Supporting Information

Synthesis of Rare Sugars with L-fuculose-1-phosphate aldolase (FucA) from *Thermus thermophilus* HB8

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Bacterial strains, plasmids, and materials.

Oligonucleotide primers were synthesized at Invitrogen (CA, USA) and listed in Table S1. Pfx DNA polymerase was purchased from Invitrogen. Restriction enzymes and T4 ligase were purchased from Fermentas (MBI, Canada). *Escherichia coli* DH5α [*lacZΔM15 hsdR recA*] was purchased from Gibco-BRL (Gaithersburg, MD). *Escherichia coli* MG1655 (rph-1, fnr) was from lab stock. *E. coli* BL21 (DE3) [F^- *ompT hsdSB*(r_B^- m_B^-) *gal dcm* (DE3)] and plasmid pET-28a were purchased from Novagen (Carlsbad, CA). *Thermus thermophiles* HB8 genomic DNA was purchased from ATCC (Manassas, VA). L-glycerol 3-phosphate bis(cyclohexylammonium) salt, DL-glycerol 3-phosphate magnesium salt, D-glyceraldehyde, glycerol phosphate oxidase, catalase, acid phosphatase from sweet potato, D-psicose, D-sorbose, isopropyl-1-thio-β-D-galactopyranoside (IPTG), imidazole, kanamycin and Ca^{2+} exchange resin were purchased from Sigma-Aldrich (St. Louis, MO). Ni^{2+}-NTA column was purchased from Qiagen (Hilden, Deutschland). L-glyceraldehyde was purchased from Capot Chemical Co (Hangzhou, China). L-tagatose and L-fructose were purchased from TCI America (Portland, OR). Amicon Ultra centrifugal filter (10 K) was purchased from Millipore (Billerica, MA). Bio gel P-2 gel, Aminex HPX-87H column (300 × 7.8 mm) and Aminex HPX-87C column (250 × 4.0 mm) were purchased from Bio-Rad Laboratories, Inc. (Hercules, CA). XK column (100 × 2.6 cm) was purchased from GE Healthcare (Piscataway, NJ). Pierce BCA Protein Assay Kit was purchased from Thermo Scientific (IL, USA).

Table S1 Primers used for plasmid construction

Primer	Sequence
pET28a-fucA$_{E.coli}$-F	5'-GCGCGGATCCATGGAACGAAATAAACTTG-3'(BamHI)
pET28a-fucA$_{E.coli}$-R	5'-GCGCAAGCTTTTACTCTTCAATTCGT-3'(HindIII)
pET28a-fucA$_{T.HB8}$-F	5'-TATAGGATCCATGCGCGCCCGGTTGTACG-3'(BamHI)
pET28a-fucA$_{T.HB8}$-R	5'-TATAAAGCTTTCATTCCCCACCCCCCAAG-3'(HindIII)
pET28a-yqaB-F	5'-GCGCCATATGTACGAGCGTTATGCAGGTT-3'(NdeI)
pET28a-yqaB-R	5'-TATACTCGAGTCACAGCAAGCGAACATCCACG-3'(XhoI)

Construction of pET28a-fucA$_{E.coli}$, pET28a-fucA$_{T.HB8}$ and pET28a-yqaB plasmids.

The genes encoding *E. coli* L-Fuculose-1-phosphate aldolase (FucA$_{E.coli}$) and YqaB phosphatase were both amplified by PCR using *E. coli* MG1655 genomic DNA as the template. The gene encoding *T. thermophiles* HB8 L-Fuculose-1-phosphate aldolase (FucA$_{T.HB8}$) was amplified by PCR using *T. thermophiles* HB8 genomic DNA as the template. The fucA$_{E.coli}$ and fucA$_{T.HB8}$ genes amplified were both digested with BamHI and HindIII then ligated into the pET-28a plasmid with the same enzymes digested. The yqaB gene amplified was digested with NdeI and XhoI then ligated into the pET-28a plasmids with the same enzymes digested. The recombinant plasmids were all transformed into DH5α strain for amplifying and sequencing.

Expression and purification of FucA$_{E.coli}$, FucA$_{T.HB8}$ *and YqaB.*

The pET28a-fucA$_{E.coli}$ plasmid was transformed into *E. coli* BL21 (DE3) to express FucA$_{E.coli}$. 20 mL overnight culture of *E. coli* BL21 (DE3) with the recombinant plasmid was inoculated to 1 L fresh LB medium containing 50 μg/mL kanamycin and cultivated at 37^{o}C, 200 rpm until the

OD_{600} reached 0.8-1.0. Then the temperature was switched to 16^{o}C and IPTG was added at a final concentration of 0.1 mM to induce the expression of $FucA_{E.coli}$ for 20 h. Cells were harvested by centrifugation at 4,000 × g for 20 min at 4^{o}C. The cell pellet was resuspended in 50 mM Tris-HCl (pH 7.5) buffer and sonicated on ice. The cell lysate was clarified by centrifugation at 15,000 × g for 30 min at 4^{o}C. The supernatant was loaded onto Ni^{2+}-NTA column equilibrated with 50 mM Tris-HCl (pH 7.5), then the column was washed with 50 mL wash buffer (50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 10 mM imidazole). Finally, $FucA_{E.coli}$ protein was eluted with elution buffer (50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 500 mM imidazole). The eluant was concentrated and desalted with Amicon Ultra (10 K) by centrifugation at 4,000 × g at 4^{o}C with 50 mM Tris-HCl buffer (pH 7.5) containing 10% glycerol to give the final volume of 2.5 mL which was stored at -20^{o}C. For $FucA_{T.HB8}$ *and YqaB,* the expression and purification were performed using similar procedures. The purity of the target proteins were determined by 12% (v/v) SDS-PAGE and the gels were stained by Coomassie Brilliant Blue (See Figure S1, Figure S2 and Figure S3). The protein concentrations were determined using Pierce BCA Protein Assay Kit according to the instructions.

