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Supporting Information

Biocatalytic Production of Amino Carbohydrates through Oxidoreductase and Transaminase Cascades

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1. Synthesis of 6-Amino-6-deoxy-D-galactopyranose trifluoroacetate salt

Chemical shifts are reported in parts per million (ppm), calibrated using residual non-deuterated solvent as internal reference [$CHCl_3$ at δ = 7.26 ppm (^{1}H NMR) and δ = 77.16 ppm (^{13}C NMR) or H_2O at δ = 4.79 ppm (^{1}H NMR)]. Infrared spectra were recorded on a Bruker ALPHA Eco-ATR spectrometer, absorption bands are reported in wave numbers [cm^{-1}].

1) Starting material for synthesis: 1,2,3,4-Di-*O*-isopropylidene-α-D-galactopyranose

2) Synthesis intermediate: 1,2,3,4-Di-*O*-isopropylidene-α-D-galactohexodialdo-1,5-pyranose

^{1}H NMR (400 MHz, $CHCl_3$): δ [ppm] = 9.60 (d, ^3J = 0.5 Hz, 1H), 5.65 (d, ^3J = 4.9 Hz, 1H), 4.63 (dd, ^3J = 7.8 Hz, ^3J = 2.5 Hz, 1H), 4.58 (dd, ^3J = 7.8 Hz, ^3J= 2.2 Hz, 1H), 4.37 (dd, ^3J = 4.9 Hz, ^3J = 2.5 Hz, 1H), 4.17 (d, ^3J = 2.2 Hz, 1H), 1.49 (s, 3H), 1.42 (s, 3 H), 1.33 (s, 3H), 1.30 (s, 3H).

^{13}C NMR (100 MHz, $CHCl_3$): δ [ppm] = 200.4, 110.1, 109.2, 96.4, 73.3, 71.8, 70.6, 70.5, 26.1, 25.9, 24.9, 24.4.

3) Synthesis intermediate: 6-Amino-6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose

^{1}H NMR (400 MHz, $CHCl_3$): δ [ppm] = 5.53 (d, ^3J = 5.1 Hz, 1 H), 4.58 (dd, ^3J = 7.9 Hz, ^3J = 2.3 Hz, 1 H), 4.29 (dd, ^3J = 5.1 Hz, ^3J = 2.3 Hz, 1 H), 4.21 (dd, ^3J = 7.9 Hz, ^3J = 1.8 Hz, 1 H), 3.70 (ddd, ^3J = 7.9 Hz, ^3J = 4.5 Hz, ^4J = 1.8 Hz, 1 H), 2.95 (dd, ^2J = 13.2 Hz, ^3J = 7.9 Hz, 1 H), 2.85 (dd, ^2J = 13.2 Hz, ^3J = 4.5 Hz, 1 H), 2.33 (br s, 2 H), 1.51 (s, 3 H), 1.43 (s, 3 H), 1.31 (s, 6 H).

^{13}C NMR (100 MHz, $CHCl_3$): δ [ppm] = 109.3, 108.6, 96.5, 71.9, 70.9, 70.7, 69.2, 42.3, 26.2, 26.1, 25.0, 24.5.

4) Synthesis product: 6-Amino-6-deoxy-D-galactopyranose trifluoroacetate salt

^{1}H NMR (400 MHz, $CHCl_3$): major isomer: δ [ppm] = 4.72 (d, ^3J = 7.2 Hz, 1 H), 4.17 (d, ^3J = 7.8 Hz, 1 H), 3.65-3.68 (m, 1 H), 3.39-3.43 (m, 1 H), 3.21-3.24 (m, 1 H), 3.09 (dd, ^3J = 9.9 Hz,^3J = 7.8 Hz, 1 H), 3.00 (d, ^3J = 7.2 Hz, 1 H); minor isomer (selected signals): δ [ppm] = 4.70 (d, ^3J = 5.1 Hz, 1 H), 3.71-3.73 (m, 1 H).

