ChemBioChem

Combining Chemistry and Biology

Chemistry Europe

European Chemical Societies Publishing

Accepted Article

Title: Discovery and Engineering of a Novel Baeyer–Villiger Monooxygenase from Gordonia sihwensis with High Normal Regioselectivity

Authors: Guang-Xiang Zhang, Zhi-Neng You, Jia-Mei Yu, Yuan-Yang Liu, Jiang Pan, Jian-He Xu, and Chun-Xiu Li

To be cited as: *ChemBioChem* 10.1002/cbic.202000478

Link to VoR: https://doi.org/10.1002/cbic.202000478

WILEY-VCH

COMMUNICATION WILEY-VCH

Discovery and Engineering of a Novel Baeyer–Villiger Monooxygenase with High Normal Regioselectivity

Guang-Xiang Zhang,[#[a]] Zhi-Neng You,[#[a]] Jia-Mei Yu,[[a]] Yuan-Yang Liu,[[a]] Jiang Pan,[[a]] Jian-He Xu,*[[a, b]] and Chun-Xiu Li*[[a]]

[a] G.-X. Zhang, Z.-N. You, J.-M. Yu, Y.-Y. Liu, Dr. J. Pan, Prof. J.-H. Xu, Dr. C.-X. Li
State Key Laboratory of Bioreactor Engineering, Shanghai Collaborative Innovation Center for Biomanufacturing, East China University of Science and Technology, Shanghai 200237 (P.R. China)
[b] Frontiers Science Center for Materiobiology and Dynamic Chemistry, East China University of Science and Technology, Shanghai 200237 (P.R. China)
E-mail: jianhexu@ecust.edu.cn; chunxiuli@ecust.edu.cn. #These authors contributed equally.

Supporting information for this article is given via a link at the end of the document.((Please delete this text if not appropriate))

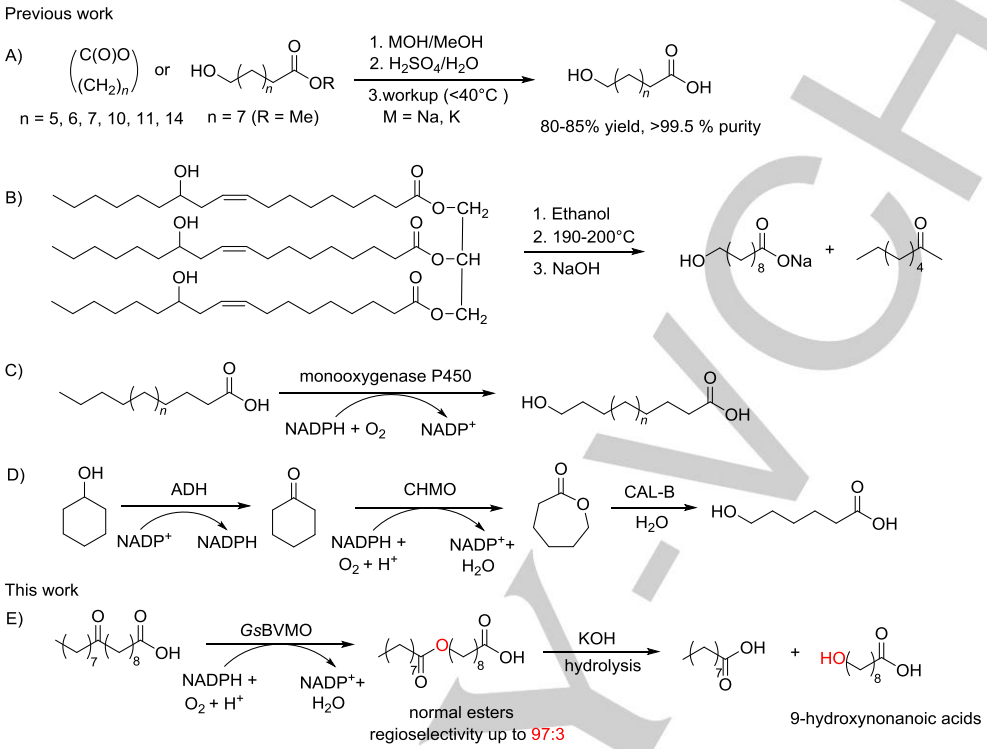
Abstract: Baeyer–Villiger monooxygenases (BVMOs) are remarkable biocatalysts for the Baeyer–Villiger oxidation of ketones to generate esters or lactones. The regioselectivity of BVMOs is essential for determining the ratio of the two regioisomeric products ("normal" and "abnormal") when catalyzing asymmetric ketone substrates. Starting from a known normal-preferring BVMO sequence from *Pseudomonas putida* KT2440 (*Pp*BVMO), a novel BVMO from *Gordonia sihwensis* (*Gs*BVMO) with higher normal regioselectivity (up to 97/3) was identified. Furthermore, protein engineering increased the specificity constant (k_{cat}/K_M) 8.9-fold to 484 s^{-1} mM^{-1} for 10-ketostearic acid derived from oleic acid. Consequently, by using the variant *Gs*BVMO$_{C308L}$ as an efficient biocatalyst, 10-ketostearic acid was efficiently transformed into 9-(nonanoyloxy)nonanoic acid, with a space-time yield of 60.5 g L^{-1} d^{-1}. This study showed that the mutant with higher regioselectivity and catalytic efficiency could be applied to prepare medium-chain ω-hydroxy fatty acids through biotransformation of long-chain aliphatic keto acids derived from renewable plant oils.

