Biocatalysis

International Edition: DOI: 10.1002/anie.201814219
German Edition: DOI: 10.1002/ange.201814219

One-Pot Bioconversion of L-Arabinose to L-Ribulose in an Enzymatic Cascade

*Litavadee Chuaboon, Thanyaporn Wongnate, Pangrum Punthong, Cholpisit Kiattisewee, Narin Lawan, Chia-Yi Hsu, Chun-Hung Lin, Uwe T. Bornscheuer, and Pimchai Chaiyen**

Abstract: *This work reports the one-pot enzymatic cascade that completely converts* L*-arabinose to* L*-ribulose using four reactions catalyzed by pyranose 2-oxidase (P2O), xylose reductase, formate dehydrogenase, and catalase. As wild-type P2O is specific for the oxidation of six-carbon sugars, a pool of P2O variants was generated based on rational design to change the specificity of the enzyme towards the oxidation of* L*-arabinose at the C2-position. The variant T169G was identified as the best candidate, and this had an approximately 40-fold higher rate constant for the flavin reduction (sugar oxidation) step, as compared to the wild-type enzyme. Computational calculations using quantum mechanics/molecular mechanics (QM/MM) molecular dynamics (MD) showed that this improvement is due to a decrease in the steric effects at the axial C4-OH of* L*-arabinose, which allows a reduction in the distance between the C2-H and flavin N5, facilitating hydride transfer and enabling flavin reduction.*

Conversion of common sugars to valuable compounds, such as rare sugars, drug components, and valuable materials is one of the key challenging topics in the field of sugar biocatalysis.[1] This technology is also instrumental to the growth of the worldwide bio-economy as it would allow common sugars, such as D-glucose, D-galactose, D-mannose, D-xylose, and L-arabinose, to be derived from abundant lignocellulosic biomass.[2] An enzyme-based approach is especially attractive for sugar conversion because enzymes display unique regio- and stereo-specific oxidation and modification of alcohol and aldehyde moieties that are common in all sugar structures.[3] Oxidations of aldoses by biocatalytic reactions are environmentally friendly and sustainable processes due to their avoidance of the use of toxic chemicals. Furthermore, their lack of a need for protecting/deprotecting steps, and the ability to perform the production processes using whole-cell platforms makes them highly valuable.[4]

The production of L-ribulose from a common sugar by biocatalytic reactions exemplifies the power of this technology and its potential contribution to the development of the bio-economy. L-Ribulose is commonly used as a precursor for the synthesis of L-nucleoside analogues, which are used as antiviral and anticancer drugs.[5] The chemical synthesis of ribulose using base-catalyzed isomerization and keto-aldol tautomerization of aldoses requires many steps and laborious purification.[6] Ribitol can also be converted to L-ribulose using enzymes present in *Acetobacter aceti* IFO 3281[7] and *G. oxydans*[8] to catalyze oxidation and dehydrogenation. However, this method is not economically viable because ribitol is a rare and expensive sugar. A more attractive route for increasing economic value is to synthesize L-ribulose (995 US$ g^{-1}) from L-arabinose (0.1 US$ g^{-1}). In the past, L-ribulose was synthesized from L-arabinose using L-arabinose isomerase (BLAI).[9] However, the uni-molecular reaction of L-arabinose isomerase cannot completely convert L-ribulose because its equilibrium is favored towards the formation of L-arabinose (90:10 of L-arabinose:L-ribulose at equilibrium).[9a] Recently, an improved process to achieve high yield L-ribulose production from L-arabinose was reported using a combination of enzymatic reactions of L-arabinose isomerase from *Thermotoga maritima* MSB8 and fructokinase (HK) from humans paired with a precipitation process using silver nitrate to push the equilibrium towards the production of L-ribulose.[10] As this process still involves the use of hazardous silver nitrate, it remains a longstanding challenge to develop an efficient biocatalytic process for the production of L-ribulose from L-arabinose.