2. Supplemental figures and tables

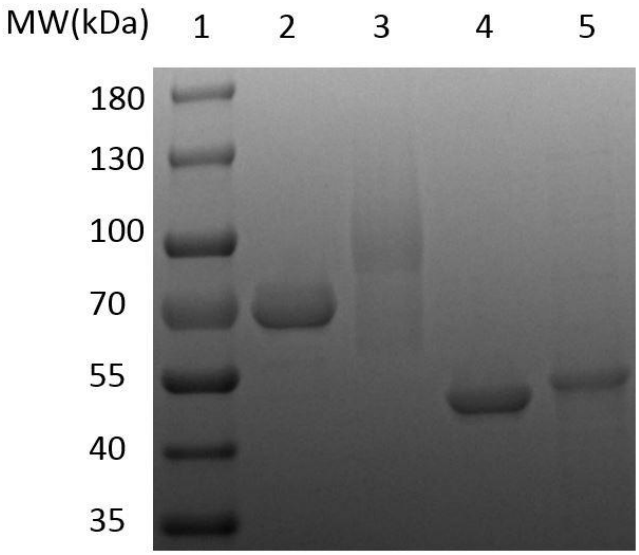


Figure S1. A 4-20 % gradient SDS-PAGE gel showing the purity of enzymes used in this study. 1) molecular weight ladder, 2) *Fgr*GaOx, 3) *Abi*PDH1, 4) *Cvi*-ω-TA and 5) *Vfl*-ω-TA. Approximately 5 μg of protein was loaded per well. The smearing seen in lane 2 was confirmed to result from glycosylation of *Ab*PDH1 (data not shown).

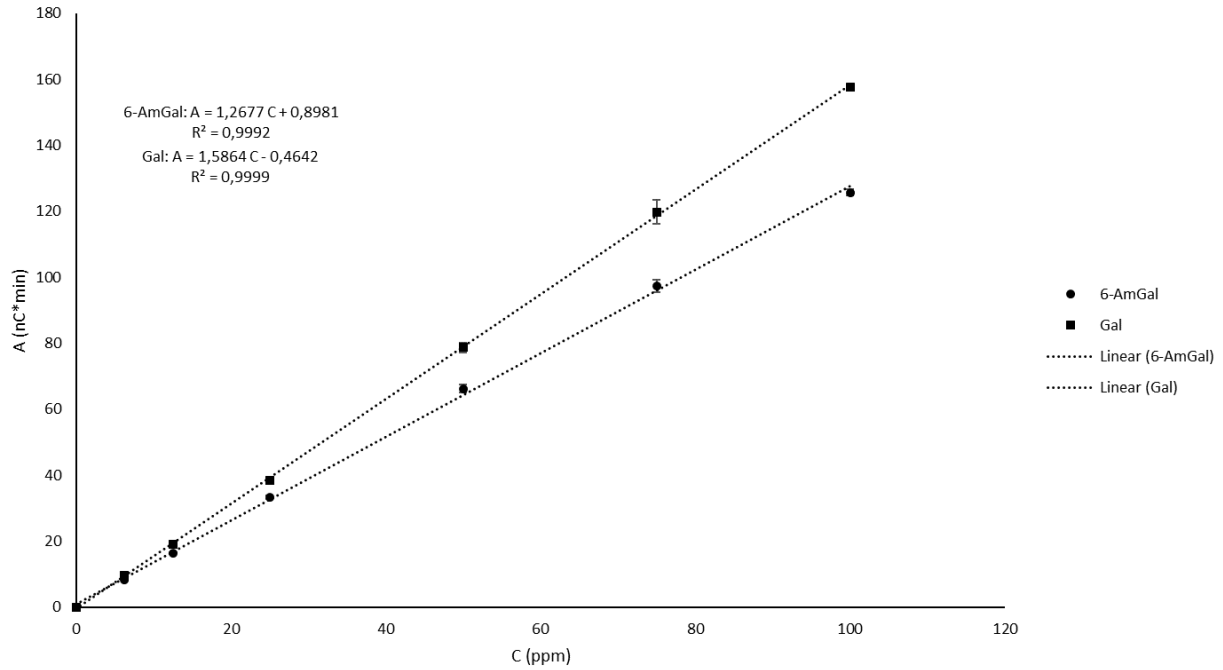


Figure S2. Calibration curves obtained by HPAEC-PAD for galactose and 6-amino-6-deoxygalactose, which were used for quantification of reaction conversions. Standards were prepared in triplicate, using 6.25, 12.5, 25, 50, 75 and 100 ppm of each compound. Error bars indicate standard deviation.