With the current depletion of nonrenewable petrochemical resources and increasing concerns regarding global environmental pollution, biobased chemicals produced from renewable and cost-effective feedstocks (such as plant oils and celluloses) are of great strategic significance.[1–3] Among various biobased chemicals, medium-chain (C_8–C_{14}) ω-hydroxy fatty acids (ω-HFAs) are important functional chemicals that possess carboxylic and hydroxyl functionalities at either terminal of the fatty acid chain, and have wide-ranging applications as fragrances, adhesives, antiseptics, and pharmaceutical intermediates.[4–6] Furthermore, ω-HFAs can be used as starting materials for the synthesis of polymer materials with superior material properties.[7] ω-HFAs can be further transformed into α,ω-dicarboxylic acids, which are important building blocks for the production of biodegradable aliphatic polymers with high resistance to heat or chemicals, nontoxicity, biocompatibility, and flexibility, such as polyesters and polyamides.[8] For example, 9-hydroxynonanoic acids can be used to synthesize nonanolactones, which are useful monomers for producing aliphatic polyesters or polylactones.[9] Currently, the most practical preparative routes for ω-hydroxy fatty acids include the catalytic hydrogenation of mixtures of saturated aliphatic dicarboxylic acids and the corresponding glycols, or reductive alkaline scission of fatty acids. However, in most cases, high temperatures and strong acids (such as H_2SO_4) and bases (NaOH, KOH) are usually indispensable conditions, both in laboratory or preparative-scale processes, resulting in environmental and safety concerns.[10]

Mohar et al. developed a simple and scalable two-step synthetic method for preparing ω-hydroxy fatty acids from the corresponding lactones or ω-hydroxycarboxylates as starting materials, achieved either by separating the ω-hydroxy acid potassium salt or through one-pot synthesis (**Scheme 1A**).[10] This operation was facile, but required strong acid (such as H_2SO_4) and a low-pressure environment (such as 8–12 mbar). Carl et al. developed a method for preparing ω-hydroxy fatty acids through alkaline cleavage of ricinoleates in ethanol (**Scheme 1B**).[11] This method avoided using a large excess of alkali and high-boiling-point alcohols as the reaction medium, and might be suitable for ricinoleic acid. However, 2–3 equiv. of NaOH and temperatures of 190–200°C were still necessary. Chandran et al. developed a biotransformation pathway for the ω-oxidation of fatty acids by monooxygenase P450, producing ω-hydroxy fatty acids, α,ω-dicarboxylic acids, and other functional chemicals (**Scheme 1C**).[12] This approach required monooxygenases with high terminal oxidation selectivity and catalytic activity. Bornscheuer et al. reported the production of ω-hydroxy fatty acids using the cyclohexanol-based multienzyme cascade biotransformation pathway (**Scheme 1D**).[13] Cyclohexanol was mainly prepared by the phenol hydrogenation method or cyclohexane oxidation method. However, both methods required either high-temperature or high-pressure conditions, and were accompanied by the formation of various byproducts.

ω-Hydroxy fatty acids can also be produced via oxidative cleavage of long-chain unsaturated fatty acids. Recently, an elegant route was developed by the Park group, comprising multistep enzymatic or chemoenzymatic cascade reactions, to convert ricinoleic acid, linoleic acid, and oleic acid into ω-hydroxy fatty acids.[14–22] *Pp*BVMO from *Pseudomonas putida* KT2440[23] and *Pf*BVMO from *Pseudomonas fluorescens* DSM 50106,[24] first reported by the Bornscheuer group, were usually employed in this route. *Pp*BVMO favored oxygen atom insertion at the higher substitution site of the asymmetric linear fatty ketone to generate a "normal" ester and the corresponding ω-hydroxy fatty acid through hydrolysis. Meanwhile *Pf*BVMO favored generating "abnormal" esters and the corresponding α,ω-dicarboxylic acids. Therefore, the regioselectivity of BVMOs, which is a traditional limitation of enzymes as catalysts in synthetic organic chemistry, is a key factor in determining the type of ester produced and downstream processing steps.[25,26] The three selected BVMOs, which were overexpressed in a soluble form in *Escherichia coli* BL21 (DE3), tended to afford 9-(nonanoyloxy)nonanoic acid from oleic acid, despite achieving maximal production of only 105 mg/L.[27] Our group discovered an "abnormal" ester-preferring BVMO from *Pseudomonas aeruginosa* (*Pa*BVMO)

with higher regioselectivity than *Pf*BVMO.[28] To our knowledge, the regioselectivity of "normal" ester-preferring BVMOs has yet to be fully investigated. BVMOs with higher regioselectivity and catalytic activity will benefit the production of medium-chain ω-hydroxy fatty acids from long-chain keto fatty acids derived from renewable oleic acid.