Herein we report a new biocatalytic route to convert L-arabinose to L-ribulose using a combination of two bimolecular enzymatic reactions, the thermodynamic favorability of which can be manipulated by controlling the concentrations of the other reactants. The first step requires a regio-specific oxidation of L-arabinose at the C2-position to generate 2-keto-arabinose, while the second step requires the specific reduction of a terminal aldehyde. As the second reaction can be achieved by using reductases that are known to specifically reduce the aldehyde functionality, the key challenge to making this new biocatalytic route successful is to construct

[*] L. Chuaboon, Prof. Dr. P. Chaiyen
Department of Biochemistry and Center for Excellence in Protein and Enzyme Technology, Faculty of Science, Mahidol University
Bangkok 10400 (Thailand)
E-mail: pimchai.chaiyen@vistec.ac.th
Dr. T. Wongnate, P. Punthong, C. Kiattisewee, Prof. Dr. P. Chaiyen
School of Biomolecular Science & Engineering, Vidyasirimedhi Institute of Science and Technology (VISTEC)
Wangchan Valley, Rayong 21210 (Thailand)
Dr. N. Lawan
Department of Chemistry, Faculty of Science, Chiang Mai University
Chiang Mai 50200 (Thailand)
C. Hsu, Prof. Dr. C. Lin
Institute of Biological Chemistry, Academia Sinica
Taipei 11529 (Taiwan)
Prof. Dr. U. T. Bornscheuer
Institute of Biochemistry, Department of Biotechnology and Enzyme Catalysis, Greifswald University
Felix-Hausdorff-Strasse 4, Greifswald (Germany)
Supporting information and the ORCID identification number(s) for the author(s) of this article can be found under: https://doi.org/10.1002/anie.201814219.

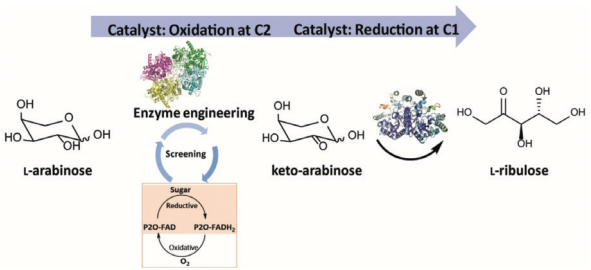


Figure 1. Biocatalytic approach for converting L-arabinose to L-ribulose.

a sugar oxidase that is specific for the oxidation of L-arabinose at the C2-position (Figure 1).

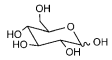
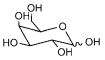
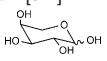
We decided to engineer pyranose 2-oxidase (P2O) as a key biocatalyst for 2-keto-arabinose production. P2O is the flavoenzyme that catalyzes the oxidation of aldopyranose sugars using oxygen to generate a keto-sugar and H_2O_2 as products.[11]

The P2O reaction is particularly useful for biocatalysis because the supply of oxygen can be increased to drive the reaction forward without creating difficulties in product isolation. Furthermore, the by-product of this reaction (H_2O_2) can be easily removed by adding catalase to generate oxygen and water. Previous studies have shown that the wild-type P2O (P2O(WT)) can use many hexoses as substrates with D-glucose being the best substrate.[11] However, the reactivity of the enzyme towards pentoses is rather low.[11]

Based on the knowledge of P2O structures and reaction mechanisms with D-glucose,[12] P2O was engineered to increase the enzyme specificity and reactivity toward L-arabinose by targeting residues in the active site (see Table S1 in the Supporting Information). The methodology described in the Supporting Information was used to screen for P2O variants enabling regio-specific oxidation of L-arabinose, the P2O variants shown in Table 1 were identified as potential candidates. To identify the variant that is the most active with L-arabinose, transient kinetics experiments were carried out by mixing the enzyme variants with various sugars in the absence of oxygen. This experiment allowed the measurement of the rate constants associated with the flavin reduction step. The results in Table 1 indicate that most of the mutants reacted poorly with L-arabinose; P2O(WT) has a reduction rate constant of only $0.05\ s^{-1}$, which is 236-fold lower compared to the native substrate D-glucose, while other variants have lower reduction rate constants than the wild-type enzyme for L-arabinose. The P2O(T169G) variant is the most active one for L-arabinose (reduction rate constant (k_{obs}) of $0.63\ s^{-1}$, 12.6 fold higher than the wild-type enzyme). Therefore, this variant was further investigated in a full cycle of transient kinetics to measure the rate constants associated with the individual steps to understand its biochemical properties and usefulness for biocatalysis.

Transient kinetics of P2O(T169G) with L-arabinose were investigated using a stopped-flow spectrophotometer (see section 4 in the Supporting Information for details). The reactions were monitored by absorption changes at 458 nm where the oxidized FAD absorbs the most. In brief, we observed a monophasic flavin reaction in which the observed

Table 1: Rate constants of enzyme-bound flavin reduction (using 50 mM sugar substrate at 4 °C, pH 7.0).