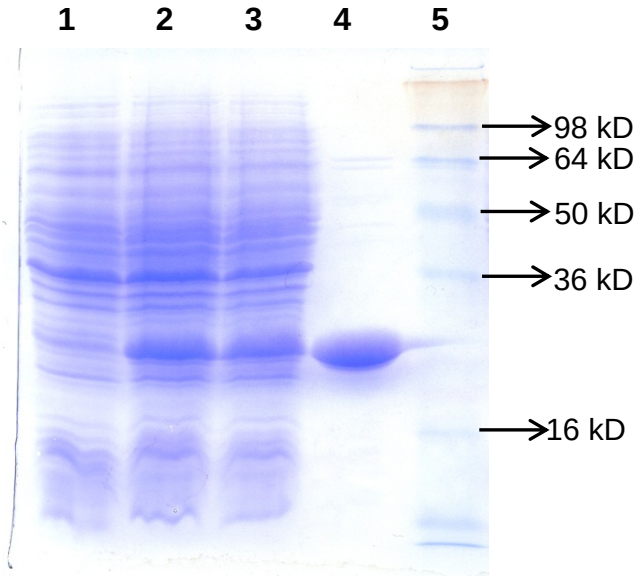


Figure S1. SDS-PAGE analysis of $FucA_{E.coli}$ expression and purification. Lanes: 1, whole cells not induced; 2, whole cells induced for 20 h; 3, supernatant of cell lysate; 4, purified $FucA_{E.coli}$; 5, protein marker.

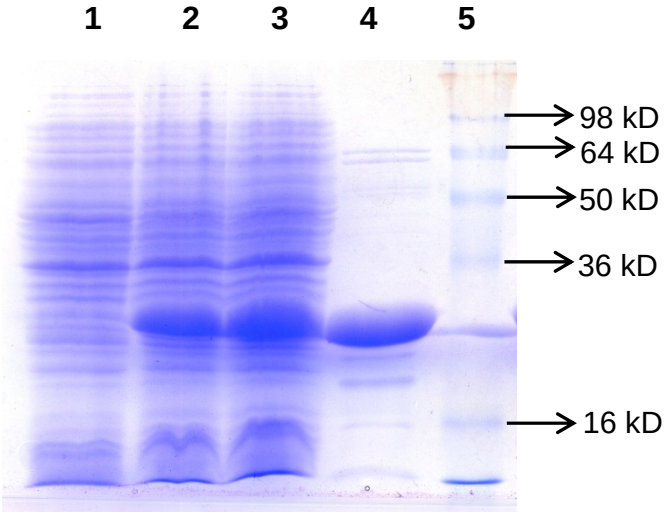


Figure S2. SDS-PAGE analysis of $FucA_{T.HB8}$ expression and purification. Lanes: 1, whole cells not induced; 2, whole cells induced for 20 h; 3, supernatant of cell lysate; 4, purified $FucA_{T.HB8}$; 5, protein marker.

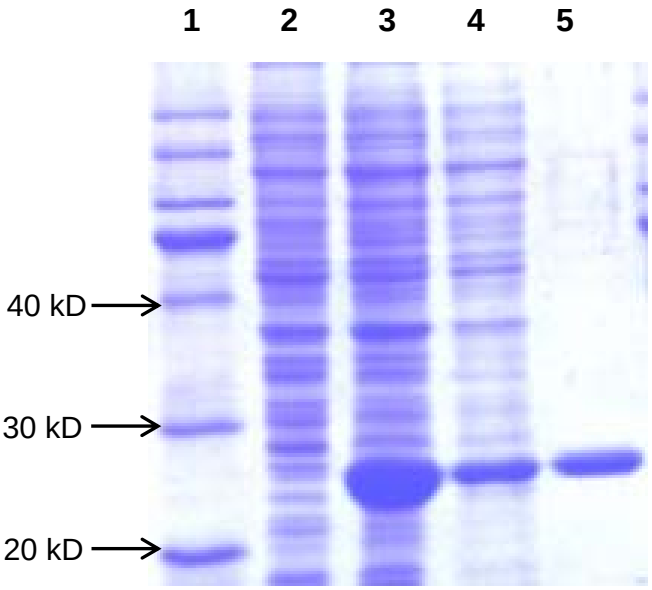


Figure S3. SDS-PAGE analysis of YqaB expression and purification. Lanes: 1, protein marker; 2, whole cells not induced; 3, whole cells induced for 20 h; 4, supernatant of cell lysate; 5, purified YqaB.

One-pot four enzyme synthesis of D-psicose with FucA ($FucA_{E.coli}$ or $FucA_{T.HB8}$) using L-glycerol 3-phosphate and D-glyceraldehyde

To a solution of L-glycerol 3-phosphate bis(cyclohexylammonium) salt (370 mg, 1.0 mmol) in 5.56 mL ddH_2O was added D-glyceraldehyde (2.44 mL, 0.5 M, 1.22 mmol) at pH 7.0, glycerol phosphate oxidase (70 U, 2 mg), catalase (1000 U, 1.18 μL) and FucA ($FucA_{E.coli}$ or $FucA_{T.HB8}$, final concentration 0.5 mg/mL). ddH_2O was added to bring the total volume to 10 mL if necessary. The mixture was shaken at rt. for 22 h and the reaction was monitored by TLC (developed by nBuOH/AcOH/H_2O 2/1/1 (v/v/v) and stained with anisaldehyde sugar stain). The pH was then adjusted to pH ~5 with 6 N HCl and 11 μL acid phosphatase (18 U) was added and the mixture was shaken at 37°C for 24 h. After cooling to rt., the pH was adjusted to 7.0 with 1 N NaOH and the mixture was diluted with methanol. The solution was filtered through Celite and washed with methanol. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (EtOAc/iPrOH/H_2O 9/3/1 (v/v/v)) to afford a pale

yellow syrup which was further purified by Bio gel P-2 column. 22 mg (12% yield) of D-psicose was obtained as the sole product with $FucA_{E.coli}$. A mixture of D-psicose and D-sorbose was obtained (121.4 mg, 67% totally, D-psicose/D-sorbose 5.3:1) with $FucA_{T.HB8}$. This mixture could be isolated by cation exchange resin column as described below.