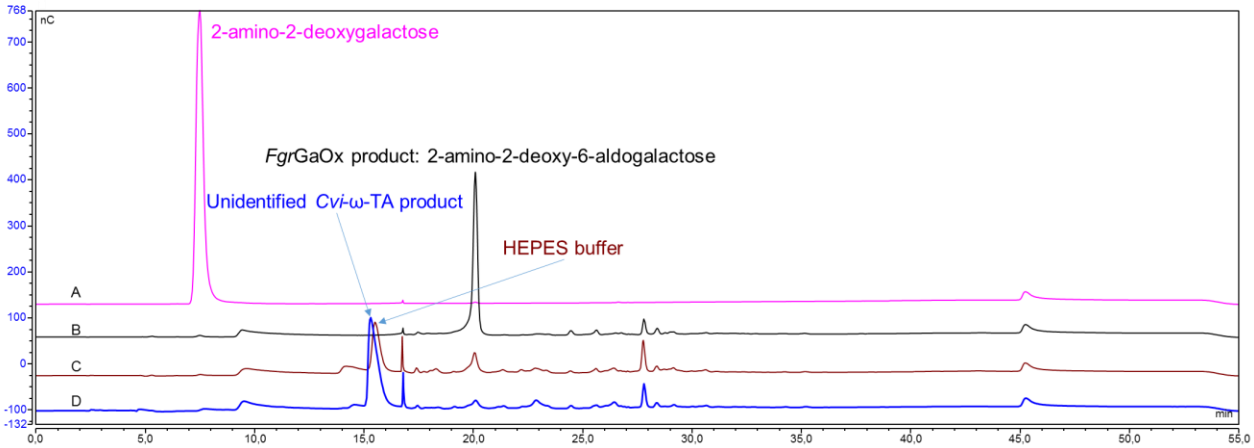
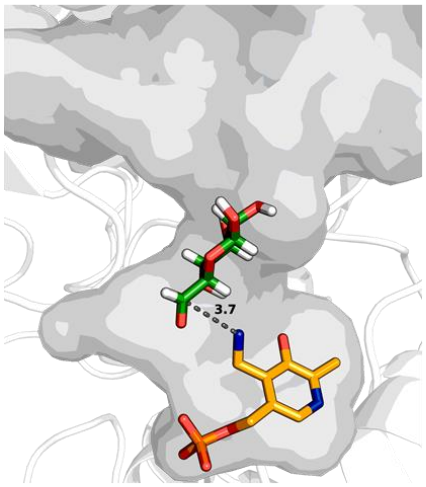
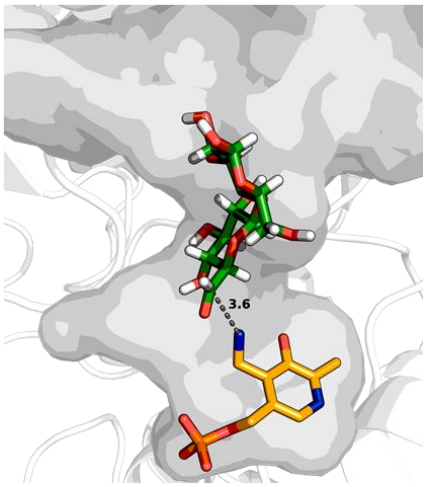


Figure S3. Staggered HPAEC-PAD chromatograms showing: A: 2-amino-2-deoxygalactose (pink); B: the product of oxidation of 2-amino-2-deoxygalactose with *Fgr*GaOx (black); C: the control reaction with the same conditions as the amination reaction but without the addition of *Cvi*-ω-TA, and D: the product of the *Cvi*-ω-TA catalyzed reaction (Unidentified product, blue). Reaction conditions were identical to the ones used for the oxidation and transamination reactions of galactose. Each reaction and analysis was conducted in triplicate at minimum. Single, representative chromatograms are shown due to negligible deviation in retention times and peak areas between replicates.

