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Bioproduction of Chiral Epoxyalkanes using Styrene Monooxygenase from *Rhodococcus* sp. ST-10 (RhSMO)

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Abstract: We describe the enantioselective epoxidation of straight-chain aliphatic alkenes using a biocatalytic system containing styrene monooxygenase from *Rhodococcus* sp. ST-10 and alcohol dehydrogenase from *Leifsonia* sp. S749. The biocatalyzed enantiomeric epoxidation of 1-hexene to (*S*)-1,2-epoxyhexane (>44.6 mM) using 2-propanol as the hydrogen donor was achieved under optimized conditions. The biocatalyst had broad substrate specificity for various aliphatic alkenes, including terminal, internal, unfunctionalized, and di- and tri-substituted alkenes. Here, we demonstrate that this biocatalytic system is suitable for the efficient production of enantioenriched (*S*)-epoxyalkanes.

Keywords: alkenes; asymmetric epoxidation; biosynthesis; enzyme catalysis; styrene monooxygenase

Introduction

Chiral epoxides are important building blocks for the synthesis of chiral target compounds, such as agrochemicals and pharmaceuticals, because they can react with a variety of nucleophiles in a highly stereoselective fashion. Asymmetric epoxidation of prochiral alkenes is an extremely powerful tool for producing optically pure epoxides. Examples of useful approaches for synthesizing enantiomerically enriched epoxides include Sharpless epoxidation and chiral metal catalysis using metal-salen complexes and fructose-derived dioxiranes.[1–6] However, further improvements are necessary in the production of chiral epoxides. For example, direct enantioselective epoxidation of unfunctionalized terminal alkenes is difficult due to their low reactivity toward electrophilic oxidation, and controlling the prochiral faces of the simply monosubstituted double bond is also challenging. Therefore, hydrolytic kinetic resolution of racemic epoxides using Co(III)-salen complexes is used to produce enantioenriched terminal epoxides.[7]

Styrene monooxygenase (SMO) catalyzes the epoxidation of styrene and is involved in styrene metabolism in microorganisms.[8–10] Several SMO coding genes have been isolated from microorganisms and metagenomes, and the enzymatic properties of SMO have been examined.[10–12] This enzyme can convert various styrene derivatives to the corresponding (*S*)-epoxides with an excellent enantiomeric excess (*ee*),[13–15] and has been studied for the bioproduction of enantioenriched epoxides.[16–18]

Recently, we have reported the isolation of an SMO coding gene from *Rhodococcus* sp. ST-10[8,19] and characterized recombinant SMO from *Rhodococcus* sp. ST-10 (RhSMO) expressed in *Escherichia coli*.[20] Furthermore, we have developed a biocatalytic process for producing enantiopure epoxides using RhSMO and alcohol dehydrogenase (LSADH) from *Leifsonia* sp. S749.[21,22] The biocatalyst can catalyze the epoxidation of various alkenes to the corresponding (*S*)-epoxides in the presence of 2-propanol as a hydrogen donor. Moreover, the biocatalyst converts straight-chain terminal alkenes (e.g., 1-hexene and 1-octene) as well as arylalkenes to (*S*)-epoxides. There are some reports of enantioselective epoxidation of terminal aliphatic alkenes to primarily (*R*)-epoxyalkanes by microorganisms that possess alkane hydroxylase, alkene monooxygenase (AMO) or toluene monooxygenase activity,[23–25] whereas epoxidation of these substrates by SMO has not been reported. AMO from *Rhodococcus rhodochrous* (formerly *Nocardia corallina*) B-276 is a three-component enzyme system that is completely different from SMO.[26] To the best of our knowledge, RhSMO is the only SMO

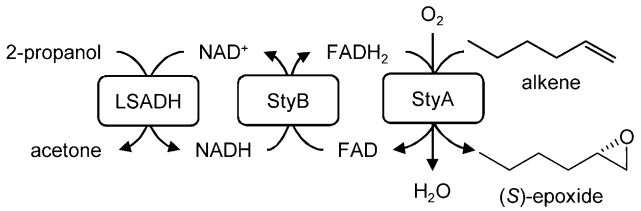
that catalyzes the epoxidation of terminal aliphatic alkenes to give (*S*)-epoxyalkanes.

