Supplementary Information

Enzymatic synthesis of novel fructosylated compounds by Ffase from *Schwanniomyces occidentalis* in green solvents

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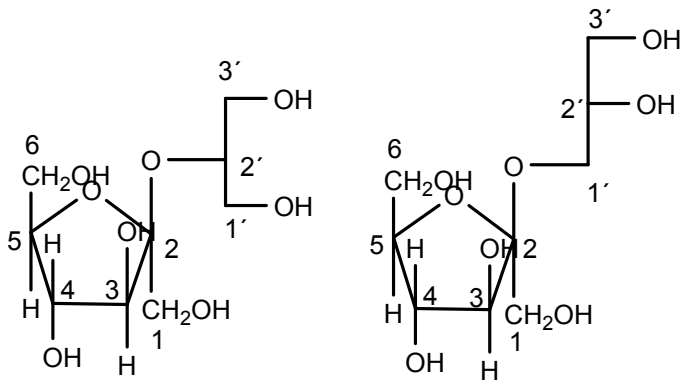
Table S1. Physical-chemical properties of the synthesized solvents considered for this study.

Solvent name	Mw (g/mol)	Density (g/mL)	LogP	Aqueous mixture behavior
GCL1	104.10	1.16	-0.97	Miscible
GCL2	118.09	1.37	-1.64	Miscible
GCL3	132.16	1.03	0.06	Miscible
GCL4	106.12	1.11*	-1.30	Miscible
GCL5	120.15	1.00*	-0.53	Miscible
GCL6	148.20	0.95*	0.35	Miscible
GCL7	162.23	0.95*	0.70	Miscible
GCL8	134.17	0.92*	-0.45	Miscible
GCL9	120.15	1.06*	-0.77	Miscible
GCL10	148.20	0.95*	0.54	Miscible
GCL11	134.17	1.02*	-0.23	Miscible
GCL12	204.31	0.91*	2.66	Floating immiscible
GCL13	204.31	0.93*	2.48	Floating immiscible
GCL14	218.33	0.89*	2.74	Floating immiscible
GCL15	174.12	1.36*	-0.52	Miscible
GCL16	216.20	1.35*	1.12	Sinking immiscible
GCL17	256.14	1.39*	1.02	Sinking immiscible
GCL18	270.17	1.30*	1.10	Sinking immiscible
GCL19	356.16	1.47*	3.64	Sinking immiscible
GCL20	456.17	1.56*	5.44	Sinking immiscible
DMA1	117.15	1.03	-1.20	Miscible
DMA2	149.19	1.03	0.44	Floating immiscible
DMA3	143.23	0.87	1.38	Floating immiscible
DMA4	171.28	0.87	2.44	Floating immiscible
DMA5	199.33	0.86	3.50	Floating immiscible
DMA6	227.39	0.86	4.57	Floating immiscible

Molecular weight (Mw), density, and octanol-water partition coefficient (LogP) values of different solvents were predicted on the basis of their chemical structure using the PhysChem Module of ACD/Labs Inc. (Toronto, Canada). Those density values marked with an asterisk were empirically determined using a DSA-5000 M vibrating tube densimeter (Anton Paar GmbH; Graz, Austria) under standard ambient conditions. The miscibility of each solvent in pure water was experimentally established by simple observation of the resultant mixture at room temperature.

Table S2. NMR (500 MHz, D_2O, δ in ppm) data of compounds 1 and 2.



	1		2	
Nº	**C-13**	**H-1**	**C-13**	**H-1**
1	62.47[a]	A: 3.70 (d, *J* = 10.0 Hz) B: 3.63 (d, *J* =10.0 Hz)	62.49[b]	A: 3.70 (d, *J* = 10.0 Hz) B: 3.63 (d, *J* =10.0 Hz)
2	103.53	-	103.47	-
3	76.84	4.13 (d, *J* = 10.0 Hz)	76.84	4.13 (d, *J* = 10.0 Hz)
4	74.59	4.06 (t, *J* = 10.0 Hz)	74.52	4.06 (t, *J* = 10.0 Hz)
5	81.08	3.85 - 3.75 (m)	81.03	3.85 - 3.75 (m)
6	62.44[a]	3.65 - 3.60 (m)	62.38[b]	3.65 - 3.60 (m)
1´	62.34[a]	3.50 - 3.45 (m)	70.82	3.70 - 3.60 (m)
2´	70.93	3.85 - 3.75 (m)	70.75	3.85 - 3.75 (m)
3´	62.34[a]	3.50 - 3.45 (m)	62.32[b]	3.65 - 3.55 (m)

[a, b] : interchanged.