Enzymes	Observed rate constant of flavin reduction [s^{-1}]			
	 D-glucose	 D-galactose	 D-xylose	 L-arabinose
P2O(WT)	11.8	0.24	4.55	0.05
V546C	16.3 (1.4 fold)[a]	0.35 (1.5 fold)	3.63	0.04
V546P	15.4 (1.3 fold)	0.38 (1.6 fold)	3.35	0.03
V546S	7.36	0.15	2.31	0.01
V546A	7.67	0.18	3.34	0.02
H167A	0.25	0.0079	0.09	0.00083
C170A	12.5 (1.1 fold)	0.34 (1.4 fold)	5.86 (1.3 fold)	0.07 (1.4 fold)
T169G	0.52	2.13 (8.9 fold)	0.35	0.63 (12.6 fold)
Y456W	19.0 (1.6 fold)	0.26	3.81	0.04

[a] The numbers in bracket are the comparison of rate constants of the flavin reduction with the P2O(WT) when the rate constant of the mutant is higher.

rate constants are dependent on the L-arabinose concentrations in a hyperbolic manner (Figure 2A). These data are consistent with a model in which the first step is the binding of L-arabinose to form the T169G:L-arabinose complex which is then followed by flavin reduction (Figure 2C). Kinetic constants associated with the individual steps are shown in Figure 2C. These values were validated by kinetic simulations (Figure 2A). When a solution of P2O(T169G) that was reduced by L-arabinose was mixed with various concentrations of oxygen, a monophasic flavin oxidation was observed (Figure 2B). The data indicate that the flavin oxidation is a single step reaction, without formation of any intermediate (Figure 2C), as in the wild-type enzyme.[13] This is consistent with a bimolecular rate constant of $118\ M^{-1}s^{-1}$. The overall reaction of P2O(T169G) can be summarized as in Figure 2C.

The results of the P2O(WT) reaction with arabinose (Supporting Information, Figure S2 and Table 2) clearly indicate that P2O(WT) reacts with arabinose with a much lower rate constant for the flavin reduction step ($0.1\ s^{-1}$) than P20(T169G). As the flavin reduction step in P20(T169G) is $4\ s^{-1}$, the reactivity towards L-arabinose was increased by about 40-fold.

Two-substrate steady-state kinetics of P2O(T169G) with various L-arabinose and oxygen concentrations were performed. Results indicate that the catalytic efficiency (k_{cat}/K_m^{sugar}) of P2O(T169G) is about 13-fold greater than that of P2O(WT) (Table 2 and section 6 in the Supporting Information). These data are consistent with the stopped-flow results that indicate that the rate of sugar oxidation was increased.

Next, the production of the oxidized sugar resulting from the reaction of P2O(T169G) with L-arabinose was studied. The reaction was first carried out under anaerobic conditions to obtain the product under single turnover conditions (Supporting Information, Figure S4). The analysis by LC/MS displayed the MS expected for keto-arabinose at 15.4 min,