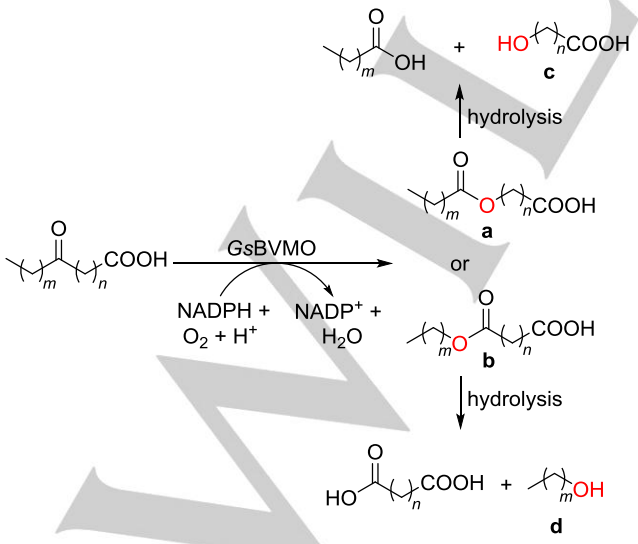
Scheme 1. Synthesis of medium-chain ω-hydroxy fatty acids by (A) chemical route from lactones or alkyl ω-hydroxycarboxylates through saponification followed by acidification and treatment at 35–40°C/8–12 mbar,[10] (B) alkaline cleavage of ricinoleates,[11] (C) the ω-oxidation pathway,[12] (D) multienzyme cascade reaction composed of ADH, CHMO, and CAL-B,[13] and (E) biotransformation using Baeyer–Villiger monooxygenase (BVMO) from *Gordonia sihwensis* (*Gs*BVMO) with high normal regioselectivity (this work). CHMO: Cyclohexanone monooxygenase; NADPH: Nicotinamide adenine dinucleotide phosphate.

Recent protein engineering by directed evolution and rational design has generated many novel BVMO mutants that display relatively high stereoselectivity, activity, thermostability, or wide substrate scopes.[29,30] Although the recent combination of directed evolution and rational design, both guided by X-ray structural data and mechanistic knowledge, has led to the regioselectivity control of BVMOs toward cyclic ketones,[31,32] the regioselectivity of BVMOs toward linear long-chain keto acids was rarely achieved.[30] Genome mining in public databases remains an effective method for discovering novel BVMOs with unique properties. For example, Fraaije et al. identified a novel CHMO from *Thermocrispum municipal* DSM 44069 (*Tm*CHMO) with superior thermostability,[33] which is a robust BVMO for producing ε-caprolactone and other valuable compounds from cyclohexanol.

This study aimed to discover a new BVMO with high normal selectivity and enhance its catalytic activity through protein engineering to efficiently convert keto fatty acids into normal esters that can easily be transformed into ω-hydroxy fatty acids by simple hydrolysis. BVMO with higher activity and regioselectivity will significantly reduce the formation of byproducts, and simplify downstream separation and purification steps. The protein sequence of *Pp*BVMO has been employed as a probe to perform pBLAST in the NCBI database.[34,35] *Pp*BVMO has shown the highest catalytic activity among the four BVMOs reported, with normal selectivity toward 10-ketostearic acid, and has been widely used in the biotransformation of unsaturated fatty acids. To increase the diversity of candidate gene sources, we selected 13 putative genes encoding various BVMOs from bacteria having 20–80% sequence identities with the *Pp*BVMO template. The 13 target BVMO sequences were successfully amplified, cloned, and heterologously expressed in *Escherichia coli* BL21 (DE3) to obtain higher catalytic activity and normal regioselectivity (**Table S1**). Whole-cell biocatalysts expressing the genes were screened against model substrate 10-ketostearic acid (**4**), which can be prepared from plant oil-derived oleic acid.[36] The functional screening reaction consisted of recombinant *E. coli* and 10-ketostearic acid. The reaction was terminated after 24 h and then subjected to pretreatment for GC analysis. The conversion of substrate 10-ketostearic acid was analyzed to determine the catalytic activity of the putative enzymes, while the analytical yields of normal (**4a**) and abnormal esters (**4b**) were analyzed to determine the regioselectivity of the putative enzymes. Each of these catalysts showed measurable monooxygenation activity toward **4**, with the monooxygenases from *Gordonia sihwensis* and *Acinetobacter radioresistens*, denoted as *Gs*BVMO and *Ar*BVMO, respectively, showing higher catalytic activity than probe *Pp*BVMO (**Table S2**). Herein, the regioselectivity of BVMO is defined as the molar ratio of **4a** to **4b**. BVMOs from *Actinoplanes missouriensis* and

Gordonia sihwensis, denoted as *Am*BVMO and *Gs*BVMO, respectively, showed higher regioselectivities for normal esters compared with probe *Pp*BVMO. These data indicated that *Gs*BVMO had the highest catalytic activity (>99% conv.) and superior regioselectivity (97:3) among the 13 putative BVMOs screened, and compared with probe *Pp*BVMO. Accordingly, *Gs*BVMO was selected as the target enzyme for further studies.

