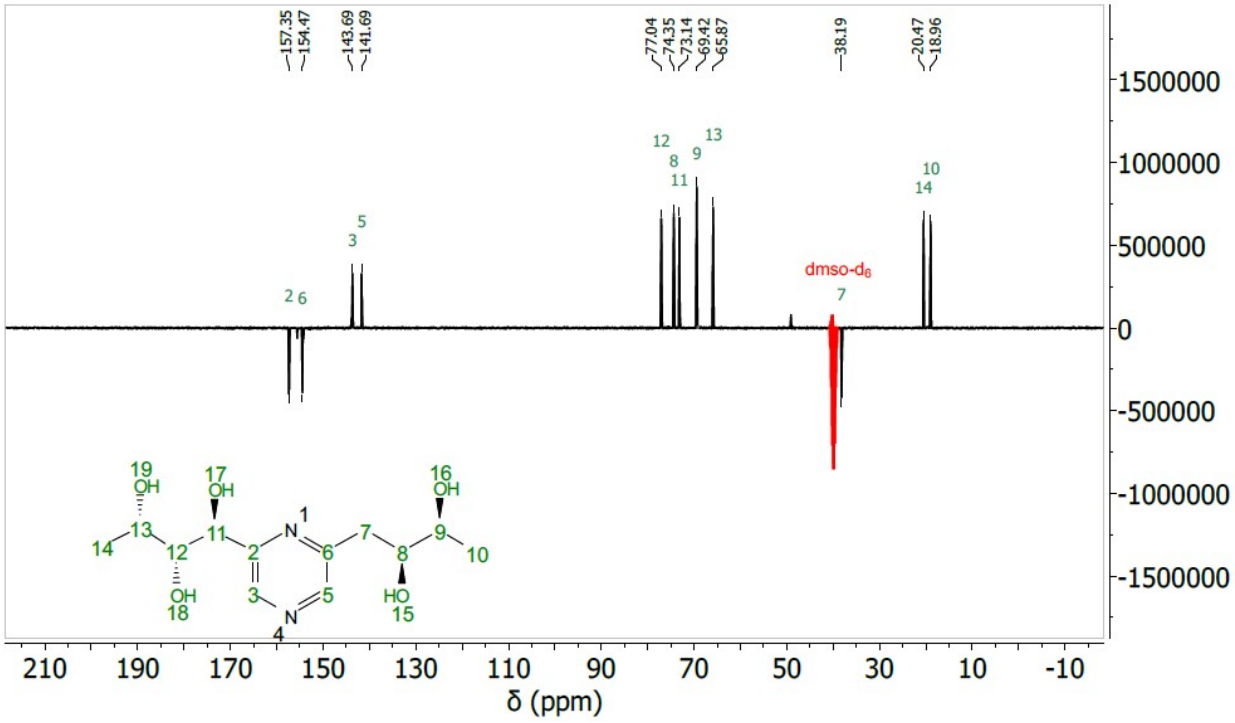
MS (ESI$^+$): Calculated for $C_{12}H_{21}N_2O_5$: 273.1445, Found: 273.1450