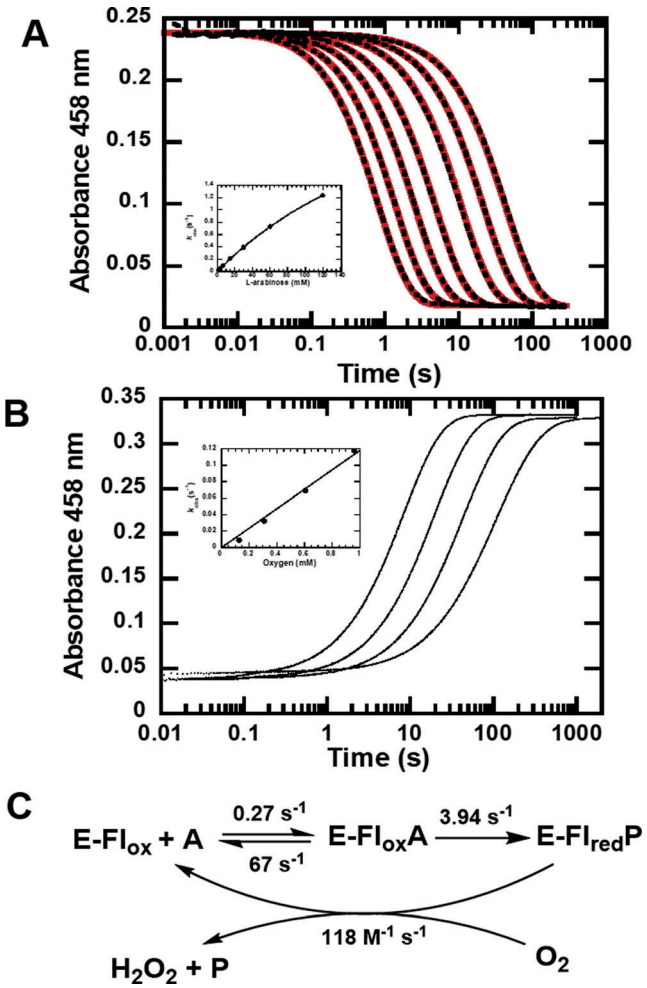


Figure 2. A) Kinetics of P2O(T169G) reduction of L-arabinose monitored at 458 nm using stopped-flow spectroscopy. A solution of oxidized P2O(T169G) (22 μM) was mixed with various concentrations of L-arabinose (1.6, 3.2, 6.4, 15, 30, 60, and 120 mM) in 50 mM sodium phosphate buffer, pH 7.0 at 4 °C. All concentrations are given after mixing. The inset shows a plot of k_{obs} of the flavin reduction (decreased absorbance 458 nm) versus L-arabinose concentration. The kinetic model and rate constant values in (C) were used for kinetic simulations using KinTek. Results of the simulations (red dotted lines) show excellent agreement with the raw kinetic data (black lines). B) Kinetics of the reduced P2O(T169G) reacting with various concentrations of oxygen. A solution of P2O(T169G) (50 μM) reduced by L-arabinose was mixed with solutions containing various concentrations of oxygen: 0.13, 0.31, 0.61, and 0.96 mM. The inset shows a plot of k_{obs} of the flavin oxidation (increased absorbance at 458 nm) versus oxygen concentrations. A bimolecular rate constant was calculated as 118 $M^{-1} s^{-1}$.

indicating that P2O(T169G) can oxidize L-arabinose to generate the desired keto-arabinose. Product analysis indicated that P2O(T169G) could produce keto-arabinose much faster than P2O(WT) (Figure 3A). Under the same conditions, P2O(T169G) completely consumed L-arabinose within 5 h (1.8 mM min^{-1}, much faster than the P2O(WT), which was not complete even after 8 h. We also noted that the reaction of P2O(WT) produced a much smaller amount of keto-arabinose and the concentration of this product also began to decrease after 150 min (Figure 3A) due to the formation of another product (Supporting Information, Figure S5). We determined that P2O, especially the wild-type enzyme, can further oxidize keto-arabinose at the C1-position to form 2-keto-arabinolactone, which can be further hydrated by water to generate 2-keto-arabinoic acid (Supporting Information, Scheme S2).

Therefore, with all the enzymatic properties investigated, P2O(T169G) is an efficient biocatalyst to generate 2-keto-arabinose which can be further reduced at the C1 aldehyde moiety to produce L-ribulose. This second reduction of the C1 aldehyde moiety can be catalyzed by aldose reductase. We selected a xylose reductase from *Hypocrea jecorina*[14] as a terminal aldehyde reductant. As the reaction of xylose reductase requires NADPH as a reductant, we coupled this 2-keto-arabinose reduction with the reaction of formate dehydrogenase to continuously generate NADPH for ribulose synthesis. As we found that production of keto-arabinose paused when the oxygen concentration was depleted, a constant concentration of O_2 was maintained by gentle shaking. The by-product H_2O_2 was removed by the addition of catalase.

A cascade of four enzymatic reactions was carried out in a one-pot reaction. Enzymes utilized included the P2O-(T169G) variant of P2O, xylose reductase, formate dehydrogenase, and catalase (Figure 3B). The results showed that this enzymatic cascade is very efficient, as it can catalyze 100% conversion of arabinose to ribulose (0.3 g L^{-1}) in less than 7 h (Figure 3C). Ribulose resulting from this reaction was easily purified, as the reaction did not generate any other sugar products and its identity was confirmed by LC-QTOF-MS analysis and NMR spectroscopy (Supporting Information, Figures S8 and S13). The final yield after purification was around 80%. This enzymatic cascade is also much more efficient than a chemo-enzymatic method in which the enzymatic reaction of P2O(T169G) with L-arabinose was combined with chemical reduction using $NaBH_4$ at 4 °C (Supporting Information, Figure S7 and Table S7). The

Table 2: Comparison of the reduction rate constants and steady state parameters from transient and steady state kinetics analysis of P2O(T169G) and P2O(WT) for reactions with L-arabinose and D-glucose.