To characterize the enzymatic properties of *Gs*BVMO, the *N*-terminal His-tagged recombinant enzyme was purified by nickel affinity chromatography and analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (**Figure S2**). The activity of *Gs*BVMO at temperatures ranging from 20 to 40°C was measured. The maximum activity was observed at 30°C, and was almost twice the enzyme activity observed at 20°C. When the temperature was higher than 30°C, the enzyme activity decreased significantly, affording only 33% of the highest activity at 40°C (**Figure S3A**). Furthermore, the effect of pH on enzyme activity was determined. The results showed that the preferred pH range of this enzyme was 7.0–8.5, and the optimal pH was 8.0 in 100 mM potassium phosphate buffer (**Figure S3B**). This showed that *Gs*BVMO preferred to catalyze the reaction under moderately alkaline conditions. The thermal stability of *Gs*BVMO was also examined, giving half-lives ($t_{1/2}$) of only 2.5 h at 30°C (**Figure S3C**), implying that the enzyme is very temperature-sensitive. Poor thermostability is common in the reported BVMOs,[37] and can be overcome using protein engineering[38,39] and enzyme immobilization.[40] Furthermore, the effect of organic solvents on the stability of *Gs*BVMO was evaluated. The residual activity was determined by adding methanol, ethanol, dimethyl sulfoxide (DMSO), acetonitrile, dimethylformamide (DMF), isopropanol, methyl benzene, or ethyl acetate (10%, v/v) to the purified enzyme, and incubating for 20 min with the blank group as a control (**Figure S4**). The results showed that *Gs*BVMO had better tolerance against DMSO among the organic solvents tested. Therefore, DMSO was used as cosolvent in subsequent experiments to increase the solubility of the long-chain aliphatic keto acids in aqueous solution.



Scheme 2. Regiodivergent Baeyer–Villiger oxidation of asymmetric long-chain keto acids: Formation of (**a**) "normal" ester and (**b**) "abnormal" ester.

Table 1. Substrate scope of *Gs*BVMO.

Category	Structure
Keto acids	**1**: *m*=3, *n*=2; **2**: *m*=5, *n*=2; **3**: *m*=4, *n*=3; **4**: *m*=7, *n*=8;
Keto esters	**5**: *m*=5, *n*=2; **6**: *m*=4, *n*=3;
	7: *m*=5, *n*=2; **8**: *m*=4, *n*=3;
Linear ketones	**9**: *n*=2; **10**: *n*=3; **11**: *n*=4; **12**: *n*=5; **13**: *n*=6; **14**: *n*=7;
	15: *n*=6; **16**: *n*=7; **17**: *n*=8; **18**: *n*=10; **19**: *n*=12; **20**: *n*=16;
Cyclic ketones	**21**: *n*=1; **22**: *n*=2; **23**: *n*=3; **24**: *n*=4;

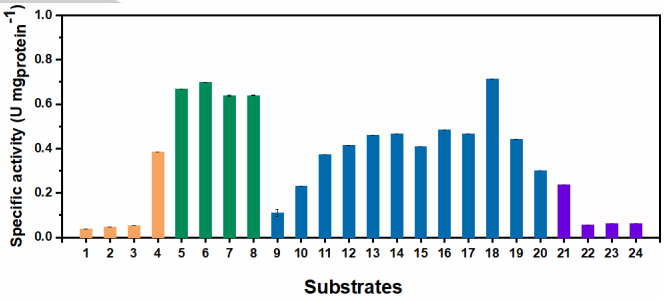


Figure 1. Catalytic activity of *Gs*BVMO toward four different groups of substrates. Specific activity was measured with linear keto acids **1**–**4** (orange bars), keto esters **5**–**8** (green bars), linear ketones **9**–**20** (blue bars), and cyclic ketones **21**–**24** (purple bars) as substrates.

To understand the substrate preference and broaden enzyme utilization, the substrate profile of *Gs*BVMO was investigated. According to the characteristics of the enzyme able to catalyze long-chain aliphatic keto acids (**Scheme 2**), the specific activity of the enzyme toward linear aliphatic keto acids, keto esters, and linear and cyclic ketones with varied chain length was determined (**Table 1**). The results clearly showed that the catalytic activity of *Gs*BVMO toward medium- and long-chain keto acids changed with varying chain length (**Figure 1**).

Medium-chain keto acids 4-oxo-octanoic acid (**1**; C_8), 4-oxo-decanoic acid (**2**; C_{10}), and 5-oxo-decanoic acid (**3**; C_{10}), *Gs*BVMO showed poor catalytic activities of only 0.04, 0.05, and 0.06 U mg^{-1}, respectively. Meanwhile, the catalytic activity toward long-chain 10-ketostearic acid (**4**; C_{18}) increased significantly, indicating that the enzyme preferred to catalyze long-chain aliphatic keto acids derived from natural long-chain