In this study, we have systematically evaluated the substrate specificity and enantioselectivity of RhSMO for various aliphatic alkenes. We demonstrate that the RhSMO-LSADH co-expression system is effective for asymmetric epoxidation of various straight-chain alkenes. The biocatalyst exhibited broad substrate specificity for functionalized, unfunctionalized, terminal, and di- or tri-substituted alkenes, and converted them into (*S*)-epoxides with fair to excellent *ee* (64–99%).

Results and Discussion

Biocatalyst Development

RhSMO requires NADH as a hydrogen donor to convert alkene substrates to the corresponding epoxides. Thus, we employed LSADH, which reduces NAD^+ to NADH using 2-propanol as a hydrogen donor, to recycle NADH (Scheme 1). We constructed several biocatalysts with different RhSMO- and LSADH-expressing plasmids, and evaluated their productivity for converting 1-hexene to (*S*)-1,2-epoxyhexane (Table 1). All the biocatalysts catalyzed the epoxidation of 1-hexene, and the *E. coli* transformant cells that carried the pET-ST10 styAB and pRSFDuet-lsadh plasmids (entry 2) showed the highest production of (*S*)-1,2-epoxyhexane. This transformant exhibited the highest StyA and StyB activities but showed the lowest LSADH activity. In contrast, the StyA activity of entry 4 was an order of magnitude lower than that of the other transformants, and produced 16.7 mM of (*S*)-1,2-epoxyhexane, which was half the value of entry 2. The results suggested that the epoxide productivity is strongly affected by the StyB activity rather than the StyA and LSADH activities. In fact, (*S*)-1,2-epoxyhexane production level responded linearly to StyB activity (Table 1). StyB catalyzes the reduction of FAD to $FADH_2$, and supplies $FADH_2$ as an electron donor for the epoxidation reaction of StyA. We found that SMO activity increased with increasing StyB activity and FAD concentration in the reaction mixture (data not shown). Furthermore, the amounts of 2-propanol consumed and (*S*)-1,2-epoxyhexane produced did not agree. Presumably, epoxide production is limited by the supply of $FADH_2$ because the majority of $FADH_2$ generates H_2O_2 through its auto-oxidative reaction with oxygen.[27–29] Therefore, the biocatalyst in entry 2 was used for further bioconversion experiments.



Scheme 1. Epoxidation of aliphatic alkenes by the RhSMO-LSADH co-expressing system using 2-propanol as a hydrogen donor.

Effect of Organic Solvent on Epoxide Productivity

The bioproduction of organic compounds often suffers from low productivity, because of the low solubility of substrates and products in the aqueous phase and their toxicity for the biocatalysts. To overcome these problems, biphasic reaction systems using water and an organic solvent are often employed for organic synthesis with biocatalysts. Biphasic reaction systems are essential for epoxide production by biocatalysis because epoxides often readily decompose in aqueous solution. We have reported that the productivity of (*S*)-styrene oxide with RhSMO in biphasic reaction systems was strictly dependent on the organic solvents used. When acetate esters (octyl, hexyl, and pentyl acetate) were used as organic solvents, the RhSMO biocatalyst exhibited good productivity of (*S*)-styrene oxide.[22] Figure 1 shows that the productivity was strongly affected by the organic solvent in the bipha-

Table 1. Comparison of (*S*)-1,2-epoxyhexane production by biocatalyst systems.