Procedure for isolation of D-sorbose and D-psicose using cation exchange resin (Ca^{2+} form)

D-Sorbose and D-psicose mixture (121.4 mg from one-pot reactions) was dissolved in 3 mL ddH_2O and applied to a cation exchange resin (Ca^{2+} form, 100 × 2.6 cm) which was preheated to 65°C using a thermostatic jacket. The column was eluted with ddH_2O (flow rate ~1.5 mL/min) and the whole isolation process was performed at 65-70°C. Fractions were collected with an automatic fraction collector and identified by HPLC. D-Sorbose was eluted off first and D-psicose was eluted off in later fractions. Fractions containing pure D-sorbose or D-psicose were pooled and lyophilized to give D-sorbose (16 mg) and D-psicose (77.8 mg).

One-pot four enzyme synthesis of D-psicose with $FucA_{T.HB8}$ using DL-glycerol 3-phosphate and D-glyceraldehyde

To a solution of DL-glycerol 3-phosphate magnesium salt (548.78 mg, 2.4 mmol) in 6.86 mL ddH_2O was added D-glyceraldehyde (2 mL, 0.5 M, 1.0 mmol) at pH 7.0, glycerol phosphate oxidase (70 U, 2 mg), catalase (1000 U, 1.18 μL) and $FucA_{T.HB8}$ (final concentration 0.5 mg/mL). ddH_2O was added to bring the total volume to 10 mL if necessary. The reaction condition and isolation procedure were similar to the process of using the substrate L-glycerol 3-phosphate as described above. After purification by Bio gel P-2 column, a mixture of D-psicose and D-sorbose

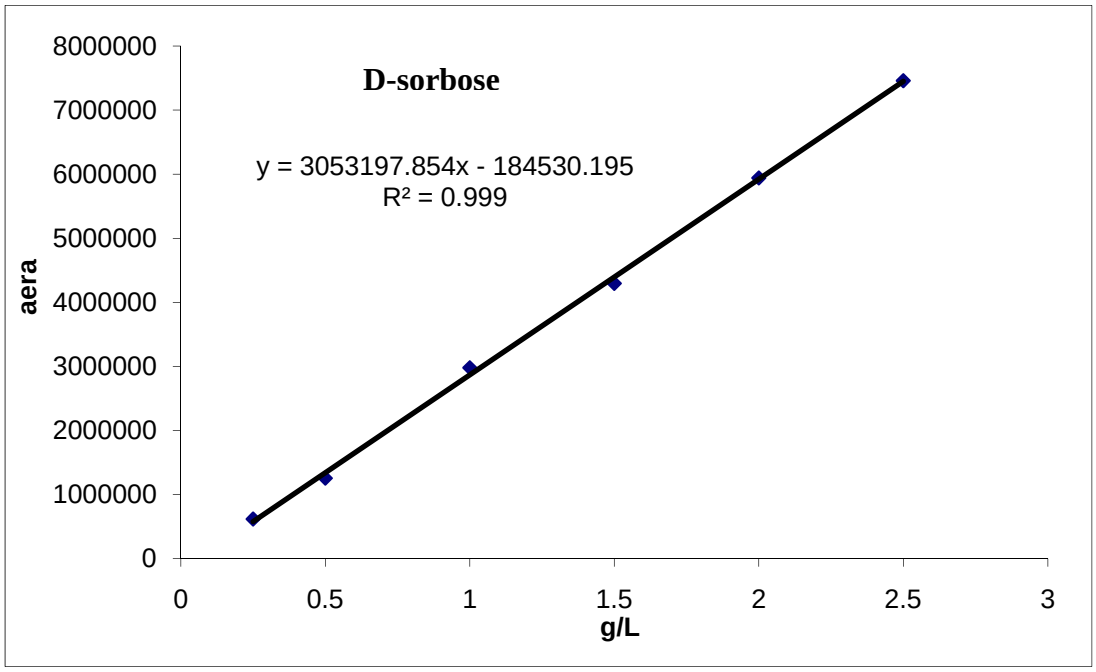
was obtained (104.2 mg, 58% totally, D-psicose/D-sorbose 8.4:1). The mixture could be further separated as described above to afford D-sorbose (15 mg) and D-psicose (74.9 mg).