fatty acids, the main components of renewable plant oils. Furthermore, the catalytic activity of this enzyme toward methyl/ethyl esters was much higher than that toward the corresponding keto acids (**2** vs. **5** & **7**; **3** vs. **6** & **8**). For example, the specific activities toward methyl 4-oxo-decanoate (**5**) and ethyl 4-oxo-decanoate (**7**) were 0.670 and 0.639 U mg^{-1}, respectively, which were 14.3 and 13.6 times higher than that toward 4-oxo-decanoic acid (**2**; 0.047 U mg^{-1}). Higher catalytic activities were also observed toward medium-chain ketones (**11**–**18**; C_8–C_{13}) compared with those toward short-chain (**9**, **10**) and long-chain ketones (**19**, **20**) among the 12 linear ketones examined. The specific activity of this enzyme toward 2-tridecanone (**18**, C_{13}) was 0.714 U mg^{-1}, which was the highest among the linear ketones tested. Notably, the enzyme showed generally poor activity toward the alicyclic ketones tested, such as cyclopentanone (**22**), cyclohexanone (**23**), and cycloheptanone (**24**), except for cyclobutanone (**21**), which are usually well accepted by BVMOs, including CPMOs and CHMOs.[13,33] Based on the substrate scope determination, *Gs*BVMO was confirmed to be more suitable for the catalysis of long-chain aliphatic keto acids and medium-chain aliphatic ketones, rather than alicyclic ketones.

Finding or designing better enzymes for a specific enzyme-catalyzed process is a major problem in enzyme technology.[41–43] Protein engineering is a common method for obtaining the desired variants under laboratory conditions.[44,45] To improve the catalytic activity and thermostability of *Gs*BVMO, we first constructed a structural model for *Gs*BVMO using homology modeling based on the crystal structure of *Rh*CHMO (PDB ID: 3UCL) in the catalytic state as a template. Second, the model substrate **4** was docked into the binding pocket of the *Gs*BVMO using the AutoDock program.[46] Then, the residue surface exposure of the obtained complex structure was analyzed for solvent accessible surface area (SASA), calculated by the GETAREA online tool.[47,48] Based on the fact that hydrogen peroxide could be generated as undesirable reactive oxygen species (ROS) in the reaction catalyzed by BVMO, and the surface-exposed methionine and cysteine could be susceptible to oxidation by the ROS.[30,49,50] Thus, the solvent-exposed residues Met209, Cys267 and Cys308 were then obtained for site-directed mutation, with the residues on the substrate binding pocket, believed to be related to the thermostability and catalytic activity of the enzyme, of special concern. Val346 was also included, because the residue at the corresponding position of Thr348 in the BVMO from *Pseudomonans putida* KT2440 was reported as a key residue for the oxidative stability.[22] The targeted residues were mutated into nonpolar amino acids of similar sizes, such as leucine and isoleucine, in order to increase hydrophobicity and oxidative stability.[22] It was found that C308L and C308I with 4.9-fold and 3.2-fold improvements in catalytic activity (**Table S4**), respectively, were identified, and their thermostabilities were essentially consistent with that of WT (**Figure S5**). Meanwhile, only M209L and V346L showed slightly improved thermostability, but accompanied by a significant decrease in catalytic activity (**Figure S5**). After further determination of variant regioselectivity, C308L was found to maintain a high normal regioselectivity (**Table S4**). In summary, the catalytic activity of variant C308L was significantly improved, while the high normal regioselectivity was maintained.

We further measured the kinetic parameters of variant C308L. The binding affinities (K_M) of C308L and WT with 10-ketostearic acid were similar, in the range of 0.016–0.017 mM. However, the first-order rate constant (k_{cat}) of C308L was 7.74 s^{-1}, which was much higher than that of WT (0.917 s^{-1}). These differences resulted in the high catalytic efficiency constant (k_{cat}/K_M) for C308L of 484 s^{-1} mM^{-1}, which was 8.9-fold higher than that of WT (**Table 2**). In the current study, engineering of Cys308, located in the substrate tunnel of *Gs*BVMO, led to a marked increase in catalytic activity of the enzyme. This may be due to the replacement of cysteine, which are susceptible to oxidative stress, with an inert amino acid leucine.[22] In addition, the C308L mutation appeared to generate two additional hydrophobic interactions with Ala200 and Thr224 (**Figure S1**), which may contribute to more favorable interactions between the enzyme and the substrate, thereby making the reaction rate faster. [22,30,51]

Investigation of the reaction performance indicated that C308L completely converted 10-ketostearic acid (3.0 mM) within 1.0 h. However, only 25.9% substrate conversion was achieved by WT after 4.0 h (**Figure S6**). Such a high normal regioselectivity and significantly improved catalytic activity made *Gs*$BVMO_{C308L}$ a promising candidate for the production of normal esters from long-chain aliphatic keto acids derived from renewable plant oils, affording medium-chain ω-hydroxy fatty acids and *n*-aliphatic carboxylic acids after simple ester hydrolysis (**Scheme 2**). Based on bio-originated fatty acids derived from renewable plant oils as starting materials, *Gs*$BVMO_{C308L}$ is considered to be highly feasible for the unnatural biotransformation of long-chain fatty acids to produce medium-chain ω-hydroxy fatty acids and other functional chemicals useful for the synthesis of biodegradable polymers. Furthermore, *Gs*BVMO can provide an effective reference for determining the mechanism of regioselectivity.

Table 2. Steady-state kinetic properties of C308L and WT toward 10-KSA (**4**).