Sugars	WT				T169G			
	k_{red} [s^{-1}]	K_m^{sugar} [mM]	k_{cat} [s^{-1}]	k_{cat}/K_m^{sugar} [$mM^{-1} s^{-1}$]	k_{red} [s^{-1}]	K_m^{sugar} [mM]	k_{cat} [s^{-1}]	k_{cat}/K_m^{sugar} [$mM^{-1} s^{-1}$]
Glucose[c]	15.3 ± 0.4[a] (15)[b]	1.13	9.7	8.6	0.7 ± 0.01[a] (ND)[b]	0.9	0.7	0.8
Arabinose	0.1 ± 0.0003[a] (0.126)[b]	90	0.1	0.001	4 ± 0.006[a] (3.94)[b]	24	0.3	0.0125

[a] Rate constants from stopped-flow experiments. [b] Rate constants from simulations. [c] Data from previous reports.[12b,13] ND = Not detected.

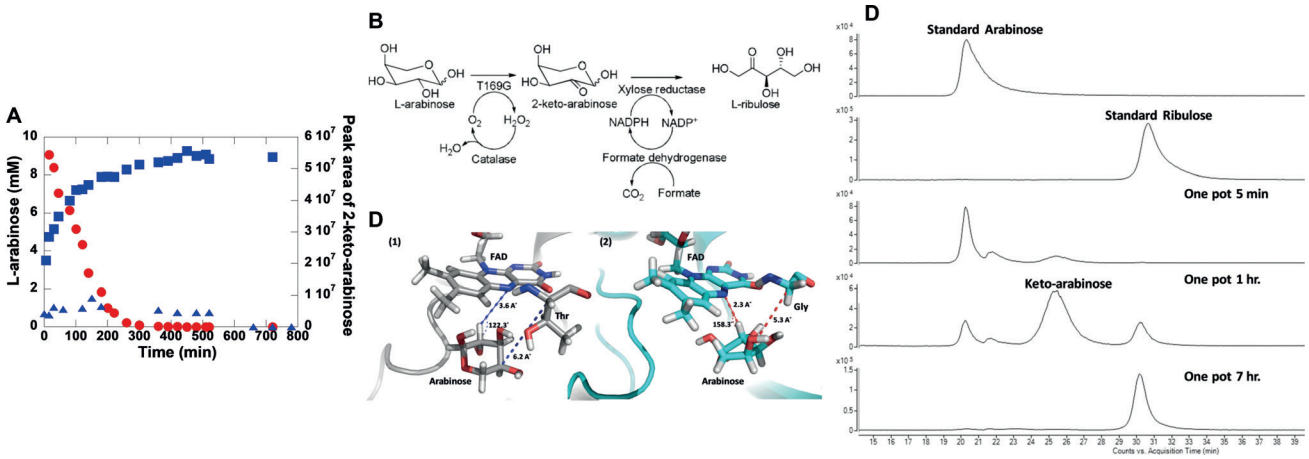


Figure 3. A) Comparison of 2-keto-arabinose production from P2O(T169G) and wild-type P2O. L-arabinose concentration is shown as red circles, 2-keto-arabinose formation by multiple turnover reactions of P2O(T169G) is shown as blue squares, and blue triangles represent the formation of 2-keto-arabinose by P2O(WT). B) Cascade reactions of P2O(T169G), xylose reductase, formate dehydrogenase, and catalase starting from L-arabinose results in complete production of L-ribulose. C) Product analysis of one-pot, multiple turnover reactions of T169G, xylose reductase, formate dehydrogenase, and catalase to convert arabinose to ribulose by HPLC/MS analysis. Total ion chromatograms of HPLC MS/MS of multiple turnover reactions of P2O(T169G), xylose reductase, formate dehydrogenase, and catalase show depletion of L-arabinose and formation of 2-keto-arabinose and L-ribulose over time. Standard arabinose (eluted at 20 min), and ribulose (eluted at 30 min) are shown as references. D) Snapshots from 40 ps QM/MM MD simulations of arabinose binding to (1) WT and (2) P2O(T169G).

chemo-enzymatic method gave only a 5% yield of L-ribulose with several by-products.