Entry	Plasmid	StyA activity [mU/mg][a]	StyB activity [mU/mg][a]	LSADH activity [U/mg][a]	(*S*)-1,2-Epoxyhexane [mM][b]
1	pETDuet-ST10styAB-lsadh	15.3	526	2.73	19.8
2	pET-ST10styAB, pRSFDuet-lsadh	45.4	1170	0.78	30.9
3	pET-ST10styAB, pRSFDuet-ST10styAB-lsadh	22.9	980	1.02	25.1
4	pRSFDuet-ST10styAB-lsadh	0.9	222	1.39	16.7

[a] Enzyme activities are reported in units (U) per milligram of protein in cell-free extract.
[b] The amount of product in the organic phase [mM] (hexyl acetate plus extract with ethyl acetate) was used to calculate the product concentration in 1 mL of the organic phase.

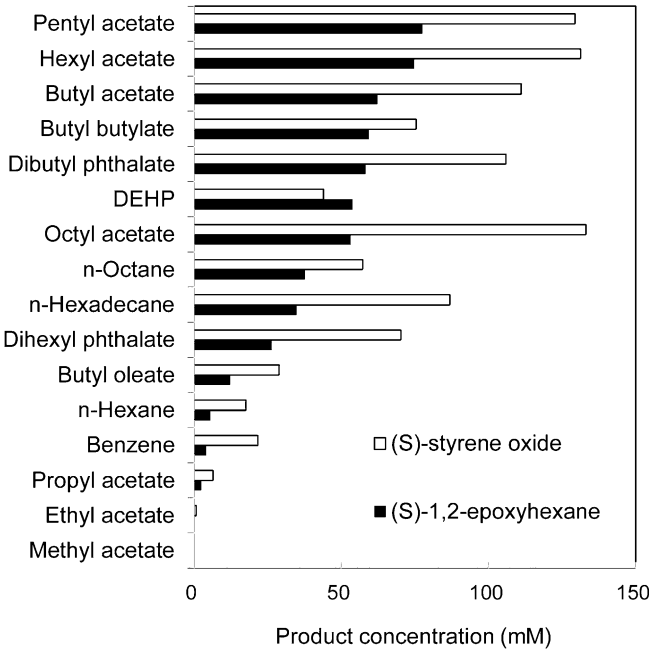


Figure 1. Effect of organic solvent on the production of (*S*)-1,2-epoxyhexane and (*S*)-styrene oxide in a biphasic reaction system. Each black bar [(*S*)-1,2-epoxyhexane] and white bar [(*S*)-styrene oxide] indicates the product concentration in the organic phase. The data for styrene are reproduced from the literature.[22]

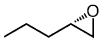
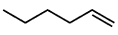
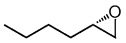
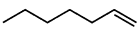
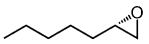
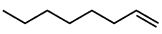
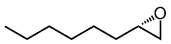
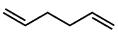
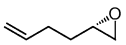
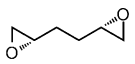
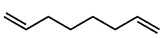
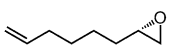
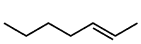
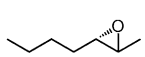
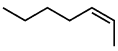
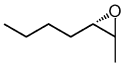
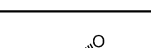
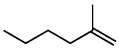
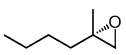
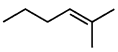
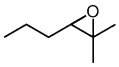
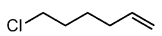
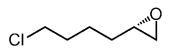
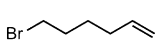
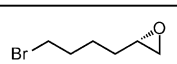
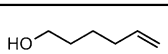
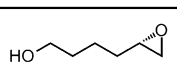
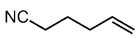
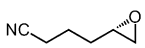
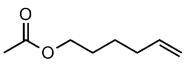
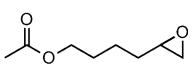
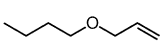
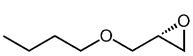
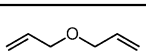
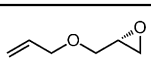
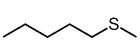
sic system. In this study, we also investigated the effect of the organic solvent on the production of (*S*)-epoxyhexane for comparison with (*S*)-styrene oxide. The highest production level of (*S*)-1,2-epoxyhexane was achieved with pentyl acetate and hexyl acetate, and was similar to the production of styrene. When more polar methyl, ethyl, and propyl acetate solvents were used, a negligible amount of the product was obtained. However, octyl acetate, which showed the highest production level for styrene oxide, produced less (*S*)-1,2-epoxyhexane than did pentyl and hexyl acetate. Bis(ethylhexyl) phthalate (DEHP) was a suitable solvent for (*S*)-1,2-epoxyhexane, whereas it resulted in low production of (*S*)-styrene oxide. These results suggest that the suitable organic solvent for the efficient bioproduction of epoxides depends on the properties and behavior of the substrate or product in the reaction system, including its solubility in the biphasic system in the case of aryl or aliphatic substrates; and on substrate or product inhibition of the biocatalyst.