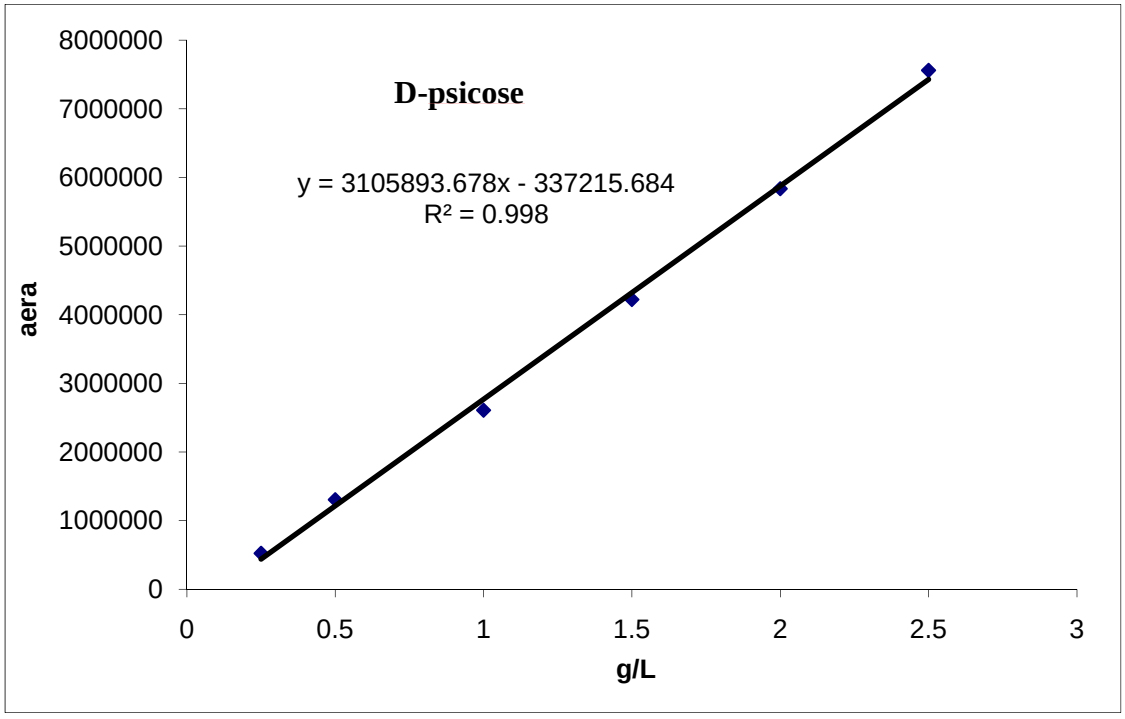
One-pot four enzyme synthesis of L-tagatose and L-fructose with $FucA_{T.HB8}$ and YqaB phosphatase using DL-glycerol 3-phosphate and L-glyceraldehyde

To a solution of DL-glycerol 3-phosphate magnesium salt (548.78 mg, 2.4 mmol) in 4.7 mL ddH_2O was added L-glyceraldehyde (3.3 mL, 0.303 M, 1.0 mmol) at pH 7.0, glycerol phosphate oxidase (70 U, 2 mg), catalase (1000 U, 1.18 μL) and $FucA_{T.HB8}$ (final concentration 0.5 mg/mL). ddH_2O was added to bring the total volume to 10 mL if necessary. The mixture was shaken at rt. for 22 h and the reaction was monitored by TLC (developed by nBuOH/AcOH/H_2O 2/1/1 (v/v/v) and stained with anisaldehyde sugar stain). Then YqaB phosphatase (final concentration 0.25 mg/mL) and $MgCl_2$ (final concentration 2.5 mM) was added and the mixture was shaken at 37 ^oC for 12 h. The purification was performed as described above. After purification by Bio gel P-2 column, a mixture of L-tagatose and L-fructose was obtained (84 mg, 47% totally). This mixture was isolated as described above to give L-fructose (34.2 mg) and L-tagatose (33.7 mg).

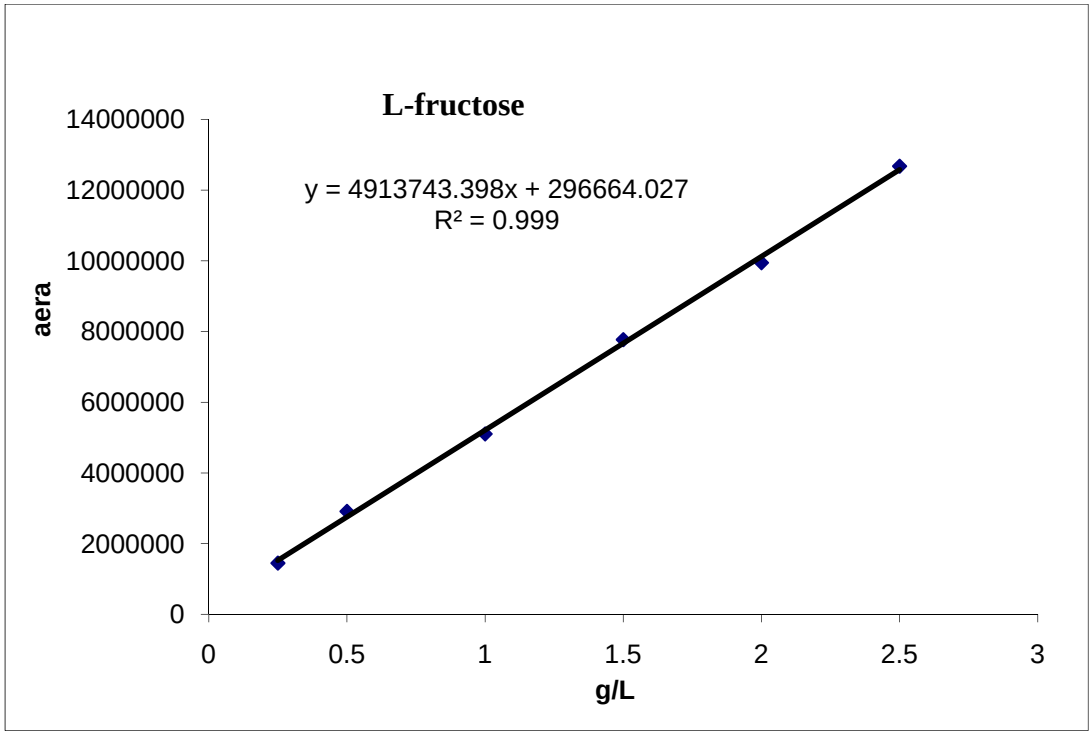
HPLC Standard curve for D-sorobse, D-psicose, L-fructose, L-tagatose quantification (peak area/concentration)