To demonstrate the wide applicability of this four-enzymatic cascade as the methodology synthetically relevant for the conversion of aldoses to specific ketoses, the bioconversions of D-galactose to D-tagatose and D-glucose to D-fructose were also performed. The results (see section 11 in the Supporting Information) indicate that this enzymatic cascade can convert D-galactose to D-tagatose (reaction using P2O(T169G)) and D-glucose to D-fructose (reaction using P2O(WT)), demonstrating that this strategy can be applied for the isomerization of aldoses to form specific ketoses (especially C2-keto sugars). The methodology is also applicable for scaling up as the same condition could be increased 10-fold in starting sugar amount to obtain similar yield and product purity (see section 10 in the Supporting Information).

To investigate the mechanistic principles underlying the success of using P2O(T169G) for L-ribulose synthesis, molecular dynamics with quantum mechanics/molecular mechanics (QM/MM MD) simulations were employed to investigate binding interactions of L-arabinose to P2O(WT) and to P2O(T169G). These QM/MM MD results suggested that P2O(T169G) has less steric hindrance for the binding of L-arabinose compared to the wild-type enzyme. The distance between C_α of residue 169 and C4 of arabinose in P20(T169G) (5.3 Å) is shorter than in P2O(WT) (6.2 Å), allowing the sugar to fit better into the active site pocket of P2O(T169G). As a result, the binding of L-arabinose (C2) is closer to the N5-position of FAD in the P2O(T169G) (2.3 Å) variant than in the wild-type (3.6 Å) enzyme (Figure 3D). As the hydride equivalent is transferred from C2–H of L-arabinose to the N5-position of FAD, a shorter distance between these atoms would allow a higher rate of sugar oxidation.

In summary, a new strategy for the synthesis of L-ribulose from L-arabinose was successfully established using a one-pot bioconversion consisting of P2O(T169G), xylose reductase, formate dehydrogenase, and catalase. The engineered P2O variant T169G displays excellent efficiency in L-arabinose oxidation because the variant allows L-arabinose to bind such that the C2-position of the sugar is closer to the FAD N5-position in the variant than in P2O(WT). The thermodynamics of this four-enzymatic cascade is very favorable towards complete yield of L-ribulose. Transient kinetics in combination with molecular dynamics simulations provided an explanation for this at the molecular level, demonstrating that the interactions between the sugar substrate and the active site residues are important for the observed selectivity. The findings reported here are important for the engineering of other sugar oxidases and should be useful for the commercial production of rare sugars.

Acknowledgements

This research was financially supported by The Thailand Research Fund through grants RTA5980001 (to P.C.) and Royal Golden Jubilee Ph.D. Program Grant PHD/0172/2556 (to L.C.) and MRG6080234 (to T.W.). We thank Mr. Pobthum Munkajohnpong and Assist. Prof. Ruchanok Tinikul from Enzmart Biotech (Thailand) for providing formate dehydrogenase. We thank Dr. Somchart Maenpuen for help with EnzFitter analysis and FRC at VISTEC for NMR and QTOF analysis.

Conflict of interest

The authors declare no conflict of interest.

Keywords: enzymatic cascades · flavoenzyme · one-pot reaction · oxidase · ribulose

How to cite: *Angew. Chem. Int. Ed.* **2019**, *58*, 2428 – 2432
Angew. Chem. **2019**, *131*, 2450 – 2454

[1] a) A. Szekrenyi, X. Garrabou, T. Parella, J. Joglar, J. Bujons, P. Clapés, *Nat. Chem.* **2015**, *7*, 724 – 729; b) R. Roldán, K. Hernandez, J. Joglar, J. Bujons, T. Parella, I. Sánchez-Moreno, V. Hélaine, M. Lemaire, C. Guérard-Hélaine, W.-D. Fessner, P. Clapés, *ACS Catal.* **2018**, *8*, 8804 – 8809; c) A. Verges, E. Cambon, S. Barbe, S. Salamone, Y. Le Guen, C. Moulis, L. A. Mulard, M. Remaud-Siméon, I. André, *ACS Catal.* **2015**, *5*, 1186 – 1198.