Bioproduction of Various Chiral Epoxides

To investigate the substrate specificity of the biocatalyst, bioconversion experiments were performed using various alkenes as a substrate (Table 2). The *E. coli* biocatalyst possessing RhSMO and LSADH had broad substrate specificity and produced epoxides with fair to excellent *ee* (64–99%). When simple terminal alkene substrates **1–5** were used, the biocatalyst exhibited the highest production level and enantioselectivity for 1-hexene **3**, followed by 1-pentene **2**, 1-heptene **4**, propylene **1**, and 1-octene **5**. Likewise, 1,5-hexadiene **6** was a better substrate than 1,7-octadiene **7**, and only traces of product were observed for dienes consisting of chains longer than C_9 (data not shown). These results indicate that the reactivity and enantioselectivity of RhSMO may be affected by the size of the substrate, which could limit the incorporation of the substrate into the enzyme's active site. In the case of 1,5-hexadiene **6**, (2*S*,5*S*)-diepoxyhexane **6b** was the major product, the minor product was (*S*)-1,2-epoxy-5-hexene **6a**, and unreacted **6** was also detected. We speculated that **6a** is more soluble in the water phase than **6**, and thus the biocatalyst may be more accessible to **6a** and produce diepoxy compound **6b**. Consequently, the second epoxidation of **6a** may proceed faster than the first epoxidation of **6**.

Disubstituted alkenes **8–11** were generally good substrates for RhSMO, with the exception of *trans*-3-heptene **10**. The relatively low yields from non-substituted linear alkenes were attributed to the lower reactivity of these substrates compared with substituted linear or arylalkenes for RhSMO, in other words, the lower reactivity of the active peroxo-/hydroxyoxoflavin intermediates in electrophilic epoxidation by SMO.[27,28] However, RhSMO exhibited the lowest enantioselectivity for *cis*-2-heptene (64.1% *ee*) of the substrates tested. A similar trend was observed for styrene derivatives; the enzyme showed slightly lower reactivity and enantioselectivity for *cis*-β-methylstyrene than for α-methyl-/*trans*-β-methylstyrene.[22] Generally, the asymmetric epoxidation of unfunctionalized and unconjugated disubstituted alkenes by chemical synthesis is difficult because of their electron deficiency and lack of stereochemical features. Therefore, there are few reports of chemical asymmetric epoxidation of non-activated *cis*-alkenes, such as *cis*-2-heptene **9**.[32] Katsuki and co-workers assumed that the enantioselectivity of the epoxidation of *cis*-alkenes depends on the stereochemistry and π-electron interaction between the catalyst and substrates.[33] Likewise, Page and co-workers reported that an iminium salt catalyst catalyzed the epoxidation of non-aryl *cis*-alkenes, although its enantioselectivity was low.[34] These studies strongly suggest that chemical reactions with synthetic catalysts are not suitable for the asymmetric epoxidation of unfunctionalized non-aryl *cis*-alkenes. On the other hand, the enantiomeric epoxidation of non-activated *cis*-alkenes using biocatalysts has been reported.[35–37] Thus, fine tuning of RhSMO's active site through protein engineering may increase

Table 2. Bioconversion of aliphatic alkenes and sulfide by SMO biocatalyst.