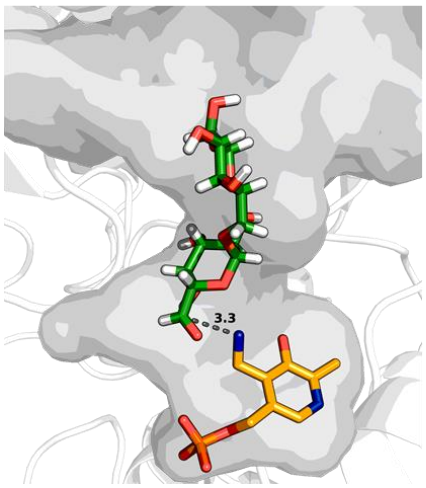
A



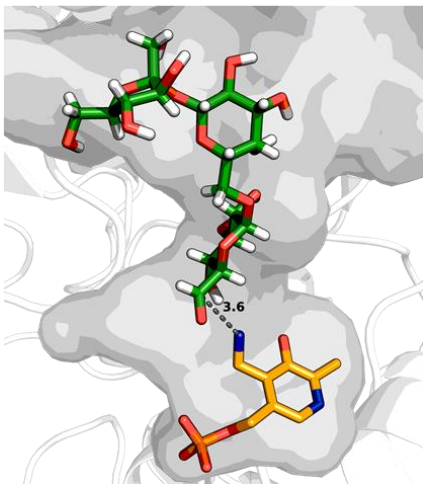
B



C



D



E

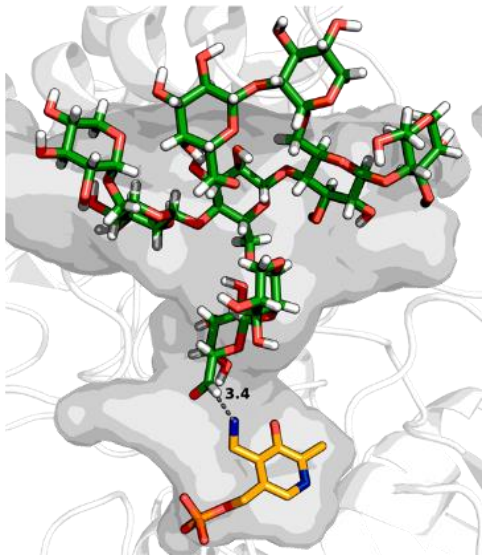


Fig S4. Docking results of the Cvi-ω-TA with 6-aldol-galactose (A), aldol-lactose (B), aldol-melibiose (C), aldol-raffinose (D), and aldol-xyloglucan oligosaccharides. The enzyme's back-bone is rendered as a white cartoon, the PMP cofactor (hydrogens not shown) is displayed in orange, the saccharides in green and the surface of the cavity of the active site and tunnel in gray. The distance in Ångström [Å] between the exocyclic nitrogen atom of PMP and the oxygen atom in the carbonyl group of the aldehyde is displayed as a dashed line. Images were created using PyMOL (The PyMOL Molecular Graphics System, Version 2.2.0 Schrödinger, LLC). In-line with the experimental results, the oligosaccharide substrates yielded plausible docking conformations with only the disaccharide branch entering the active site tunnel of the enzyme.