Table S3. Commercial solvents, media and chemical compounds used throughout this work and suppliers.

Beneo Ibérica S.L. (Barcelona, Spain)		
Inulin extract (chicory root, Orafti®)		
Fisher Scientific Ltd. (Loughborough, UK)		
Acetonitrile (anhydrous)	Agar (granulated, BD Difco™)	Dimethyl sulfoxide [DMSO]
Ethanol [EtOH] (absolute)	*n*-Hexane	Methanol [MeOH]
Peptone (bacteriological, BD Difco™)	Yeast extract [YE] (BD Difco™)	Yeast nitrogen base without amino acids [YNB w/o AA] (BD Difco™)
Merck KGaA (Darmstadt, Germany)		
Ammonia [NH_3] 25% (aqueous)	1-Butyl-3-methylimidazolium tetrafluoroborate [BMIM-BF_4]	1-Butyl-3-methylimidazolium tris(penta fluoroethyl)trifluorophosphate [BMIM-FAP]
1-Butyl-3-methylimidazolium methylsulfate [BMIM-$MeSO_4$]	1-Butyl-3-methylimidazolium hexafluorophosphate [BMIM-PF_6]	1-Butyl-3-methylimidazolium bis (trifluoromethylsulfonyl)imide [BMIM-Tf_2N]
Caprylic acid	1-Ethyl-3-methylimidazolium tetrafluoroborate [EMIM-BF_4]	1-Ethyl-3-methylimidazolium methylsulfate [EMIM-$MeSO_4$]
D-Fructose	D-Galactose	D-Glucose
1-Hexyl-3-methylimidazolium bis (trifluoromethylsulfonyl)imide [HMIM-Tf_2N]	Hydrochloric acid [HCl] 37% (fuming)	β-Mercaptoethanol
1-Octyl-3-methylimidazolium hexafluorophosphate [OMIM-PF_6]	Propylene glycol	Sucrose
Sigma-Aldrich Corp. (St. Louis, USA)		
Acetic acid (glacial)	*t*-Butanol	Diethylene glycol dimethyl ether [Diglyme]
Dinitrosalicylic acid [DNS]	1-Ethyl-3-methylimidazolium chloride [EMIM-Cl]	Ethyl valerate
Ethylene glycol	Glycerol [GCL]	L-Glycine
L-Histidine	L-Leucine	L-Methionine
Polyethylene glycol 600 [PEG 600]	2-Propanol	Sodium acetate (anhydrous)
Sodium dodecyl sulfate [SDS]	Sorbitan trioleate (Span®)	Sulfuric acid [H_2SO_4]
Tetrahydrofuran [THF]	Trioctylmethylammonium bis(trifluoro methylsulfonyl)imide [TROMA-Tf_2N]	Tris(hydroxymethyl)aminomethane [Tris] (Trizma®)
γ-Valerolactone	Tributyrin	Vynil laurate
TCI-Europe N.V. (Zwijndrecht, Belgium)		
Bromophenol blue	1-Kestose	Nystose

Highest available purity was employed.