	Substrate		Product	Product [mM][a]/ Conversion [%][b]	Yield [%][c]	Absolute configuration, *ee* [%]
1		**1a**		2.5/ND	ND	ND
2		**2a**		23.6/11.8	3.2	(*S*), 77.8[d]
3		**3a**		44.6/22.3	9.5	(*S*), 94.8
4		**4a**		15.4/7.7	4.8	(*S*), 84.0
5		**5a**		1.7/0.9	<0.1	(*S*), 86.5
6		**6a**		19.4/9.7	3.3	(2*S*), 98.9[d]
		6b		41.4/20.7	15.4	(2*S*,5*S*), 97.6
7		**7a**		8.6/4.3	2.7	(*S*), 91.9
8		**8a**		94.2/47.1	12.1	(2*S*,3*S*), >99.9[e]
9		**9a**		51.8/25.9	5.6	(2*R*,3*S*), 64.1[e]
10		**10a**		3.2/1.6	<0.1	(3*S*,4*S*), 80.3[e]
11		**11a**		24.2/12.1	7.3	(*S*), 90.2[f]
12		**12a**		105.7/52.9	26.5	ND, 90.4
13		**13a**		35.8/17.9	12.4	(*S*), 95.8
14		**14a**		18.6/9.3	7.5	(*S*), 98.8
15		**15a**		55.9/27.9	21.0	(*S*), >99.9
16		**16a**		18.2/9.1	5.8	(*S*), 99.8
17		**17a**		1.2/0.6	<0.1	ND
18		**18a**		0.8/0.4	<0.1	(*S*), 87.4
19		**19a**		34.1/17.1	13.2	(*S*), 91.0
20		**20a**		60.3/30.1	24.7	(*S*), 21.0[f,g]

the productivity and stereoselectivity of the epoxidation of non-aryl *cis*-alkenes.

1,1-Di- and tri-substituted alkenes **11** and **12** were also converted to the corresponding epoxides by RhSMO with good enantioselectivity. In addition to unfunctionalized alkenes, RhSMO converted various functionalized alkenes (**13**–**19**) with good enantioselectivity. 5-Hexen-1-ol **15** exhibited the highest reactivity and enantioselectivity for RhSMO. In contrast, halogenated alkenes **13** and **14** showed slightly lower reactivities and enantioselectivities. Furthermore, RhSMO showed negligible reactions with 5-hexenyl acetate **17** and butyl allyl ether **18**, presumably because these compounds did not fit into the active site of RhSMO. From these results, we surmised that aliphatic alkenes with electronegative, polar, and large substituents affect the reactivity and enantioselectivity of RhSMO. Interestingly, RhSMO also catalyzed the enantioselective sulfoxidation of sulfide **20** to (*S*)-sulfoxide, although the *ee* of the product was lower than that of the epoxyalkanes.

Compared with previously reported biocatalysts, the RhSMO system shows remarkable versatility in converting straight-chain alkenes to enantioenriched (*S*)-epoxyalkanes with high yields. To our knowledge, there are no reports of asymmetric epoxidation of aliphatic alkenes by SMO to the corresponding (*S*)-epoxides. Therefore, RhSMO shows superior catalytic properties to previously reported SMOs. We have demonstrated that the biocatalytic RhSMO system is a powerful tool for producing various enantiopure (*S*)-epoxyalkanes. Moreover, our biocatalytic system is environmentally friendly because it requires molecular oxygen as an oxidant and 2-propanol as a hydrogen donor instead of metals and oxidizing agents, such as peroxides, which are generally used in the chemical synthesis of epoxides.