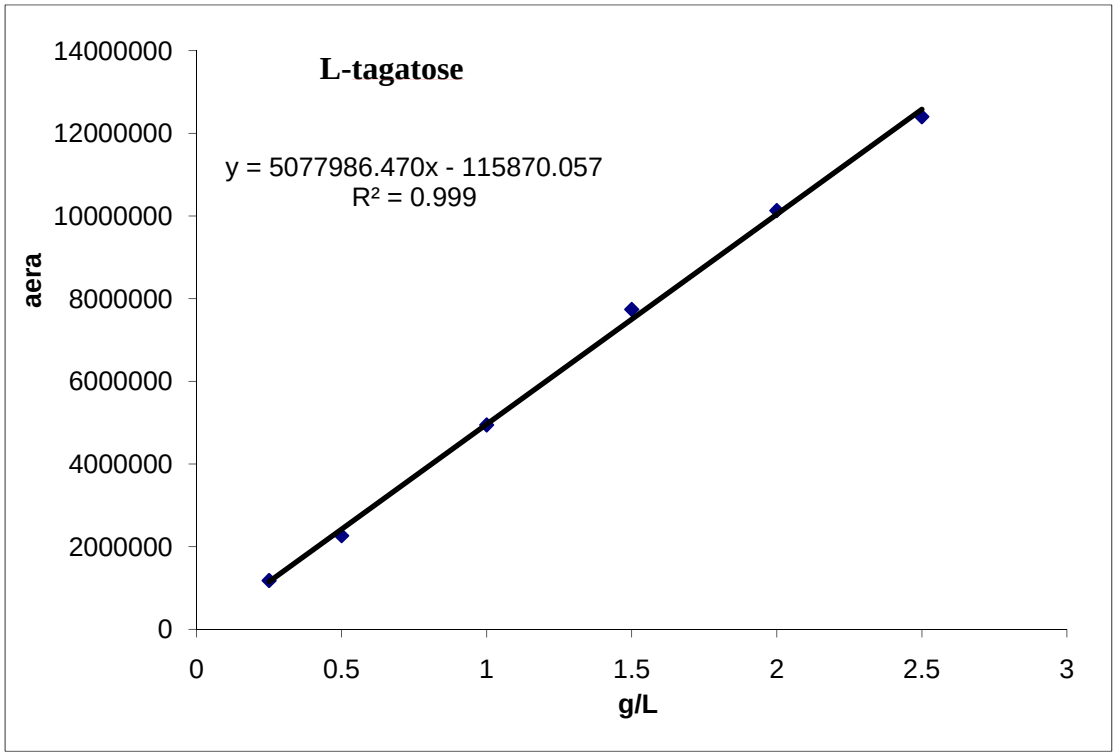
Aminex HPX-87H column (hydrogen form, sulfonated divinyl benzene-styrene copolymer support and eluted with 5 mM H_2SO4) for quantification of D-sorbose and D-psicose