Enzyme variant	K_M (mM)	k_{cat} (s^{-1})	k_{cat}/K_M (s^{-1} mM^{-1})
WT	0.017 ± 0.003	0.917	54.0
C308L	0.016 ± 0.005	7.74	484

Finally, 10-ketostearic acid (**4**) was selected for the biosynthesis of 9-(nonanoyloxy)nonanoic acid to verify the feasibility of *Gs*$BVMO_{C308L}$ in the enzymatic synthesis of ω-hydroxy fatty acids from long-chain aliphatic keto acids. The reaction conditions were optimized to obtain a higher yield. First, the Baeyer–Villiger oxidation was conducted at 20, 25, and 30°C, with the highest conversion of 10-ketostearic acid obtained at 25°C (**Figure S7**). As the cell membrane obstructs the mass transfer of long-chain fatty ketoacids and catalyst stability, lyophilized powders of the cell-free extract were used as catalyst for subsequent preparative-scale reactions (**Figure S8**). Further investigation resulted in a higher conversion in potassium phosphate buffer (pH 8.0, 100 mM) (**Figure S9**). Commercially expensive flavin adenine dinucleotide (FAD) contained in the lyophilized enzyme powder, which acts as a redox-active cofactor in the Baeyer–Villiger oxidation, was proven to be sufficient (**Figure S10**). Furthermore, considering the low solubility of long-chain aliphatic keto acids in aqueous solution, it was necessary to add some surfactants, such as Tween-80, to

promote substrate dispersion in the reaction system and accelerate the reaction.

Based on the optimized reaction conditions, a preparative-scale synthesis of 9-(nonanoyloxy)nonanoic acid was performed. As shown in **Figure 2**, the oxidation of substrate **4** was complete within 4.0 h using a fed-batch strategy. The molar ratio of **4a** to **4b** was 97:3, as determined by GC analysis of the fully hydrolyzed products, 9-hydroxynonanoic acid and *n*-octanol, respectively. The space–time yield of 9-(nonanoyloxy)nonanoic acid was approximately 60.5 g L^{-1} d^{-1}. Compared with using insufficiently regioselectivity *Pp*BVMO to prepare ω-hydroxy fatty acids, highly regioselective *Gs*BVMO allowed improved substrate utilization and reduced byproduct formation to simplify the downstream separation and purification steps. Overall, the discovery and engineering of *Gs*BVMO expands the BVMO toolbox for the biotransformation of long-chain aliphatic keto acids, and provides further options for the green and efficient synthesis of ω-hydroxy fatty acids from renewable feedstocks.

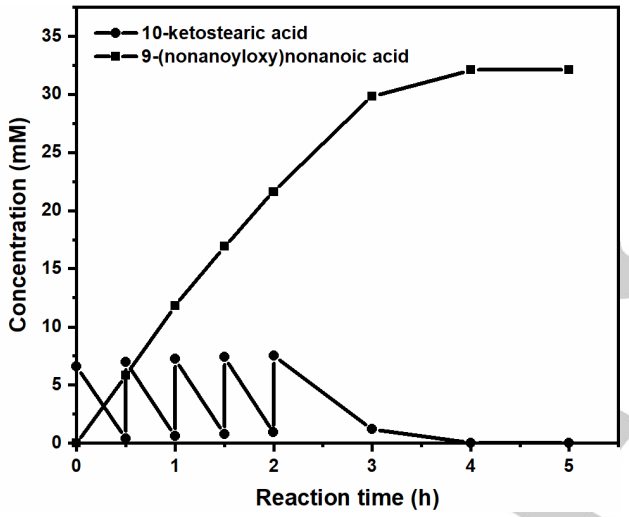


Figure 2. Time-dependent conversion of 10-ketostearic acid (**4**; 10.0 g L^{-1}, feed rate: 6.67 mM/0.5 h) into 9-(nonanoyloxy)nonanoic acid (**4a**) using a fed-batch strategy with lyophilized powder of cell-free *Gs*$BVMO_{C308L}$ (5.0 g L^{-1}) at 25°C in potassium phosphate (pH 8.0, 100 mM).

In conclusion, a novel BVMO from *Gordonia sihwensis* with high normal regioselectivity was discovered through genome mining. Substrate scope investigation indicated that *Gs*BVMO was more suitable for the catalysis of long-chain aliphatic keto acids and medium-chain aliphatic ketones. Enzymatic characterization of the purified enzymes provided additional knowledge regarding the catalytic properties of long-chain aliphatic keto acids. Furthermore, Protein engineering of *Gs*BVMO resulted in a significant enhancement of catalytic performance, along with successful maintenance of the excellent normal regioselectivity.

The engineered enzyme and optimized reaction conditions enabled efficient production of medium-chain ω-hydroxy fatty acids from long-chain aliphatic keto acids. This shows great potential for replacing traditional chemical synthesis processes and reducing the formation of byproducts due to insufficient regioselectivity of the native BVMO. This highly regioselective BVMO can be easily integrated as an elemental enzyme into the already developed unnatural biotransformation routes of bio-originated fatty acids, which might enhance the feasibility of taking advantage of renewable raw materials and contribute to the sustainability of the chemical industry.