Conclusions

We have successfully developed a biocatalytic system for synthesizing enantioenriched (*S*)-epoxides from aliphatic alkenes using molecular oxygen as an oxidant and 2-propanol as a hydrogen donor. The enzymatic activities of RhSMO and LSADH and the organic solvent in the biphasic reaction system were optimized for the production of epoxyalkanes. The biocatalytic system catalyzed the epoxidation of various straight-chain aliphatic alkenes (less than C_9), including those substituted with functional groups, with good enantioselectivity. Further improvement of the process would make it suitable for producing various aliphatic (*S*)-epoxyalkanes.

Experimental Section

Chemicals

Propylene was purchased from Sumitomo Seika. *rac*-Propylene oxide was purchased from Nacalai. 1,2-Epoxyhexane was purchased from Merck. 1-Pentene, *rac*-1,2-epoxypentane, 1,5-hexadiene, 1,7-octadiene, 2-methyl-1-hexene, *cis*-2-heptene, *trans*-3-heptene, 2-methyl-2-hexene, 6-bromo-1-hexene, 5-hexenyl acetate, allyl ether, *meta*-chloroperbenzoic acid, *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA), chlorotrimethylsilane, and Chirabite-AR were purchased from Tokyo Chemical Industry. (*R*)-1,2-Epoxyhexane, (*S*)-1,2-epoxyoctane, (*S,S*)-(−)-*N,N′*-bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexanediaminocobalt(II), and (*R,R*)-(−)-*N,N′*-bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexanediaminocobalt(II) were purchased from Sigma. 1-Hexene, 1-heptene, 1-octene, 1,2-epoxyoctane, *rac*-1,2-epoxy-5-hexene, *rac*-1,2,5,6-diepoxyhexane, *rac*-1,2-epoxy-7-heptene, *rac*-1,2,7,8-diepoxyoctane, *trans*-2-heptene, 6-chloro-1-hexene, 5-hexenenitrile, 5-hexen-1-ol, amyl methyl sulfide, allyl butyl ether, *n*-butyl glycidyl ether, allyl glycidyl ether, and all other chemicals were purchased from Wako Pure Chemical Industries.

HPLC Analysis

To determine the absolute configuration of the epoxidation products of 1-pentene and 1,5-hexadiene, the purified products were ring-opened with 2-naphthalenethiol by using the method reported by Kulkarni et al.[38] Chiral HPLC analysis was performed on an LC-10 HPLC system (Shimadzu) equipped with a Chiracel OD-H column (Diacel). The mobile phase was hexane/2-propanol with a flow rate of 1 mL min^{-1} (see the Supporting Information). The column

[a] Bioconversion was performed in a biphasic reaction system with hexyl acetate. The amount of product in the organic phase [mM] (hexyl acetate plus extract with ethyl acetate) was used to calculate the product concentration in 1 mL of the organic phase.
[b] Conversion [%] = [mol (product in organic phase)/mol (substrate)] × 100.
[c] Yield [%] = [mol (purified product)/mol (substrate)] × 100.
[d] Absolute configurations and *ee* were estimated by HPLC analysis of the 2-naphthyl sulfide derivatives of product. ND: not determined.
[e] Products were reduced by $LiAlH_4$ and absolute configurations were determined by the comparison with the corresponding 2- or 3-heptanol.[30]
[f] Absolute configurations were determined by comparing the optical rotations with reported values.
[g] Enantiomeric excess values were estimated from NMR spectra in the presence of the chiral shift reagent Chirabite-AR.[31]

was kept at 40 °C and the products were monitored at 254 nm.

GC-MS Analysis

GC-MS analysis was performed on a QP-5000 mass spectrometer (Shimadzu) equipped with a GC-17 A GC system (Shimadzu). The sample was injected into a TC-70 capillary column (0.25 mm × 60 m, 0.25 μm film, GL Science). The carrier gas was He at a flow rate of 1.7 mL min^{-1} (14.5 psi), the split ratio was 26, and the linear velocity was 47.4 cm s^{-1}.