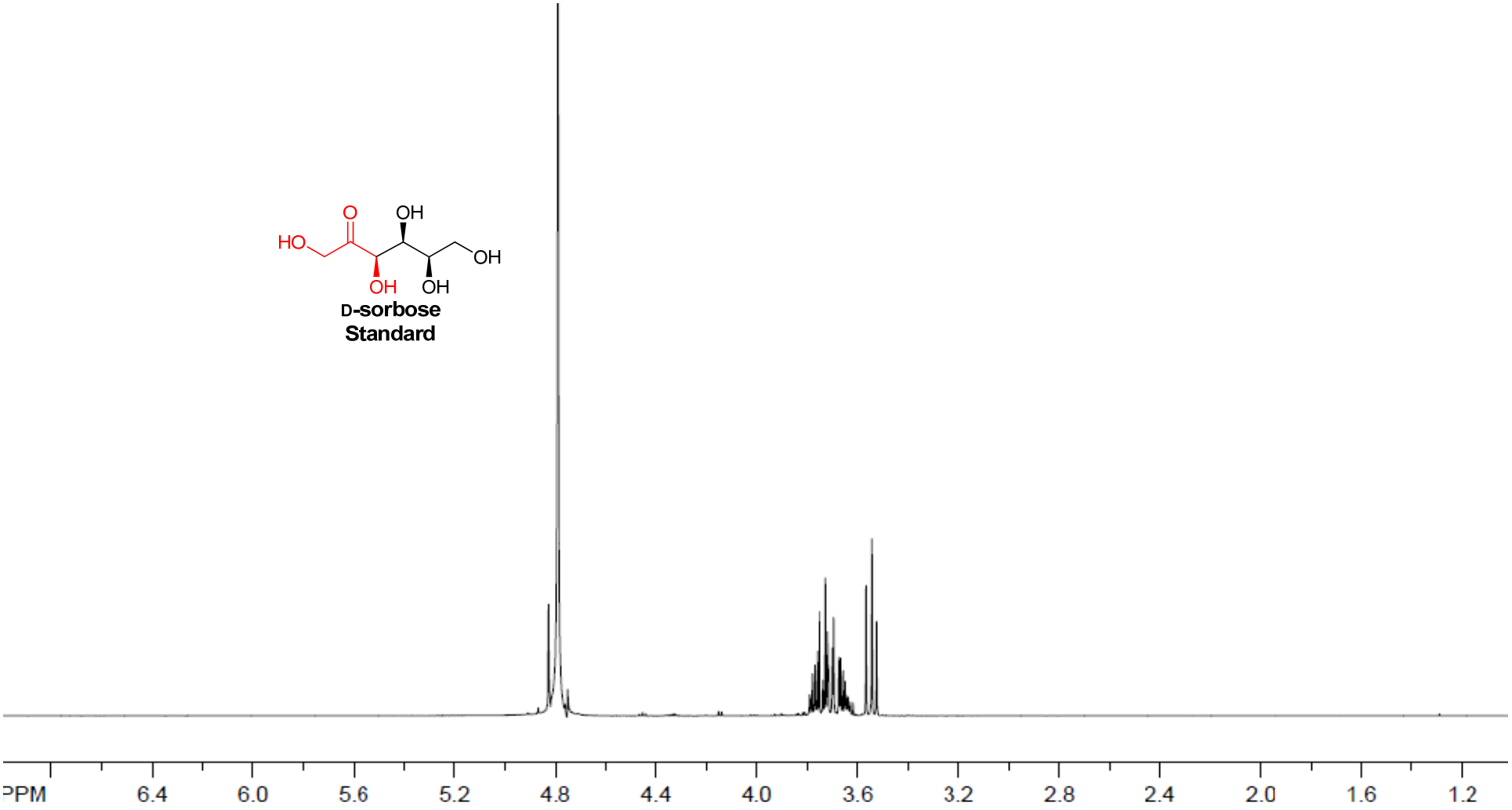




Aminex HPX-87C column (Calcium form, sulfonated divinyl benzene-styrene copolymer support and eluted with H_2O) for quantification of L-fructose and L-tagatose