Acknowledgements

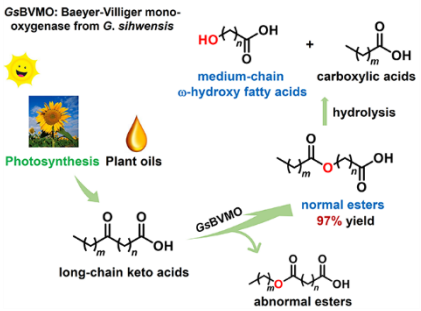
This work was financially sponsored by the National Natural Science Foundation of China (Nos. 21536004, 21776085, 21871085 & 31971381), the National Key Research and Development Program of China (2019YFA09005000 & 2018YFC1706204), and the Fundamental Research Funds for the Central Universities (22221818014).

Keywords: Baeyer–Villiger monooxygenase • biocatalysis • oxidation • regioselectivity • renewable resources

[1] T. Willke, K. D. Vorlop, *Appl. Microbiol. Biotechnol.* **2004**, *66*, 131-142.
[2] U. Biermann, W. Friedt, S. Lang, W. Lühs, G. Machmüller, J. O. Metzger, M. R. Klaas, H. J. Schäfer, M. P. Schneider, *Angew. Chem. Int. Ed.* **2000**, *39*, 2206–2224.
[3] U. Biermann, U. Bornscheuer, M. A. R. Meier, J. O. Metzger, H. J. Schäfer, *Angew. Chem. Int. Ed.* **2011**, *50*, 3854–3871.
[4] S. H. Malca, D. Scheps, L. Kuhnel, E. Venegas-Venegas, A. Seifert, B. M. Nestl, B. Hauer, *Chem. Commun.* **2012**, *48*, 5115–5117.
[5] D. Scheps, S. H. Malca, S. M. Richter, K. Marisch, B. M. Nestl, B. Hauer, *Microb. Biotechnol.* **2013**, *6*, 694–707.
[6] W. H. Lu, J. E. Ness, W. C. Xie, X. Y. Zhang, J. Minshull, R. A. Gross, *J. Am. Chem. Soc.* **2010**, *132*, 15451–15455.
[7] W. R. Kang, M. J. Seo, K. C. Shin, J. B. Park, D. K. Oh, *Biotechnol. Bioeng.* **2017**, *114*, 74–82.
[8] C. H. Bowen, J. Bonin, A. Kogler, C. Barba-Ostria, F. Z. Zhang, *ACS Synth. Biol.* **2016**, *5*, 200–206.
[9] G. Liu, X. Kong, H. Wan, S. Narine, *Biomacromolecules* **2008**, *9*, 949–953.
[10] M. M. S. Stephan, B. Mohar, *Org. Process Res. Dev.* **2006**, *10*, 481–483.
[11] C. A. Elliger, M. J. Diamond, *J. Am. Oil Chem. Soc.* **1972**, *49*, 278–278.
[12] C. Sathesh-Prabu, S. K. Lee, *J. Agric. Food Chem.* **2015**, *63*, 8199–8208.
[13] V. S. T. Srinivasamurthy, D. Böttcher, U. T. Bornscheuer, *Zeitschrift für Naturforschung C* **2019**, *74*, 71.
[14] J. W. Song, E. Y. Jeon, D. H. Song, H. Y. Jang, U. T. Bornscheuer, D. K. Oh, J. B. Park, *Angew. Chem. Int. Ed.* **2013**, *52*, 2534–2537.
[15] H. Y. Jang, E. Y. Jeon, A. H. Baek, S. M. Lee, J. B. Park, *Process Biochem.* **2014**, *49*, 617–622.
[16] H. J. Oh, S. U. Kim, J. W. Song, J. H. Lee, W. R. Kang, Y. S. Jo, K. R. Kim, U. T. Bornscheuer, D. K. Oh, J. B. Park, *Adv. Synth. Catal.* **2015**, *357*, 408–416.
[17] H. Y. Jang, K. Singha, H. H. Kim, Y. U. Kwon, J. B. Park, *Green Chem.* **2016**, *18*, 1089–1095.
[18] E. Y. Jeon, J. H. Seo, W. R. Kang, M. J. Kim, J. H. Lee, D. K. Oh, J. B. Park, *ACS Catal.* **2016**, *6*, 7547–7553.
[19] S. Koppireddi, J. H. Seo, E. Y. Jeon, P. S. Chowdhury, H. Y. Jang, J. B. Park, Y. U. Kwon, *Adv. Synth. Catal.* **2016**, *358*, 3084–3092.
[20] H. J. Cha, E. J. Seo, J. W. Song, H. J. Jo, A. R. Kumar, J. B. Park, *Adv. Synth. Catal.* **2018**, *360*, 696–703.
[21] E. J. Seo, Y. J. Yeon, J. H. Seo, J. H. Lee, J. P. Bongol, Y. Oh, J. M. Park, S. M. Lim, C. G. Lee, J. B. Park, *Bioresour. Technol.* **2018**, *251*, 288–294.
[22] E. J. Seo, C. W. Kang, J. M. Woo, S. Jang, Y. J. Yeon, G. Y. Jung, J. B. Park, *Metab. Eng.* **2019**, *54*, 137–144.
[23] J. Rehdorf, A. Kirschner, U. T. Bornscheuer, *Biotechnol. Lett.* **2007**, *29*, 1393–1398.