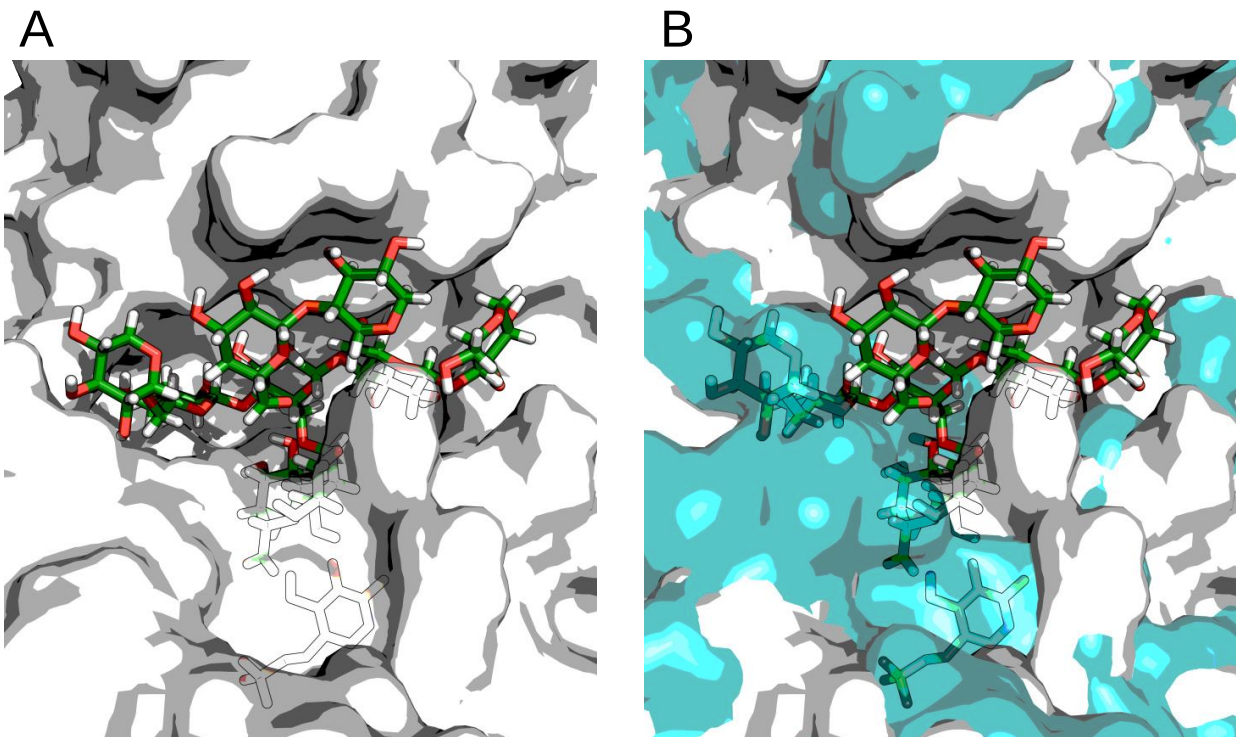


Figure S5. Visualization of the protein surface to assess the entrance tunnel to the active site of the *Cvi*-ω-TA (A) and a comparison between *Cvi*-ω-TA (white) and the crystal structure (PDB ID: 4E3Q, blue) of *Vfl*-ω-TA (B) with the docked aldol-XLLG substrate. Images were created using PyMOL (The PyMOL Molecular Graphics System, Version 2.2.0 Schrödinger, LLC).