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OH
OH
D-sorbose
Standard
PPM
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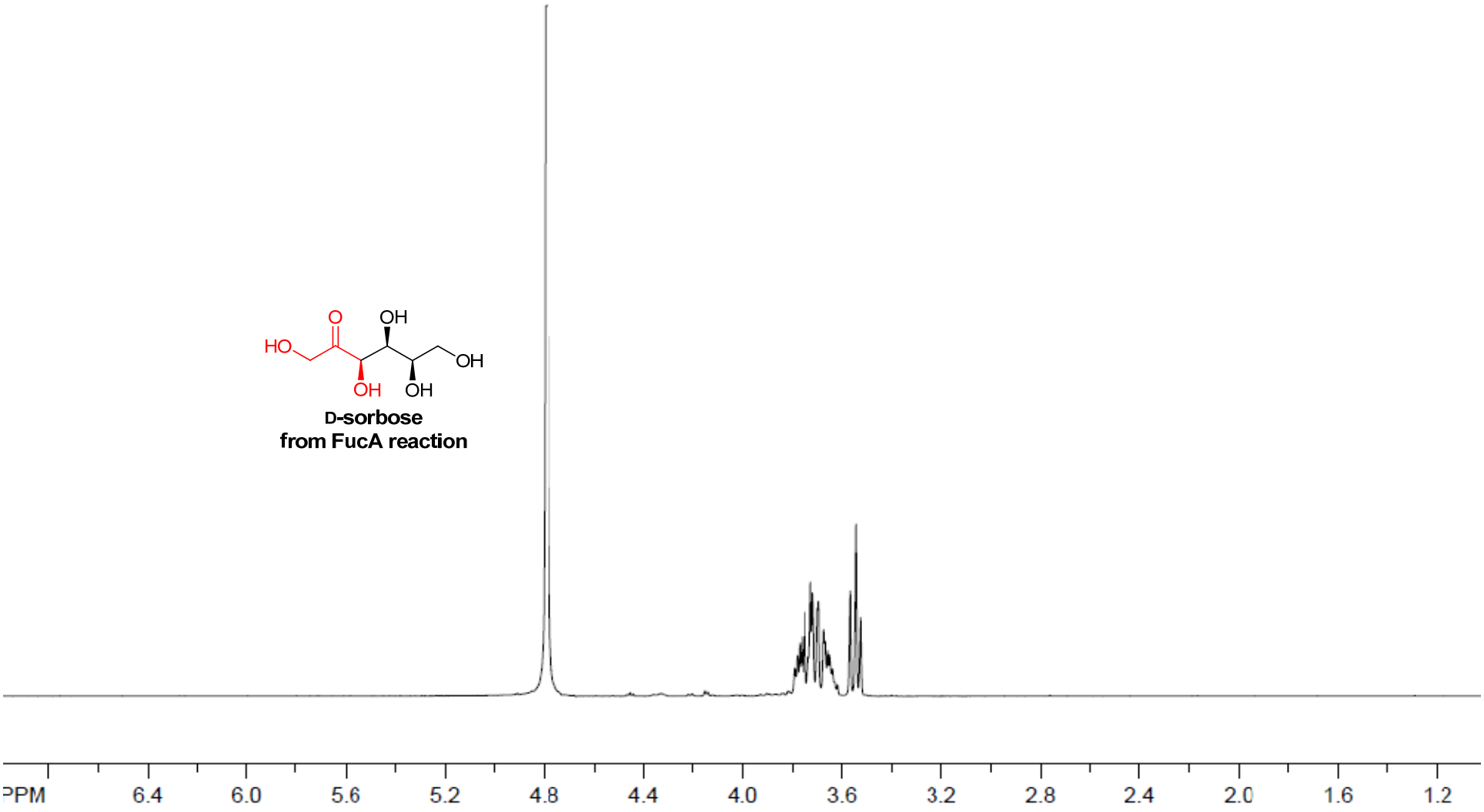
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D-sorbose
from FucA reaction
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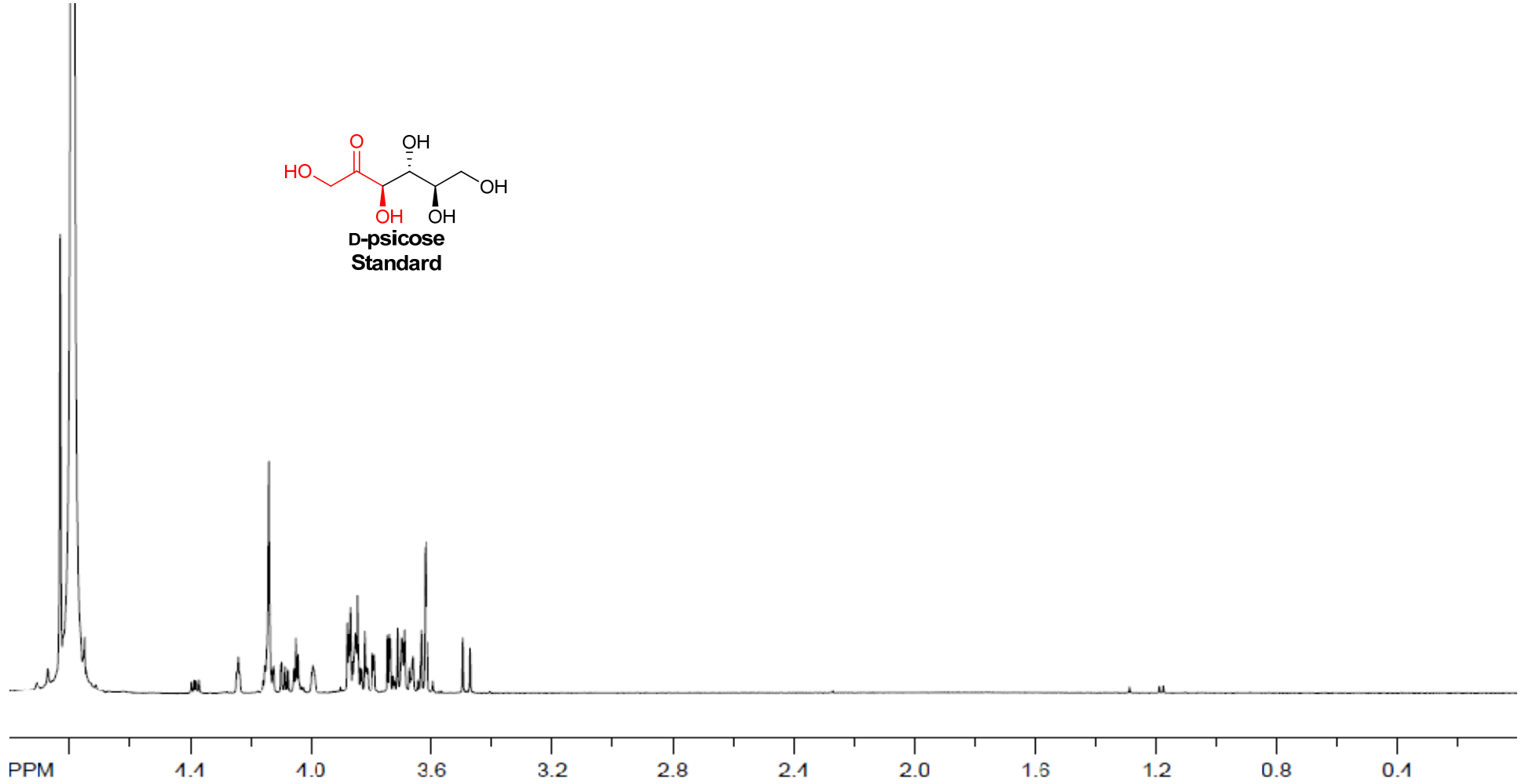
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D-psicose
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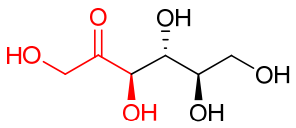
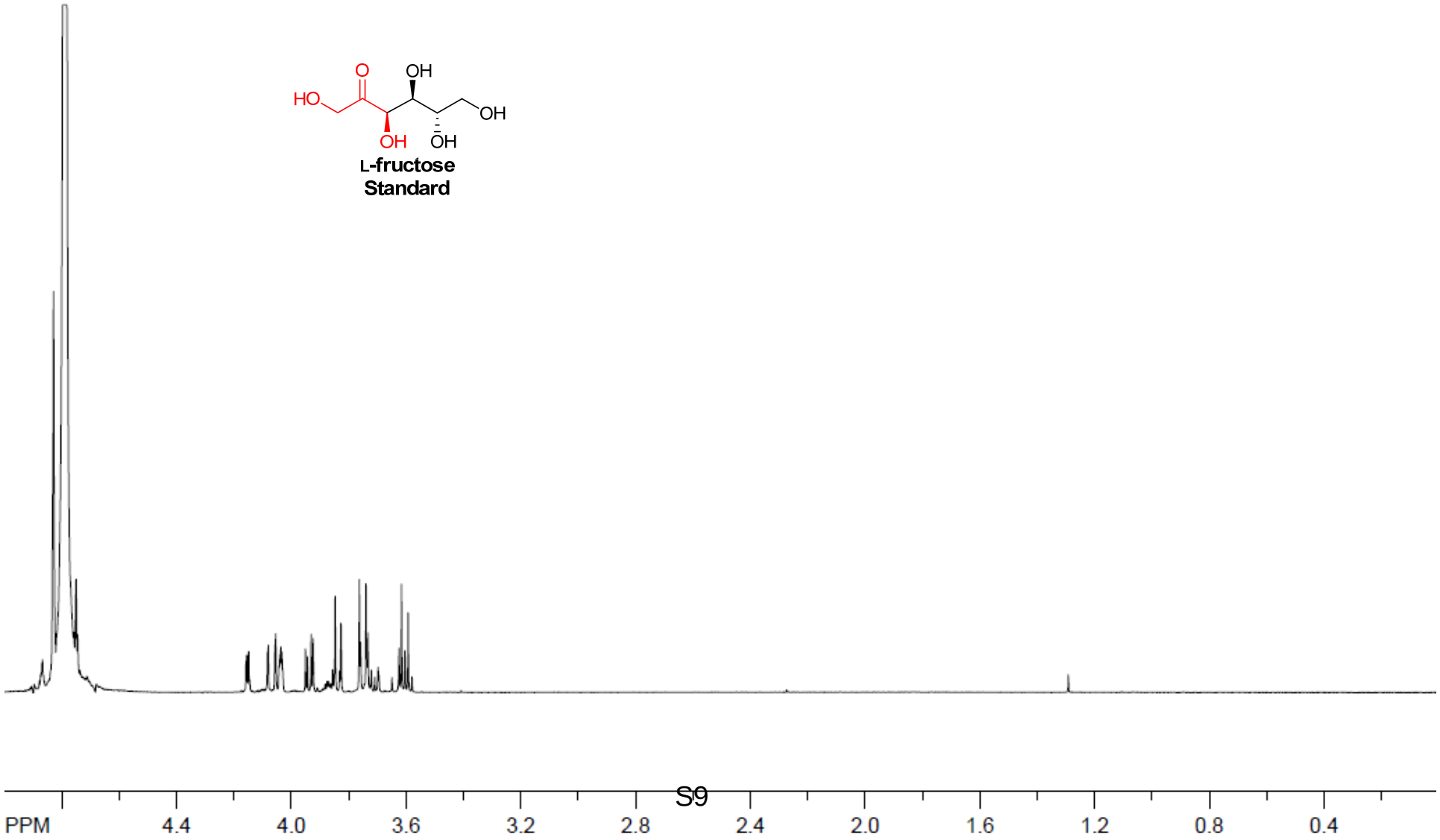