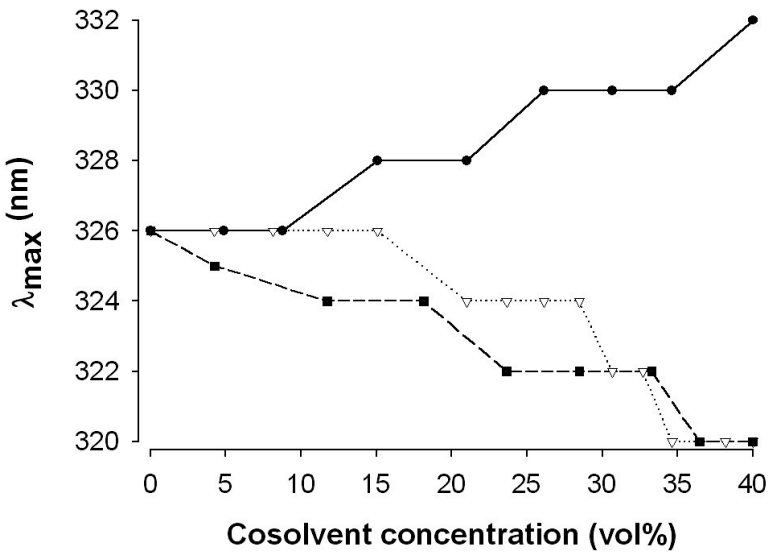


Figure S1. Maximum emission intensity of Ffase after excitation at 295 nm. Fluorescence values obtained with solvents GCL5 (-●-), GCL12 (-▽-), and TROMA-Tf_2N (-■-) are presented. Excitation wavelength was selected for matching with the maximum protein absorption due primarily to tryptophan aromatic amino acids. Emission spectra with 0.1 s integration time were recorded from 310 to 500 nm in quartz Hellma® cuvettes of 2-mm path length through QuantaMaster 40™ (Photon Technology International Inc.; Birmingham, UK) maintained at 25 °C. Emission and excitation slits were kept constant at 4 and 2 nm, respectively. Pure Ffase (0.18 mg/mL) in 0.2 M sodium acetate buffer pH 5.5 was employed and successive aliquots of each particular solvent (>99% purity) transferred while maintaining the enzymatic concentration. Tryptophan content of Ffase is around 2.2% what is within an acceptable range for measurement conditions. Readings were performed twice and averaged.