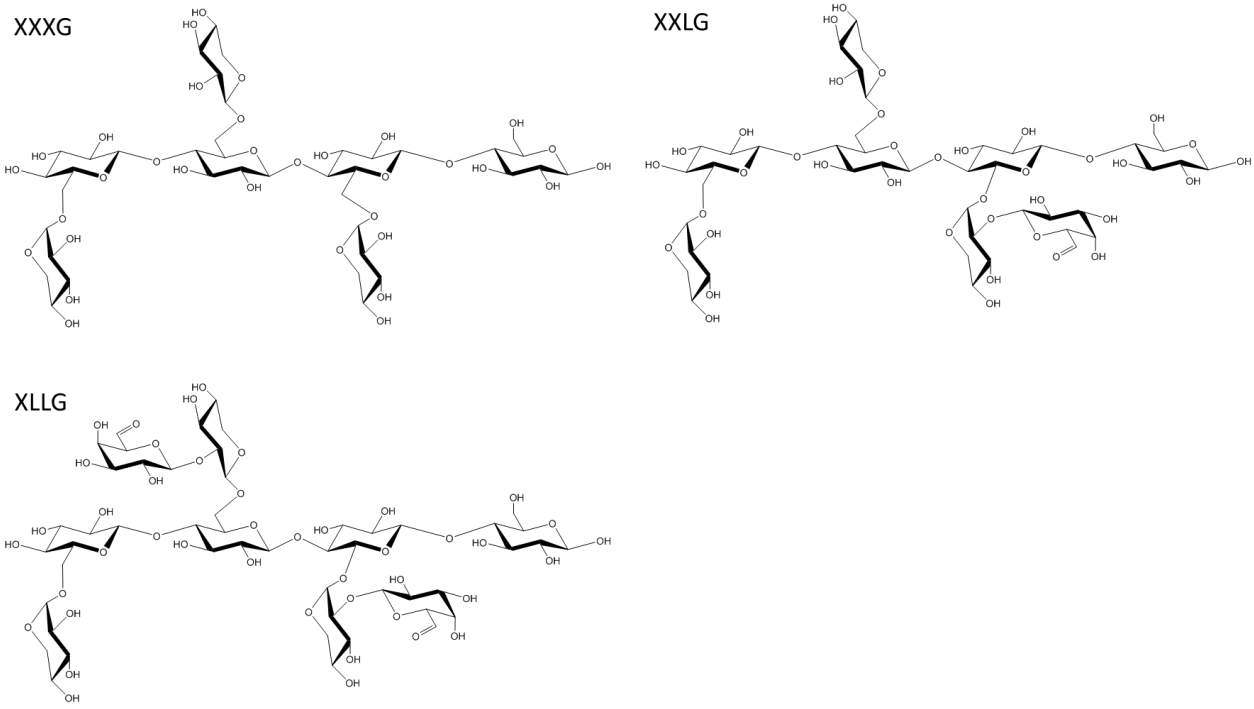
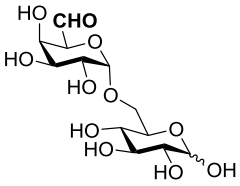
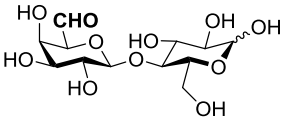
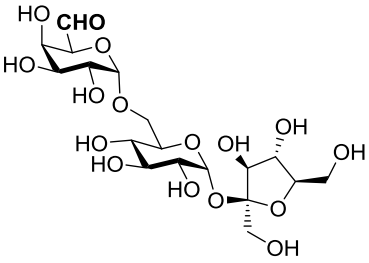
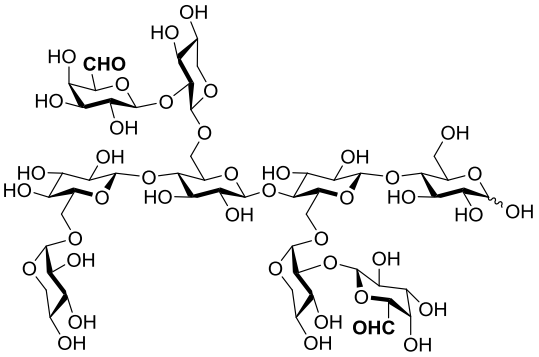


Figure S6. Structures of *Fgr*GaOx-oxidized xyloglucan hepta, octa and nonasaccharides (XXXG, XXLG and XLLG).

Table S1. Oxidized oligosaccharides generated using *Fgr*GaOx

Substrate	Structure
Aldo-Melibiose (**3b)**	
Aldo-Lactose (**4b)**	
Aldo-Raffinose **(5b)**	
Oxidized Xyloglucan oligosaccharides[a] **(6b)**	

[a]Only the structure of XLLG is depicted. Other xyloglucan oligosaccharides included in the oligosaccharide mixture were XXXG and XXLG (Figure S6)

Table S2. Energies from the log files of the run_ensemble.mcr docking macro. Additionally, the obtained distances in Ångström [Å] between PMP's exocyclic nitrogen atom and the carbonyl atom of the aldehyde are presented.

Ligand	**Binding energy [kcal mol^{-1}]**	**Dissociation constant [pM]**	**Distance [Å]**
Aldol-galactose	5.1340	172475776.0000	3.7
Aldol-lactose	5.9360	44550676.0000	3.6
Aldol-raffinose	8.0540	1248359.6250	3.6
Aldol-melibiose	8.6260	475398.8750	3.3
Aldol-XLLG	8.6260	475398.8750	3.4