[2] N. Mosier, C. Wyman, B. Dale, R. Elander, Y. Y. Lee, M. Holtzapple, M. Ladisch, *Bioresour. Technol.* **2005**, *96*, 673 – 686.

[3] a) K. Beerens, T. Desmet, W. Soetaert, *J. Ind. Microbiol. Biotechnol.* **2012**, *39*, 823 – 834; b) A. L. Concia, C. Lozano, J. A. Castillo, T. Parella, J. Joglar, P. Clapes, *Chem. Eur. J.* **2009**, *15*, 3808 – 3816.

[4] a) J. Muschiol, C. Peters, N. Oberleitner, M. D. Mihovilovic, U. T. Bornscheuer, F. Rudroff, *Chem. Commun.* **2015**, *51*, 5798 – 5811; b) F. Rudroff, M. D. Mihovilovic, H. Gröger, R. Snajdrova, H. Iding, U. T. Bornscheuer, *Nat. Catal.* **2018**, *1*, 12 – 22; c) W.-D. Fessner, *New Biotechnol.* **2015**, *32*, 658 – 664.

[5] a) B. H. Cho, J. H. Kim, H. B. Jeon, K. S. Kim, *Tetrahedron* **2005**, *61*, 4341 – 4346; b) M. Yun, H. R. Moon, H. O. Kim, W. J. Choi, Y.-C. Kim, C.-S. Park, L. S. Jeong, *Tetrahedron Lett.* **2005**, *46*, 5903 – 5905; c) K. Vanhessche, C. G. Bello, M. Vandewalle, *Synlett* **1991**, 921 – 922.

[6] a) D. Ekeberg, S. Morgenlie, Y. Stenstrøm, *Carbohydr. Res.* **2002**, *337*, 779 – 786; b) G. Meher, R. Krishnamurthy, *Carbohydr. Res.* **2011**, *346*, 703 – 707.

[7] A. K. Kylmä, T. Granström, M. Leisola, *Appl. Microbiol. Biotechnol.* **2004**, *63*, 584 – 591.

[8] C. De Muynck, C. Pereira, W. Soetaert, E. Vandamme, *J. Biotechnol.* **2006**, *125*, 408 – 415.

[9] a) Z. Ahmed, T. Shimonishi, S. H. Bhuiyan, M. Utamura, G. Takada, K. Izumori, *J. Biosci. Bioeng.* **1999**, *88*, 444 – 448; b) Y.-W. Zhang, M. Jeya, J.-K. Lee, *Appl. Microbiol. Biotechnol.* **2010**, *87*, 1993 – 1999.

[10] L. Wen, K. Huang, M. Wei, J. Meisner, Y. Liu, K. Garner, L. Zang, X. Wang, X. Li, J. Fang, H. Zhang, P. G. Wang, *Angew. Chem. Int. Ed.* **2015**, *54*, 12654 – 12658; *Angew. Chem.* **2015**, *127*, 12845 – 12849.

[11] C. Leitner, J. Volc, D. Haltrich, *Appl. Environ. Microbiol.* **2001**, *67*, 3636 – 3644.

[12] a) B. M. Hallberg, C. Leitner, D. Haltrich, C. Divne, *J. Mol. Biol.* **2004**, *341*, 781 – 796; b) M. Prongjit, J. Sucharitakul, T. Wongnate, D. Haltrich, P. Chaiyen, *Biochemistry* **2009**, *48*, 4170 – 4180; c) J. Sucharitakul, T. Wongnate, P. Chaiyen, *Biochemistry* **2010**, *49*, 3753 – 3765; d) T. Wongnate, P. Surawatanawong, S. Visitsatthawong, J. Sucharitakul, N. S. Scrutton, P. Chaiyen, *J. Am. Chem. Soc.* **2014**, *136*, 241 – 253.

[13] W. Pitsawong, J. Sucharitakul, M. Prongjit, T.-C. Tan, O. Spadiut, D. Haltrich, C. Divne, P. Chaiyen, *J. Biol. Chem.* **2010**, *285*, 9697 – 9705.

[14] B. Seiboth, C. Gamauf, M. Pail, L. Hartl, C. P. Kubicek, *Mol. Microbiol.* **2007**, *66*, 890 – 900.

Manuscript received: December 14, 2018
Accepted manuscript online: January 3, 2019
Version of record online: January 29, 2019