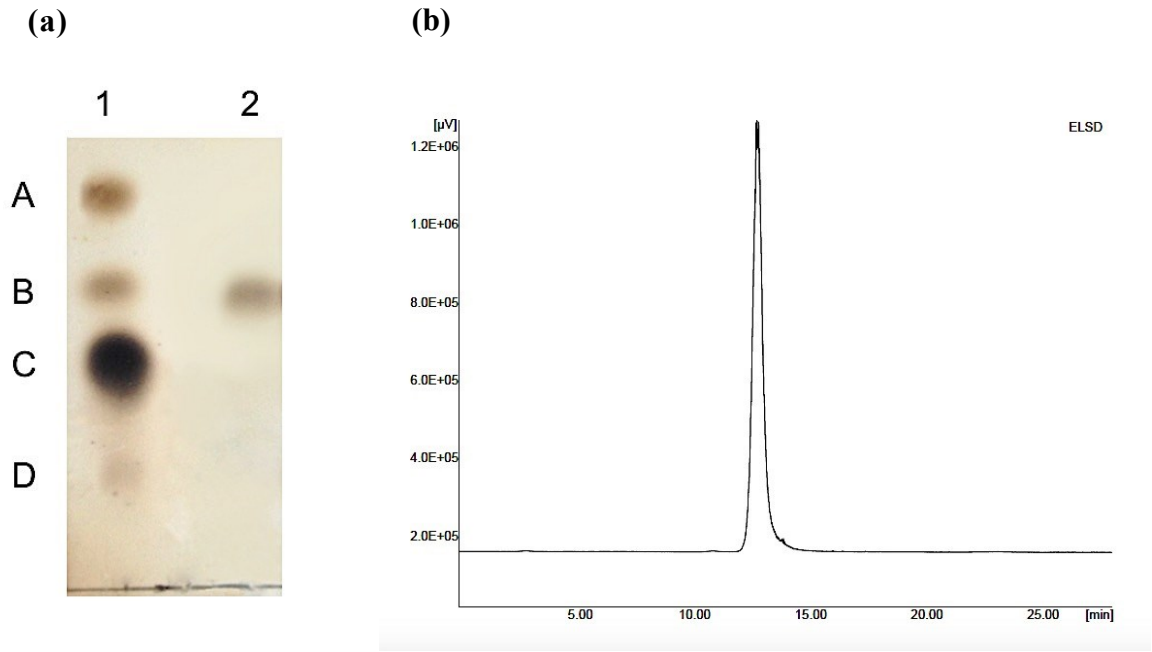


Figure S2. TLC (a) and HPLC (b) analysis of the purified sample containing the new glycerol-acceptor transfer product. (a) Lane 1: Reaction of 10 U/mL Ffase with 400 g/L sucrose and 400 g/L glycerol. Lane 2: Purified sample. References: Glycerol (A); fructo-conjugate from glycerol (B); fructose, glucose and sucrose (C); 1-kestose (D). (b) Chromatogram of the purified compound.

(a)