[24] A. Kirschner, J. Altenbuchner, U. T. Bornscheuer, *Appl. Microbiol. Biotechnol.* **2007**, *73*, 1065–1072.
[25] E. Romero, J. R. Gomez Castellanos, G. Gadda, M. W. Fraaije, A. Mattevi, *Chem. Rev.* **2018**, *118*, 1742–1769.
[26] G. de Gonzalo, M. D. Mihovilovic, M. W. Fraaije, *ChemBioChem* **2010**, *11*, 2208–2231.
[27] P. D. V. N. Sudheer, J. Yun, S. Chauhan, T. J. Kang, K.-Y. Choi, *Biotechnol. Bioprocess Eng.* **2017**, *22*, 717–724.
[28] J. M. Yu, Y. Y. Liu, Y. C. Zheng, H. Li, X. Y. Zhang, G. W. Zheng, C. X. Li, Y. P. Bai, J. H. Xu, *ChemBioChem* **2018**, *19*, 2049–2054.
[29] Z. G. Zhang, L. P. Parra, M. T. Reetz, *Chem. Eur. J.* **2012**, *18*, 10160–10172.
[30] K. Balke, A. Beier, U. T. Bornscheuer, *Biotechnol. Adv.* **2018**, *36*, 247–263.
[31] J. b. Wang, G. Li, M. T. Reetz, *Chem. Commun.* **2017**, *53*, 3916-3928.
[32] K. Balke, M. Baumgen, U. T. Bornscheuer, *ChemBioChem* **2017**, *18*, 1627–1638.
[33] E. Romero, J. R. G. Castellanos, A. Mattevi, M. W. Fraaije, *Angew. Chem. Int. Ed.* **2016**, *55*, 15852-15855;
[34] H. Li, G. X. Zhang, L. M. Li, Y. S. Ou, M. Y. Wang, C. X. Li, G. W. Zheng, J. H. Xu, *ChemCatChem* **2016**, *8*, 724-727.
[35] S. C. Shi, Z. N. You, K. Zhou, Q. Chen, J. Pan, X. L. Qian, J. H. Xu, C. X. Li, *Adv. Synth. Catal.* **2019**, *361*, 4661–4668.
[36] Y. X. Wu, J. Pan, H. L. Yu, J. H. Xu, *J. Biotechnol.: X* **2019**, *2*, 100008.
[37] M. J. L. J. Fürst, A. Gran-Scheuch, F. S. Aalbers, M. W. Fraaije, *ACS Catal.* **2019**, *9*, 11207–11241.
[38] M. J. L. J. Fürst, M. Boonstra, S. Bandstra, M. W. Fraaije, *Biotechnol. Bioeng.* **2019**, *116*, 2167–2177.
[39] Z. N. You, Q. Chen, S. C. Shi, M. M. Zheng, J. Pan, X. L. Qian, C. X. Li, J. H. Xu, *ACS Catal.* **2019**, *9*, 466–473.
[40] H. Cheng, Y. L. Zhao, X. J. Luo, D. S. Xu, X. Cao, J. H. Xu, Q. Dai, X. Y. Zhang, J. Ge, Y. P. Bai, *Bioresour. Bioprocess.* **2018**, *5*, 49.
[41] F. H. Arnold, *Angew. Chem. Int. Ed.* **2018**, *57*, 4143–4148.
[42] C. A. Denard, H. Ren, H. Zhao, *Curr. Opin. Chem. Biol.* **2015**, *25*, 55–64.
[43] U. T. Bornscheuer, M. Pohl, *Curr. Opin. Chem. Biol.* **2001**, *5*, 137–143.
[44] N. Turner, *Nat. Chem. Biol.* **2009**, *5*, 567–573.
[45] V. J. Frauenkron-Machedjou, A. Fulton, J. Zhao, L. Weber, K. E. Jaeger, U. Schwaneberg, L. Zhu, *Bioresour. Bioprocess*. **2018**, *5*, 2.
[46] O. Trott, A. J. Olson, *J. Comput. Chem.* **2010**, *31*, 455–461.
[47] R. Fraczkiewicz, W. Braun, *J. Comput. Chem*. **1998**, *19*, 319–333.
[48] R. Fraczkiewicz, W. Braun, *J. Comput. Chem*. **1998**, *19*, 319–326.
[49] W. P. Dijkman, G. de Gonzalo, A. Mattevi, M. W. Fraaije, *Appl. Microbiol. Biotechnol.* **2013**, *97*, 5177–5188.
[50] D. Holtmann, F. Hollmann, *ChemBioChem* **2016**, *17*, 1391–1398.
[51] D.J. Opperman, M.T. Reetz, *ChemBioChem* **2010**, *11*, 2589–2596.

Entry for the Table of Contents



Structure controllable synthesis: Regioselectivity is essential in Baeyer–Villiger monooxygenase - mediated regiodivergent conversions of asymmetric ketones to generate esters in organic synthesis. A novel normal-preferring BVMO from *Gordonia sihwensis* (*Gs*BVMO) with superior regioselectivity (97:3) was identified, engineered, and employed to efficiently synthesize 9-(nonanoyloxy)nonanoic acid from 10-ketostearic acid derived from renewable plant oils.