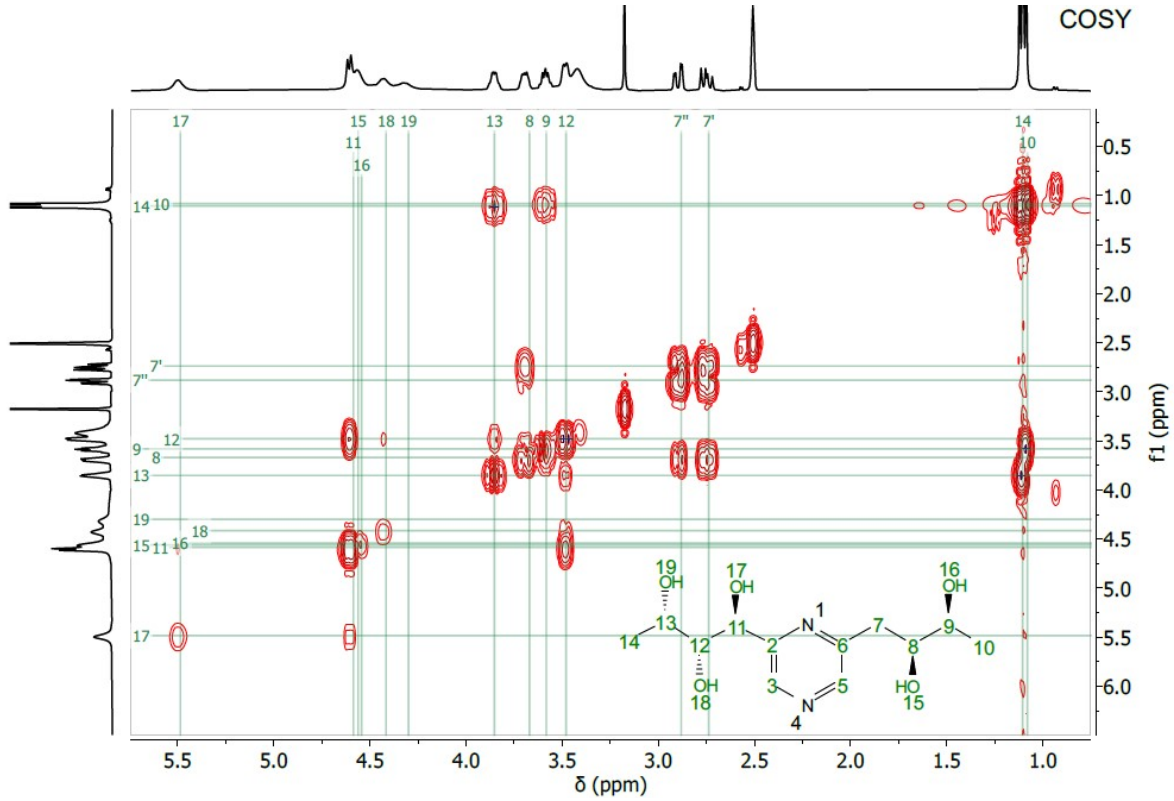
^{1}H -NMR (400MHz, DMSO-d_6) for 2,6-DOFu from fucose



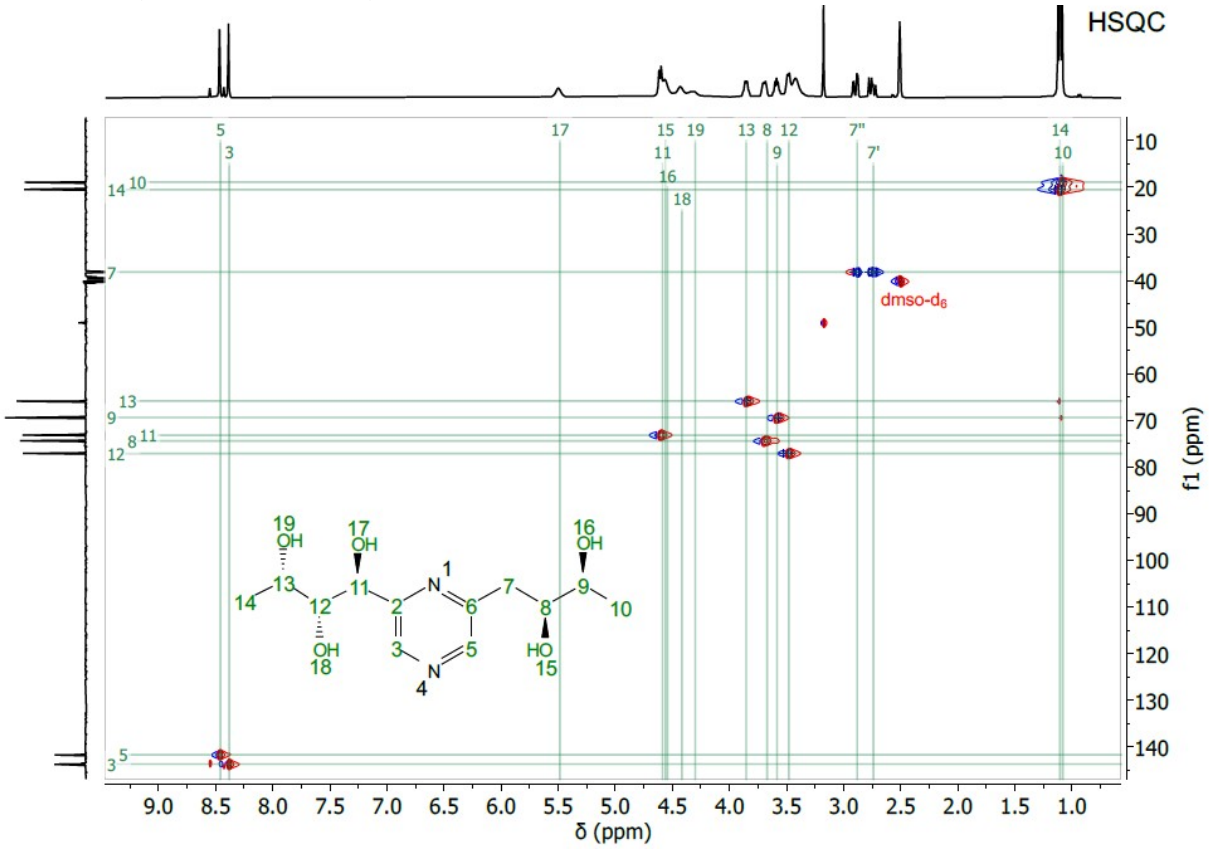
^{13}C -NMR (400MHz, DMSO-d_6) for 2,6-DOFu from fucose