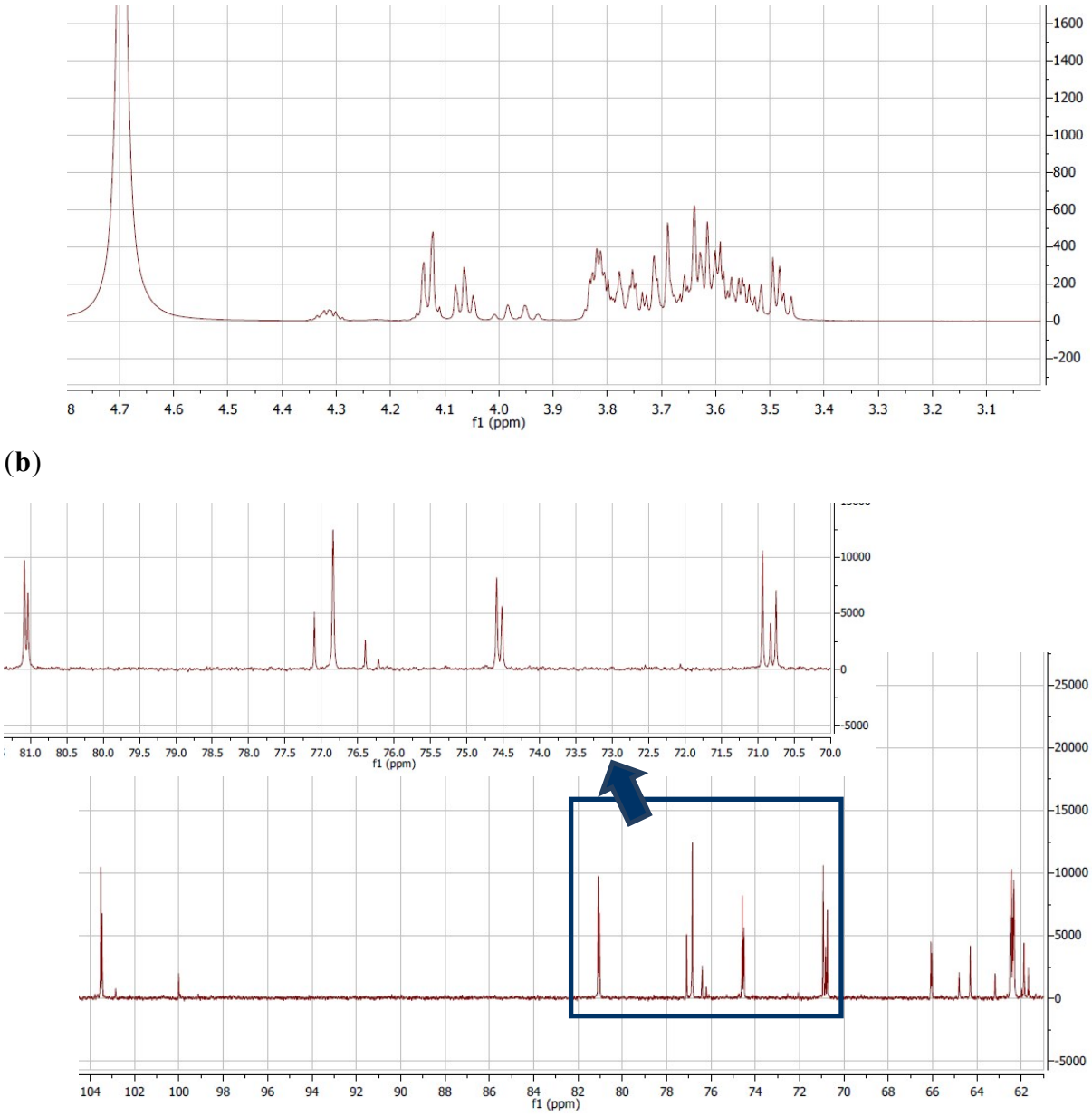


Figure S3. NMR spectra of **1** and **2** (a) ^{1}H NMR and (b) ^{13}C NMR).

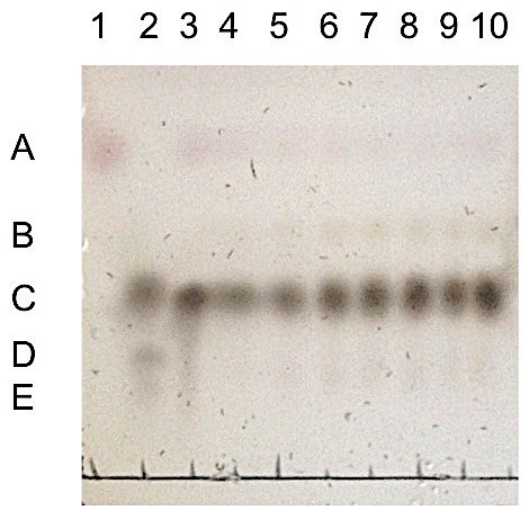


Figure S4. TLC monitoring of the progression of the glycerol transfructosylating reaction. Lanes 1-2: Standards. Lanes 3-10: Reaction of 10 U/mL Ffase with 200 g/L sucrose and 200 g/L glycerol after, respectively, 0, 60, 90, 120, 150, 180, 240 and 300 min. References: Glycerol (A); fructo-conjugate from glycerol (B); fructose, glucose and sucrose (C); 1-kestose (D); nystose (E). The obtained fructo-conjugate reached the maximum after 120 min of reaction (Lane 6), and then it remained apparently stable (Lanes 7-10).

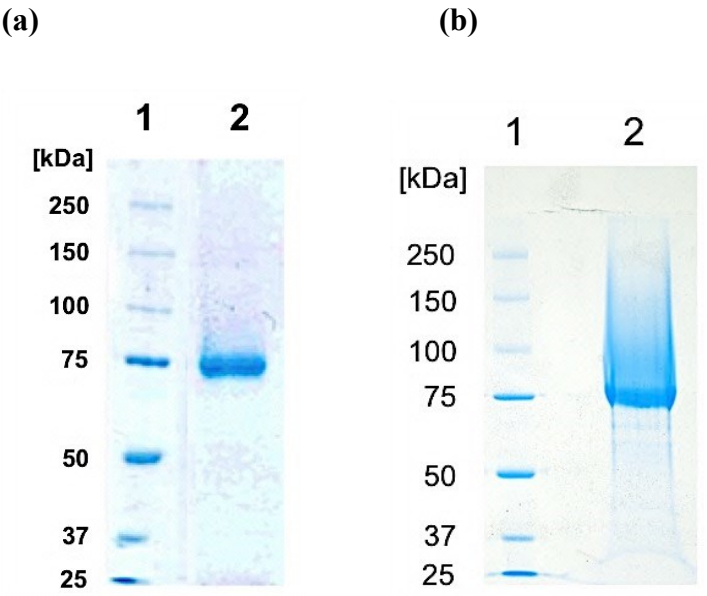


Figure S5. SDS-PAGE analysis of the protein purity of Ffase (a) and Ffase-Leu196 (b) variants. Lane 1: Precision Plus Protein™ 25-250 kDa unstained standards was used for molecular weight estimations. Lane 2: 0.5 µl of purified enzyme. Numbers at the left indicate the positions of molecular mass standards in kDa.