D-psicose
from FucA reaction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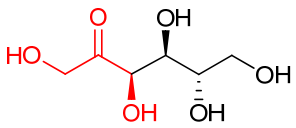
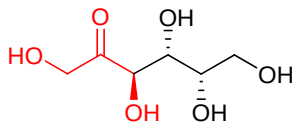
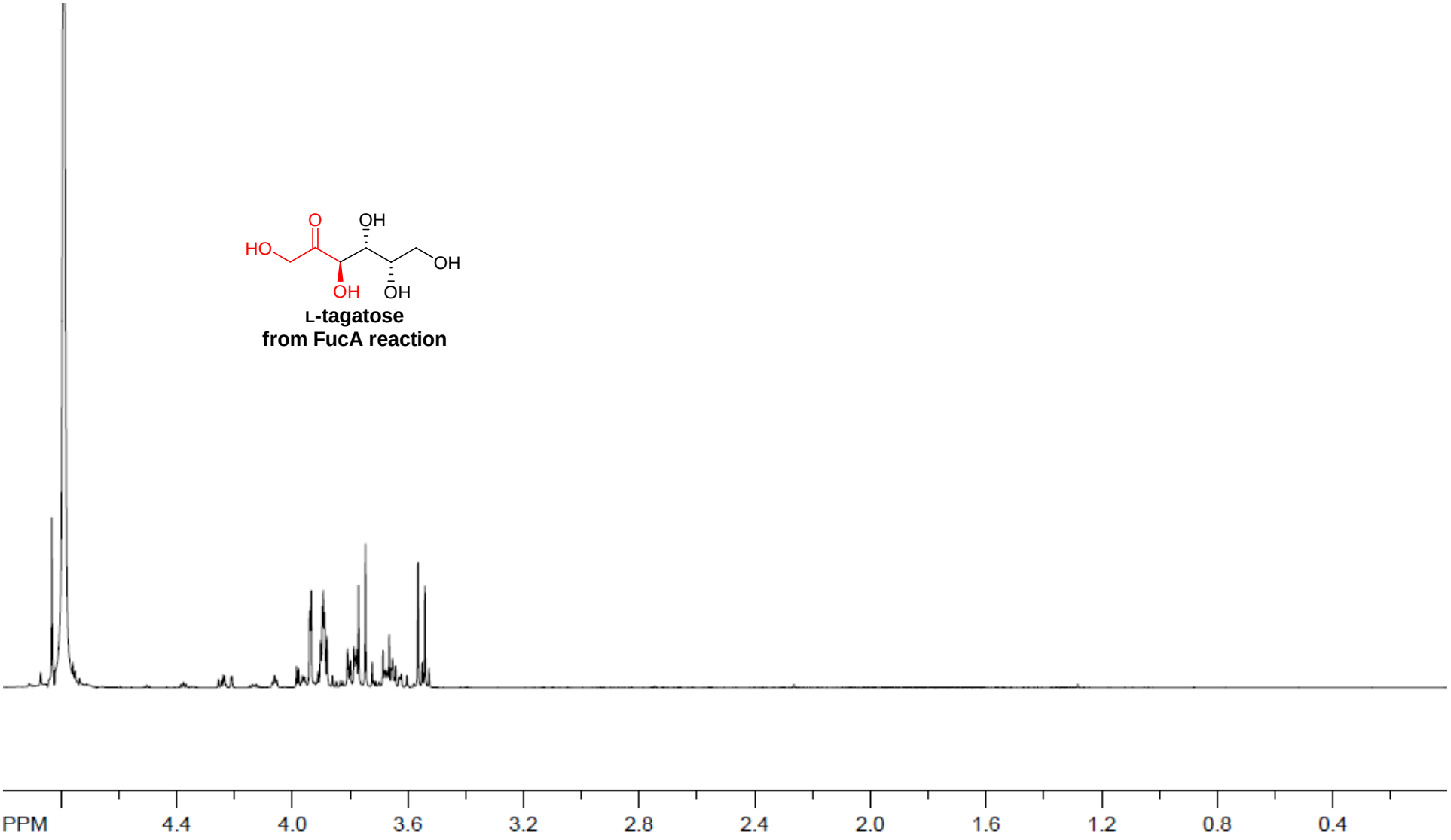


L-fructose
from FucA reaction



L-tagatose standard



L-tagatose
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