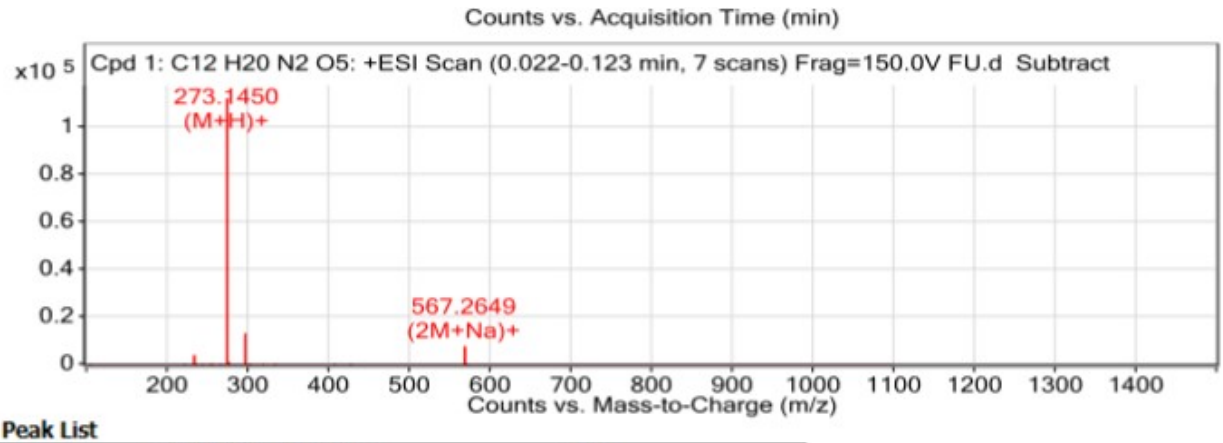
^{13}C-APT-NMR (400MHz, DMSO-d_6) for 2,6-DOFu from fucose



COSY-NMR (400MHz, DMSO-d_6) for 2,6-DOFu from fucose



HSQC-NMR (400MHz, DMSO-d_6) for 2,6-DOFu from fucose



Peak List

m/z	z	Abund	Formula	Ion
255.1345	1	325	C12 H19 N2 O4	(M+H)+[-H2O]
273.145		114665	C12 H21 N2 O5	(M+H)+
273.3488		2166		
274.1479		11320	C12 H21 N2 O5	(M+H)+
275.15		1459	C12 H21 N2 O5	(M+H)+
295.1274	1	13940	C12 H20 N2 Na O5	(M+Na)+
296.1309	1	1815	C12 H20 N2 Na O5	(M+Na)+
549.2513	1	79	C24 H38 N4 Na O9	(2M+Na)+[-H2O]
567.2649	1	8131	C24 H40 N4 Na O10	(2M+Na)+
568.2676	1	1988	C24 H40 N4 Na O10	(2M+Na)+

HR-MS for 2,6-DOFu from fucose

References

1. Wong Weng R, Oliver Allen G, Linington Roger G. Development of Antibiotic Activity Profile Screening for the Classification and Discovery of Natural Product Antibiotics. Chemistry & Biology. 2012;19(11):1483-95.
2. Hawkins PM, Hoi DM, Cheung C-Y, Wang T, Quan D, Sasi VM, et al. Potent Bactericidal Antimycobacterials Targeting the Chaperone ClpC1 Based on the Depsipeptide Natural Products Ecumicin and Ohmyungsamycin A. Journal of Medicinal Chemistry. 2022;65(6):4893-908.