NMR and Optical Rotation Analysis

^{1}H NMR spectra were recorded on an AV400 spectrometer (Bruker). All signals are expressed as parts per million (ppm) with TMS or residual undeuterated solvent as references. Optical rotations were measured with a P-1030 polarimeter (JASCO).

Synthesis of Authentic Compounds

All racemic epoxides used for product identification were synthesized using *meta*-chloroperbenzoic acid (*m*-CPBA) through the method reported by Sharma et al.[39] Chiral epoxides used for identifying the absolute configuration of the bioproduced epoxides were prepared as previously reported.[6,7] The authentic epoxides were purified by silica gel chromatography and used for the following analyses.

Preparation of the Biocatalyst

The genes coding RhSMO (StyA and StyB) and LSADH were amplified by PCR and treated with restriction endonucleases. Each DNA fragment was purified by agarose gel electrophoresis and cloned into its expression vector. The resulting plasmids were introduced into *E. coli* BL21(DE3), which contained the chaperone carrying plasmid pG-KJE8 (TaKaRa Bio Inc.), and the *E. coli* biocatalyst was prepared according to our previously reported method.[22]

Measurement of Enzyme Activities

Recombinant *E. coli* cells were harvested by centrifugation at 33,800 g for 10 min. Cell pellets were resuspended in 50 mM potassium phosphate buffer (KPB, pH 7.5) and disrupted by sonication for 10 min on ice. The cell lysates were centrifuged at 33,800 g for 10 min, and cell-free extracts were recovered. Cell-free extracts were used for measuring enzyme activities. StyA and StyB activities were measured according to our previously reported methods.[20] One unit (U) of StyA or StyB activities were defined as the amount of the enzyme that converted 1 μmol of styrene or NADH to (*S*)-styrene oxide or NAD^+ per minute at 30 °C.

Biotransformation Experiment

The biocatalysts were resuspended (20% wet w/v) in 50 mM KPB (1 mL, pH 7.5) containing 1 mM NAD^+ and 0.2 mM FAD. The cell suspension (1 mL), a 200 mM organic solvent solution of the substrate (1 mL), and 2-propanol (0.12 mL) were transferred to a 50-mL tube. The tube was filled with pure oxygen gas and tightly sealed. The reaction was performed with vertical shaking (300 rpm) at 25 °C for 24 h, according to our previously reported method.[22] Propylene was dissolved in the organic solvent by blowing, and used as a substrate. After the reaction ended, the organic phase was isolated by centrifugation and the remaining product in the water phase was extracted with ethyl acetate. The organic phase and extracted product were collected and dried with anhydrous Na_2SO_4. The amounts of product and substrate were measured by GC or HPLC.

Analytical Data for Purified Products

After the biotransformation, the recovered product was purified by silica gel chromatography (0.9 × 10 cm), and the respective eluates concentrated under vacuum. The products were analyzed by GC-EI-MS, ^{1}H NMR, and polarimetry.

(*S*)-1,2-Epoxypentane (2a): Colorless liquid, (77.8% *ee*); $[\alpha]_D^{24.5}$: −9.6 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.94–2.89 (m, 1H), 2.75 (dd, *J* = 4.0, 5.0 Hz, 1H), 2.47 (dd, *J* = 2.8, 5.0 Hz, 1H), 1.57–1.42 (m, 4H), 0.97 (t, *J* = 7.2 Hz, 3H); GC-EI-MS: *m*/*z* (%) = 85 (2), 71 (49), 56 (16), 41 (100), 29 (53).

(*S*)-1,2-Epoxyhexane (3a): Colorless liquid, (94.8% *ee*); $[\alpha]_D^{24.5}$: −9.1 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.91 (ddt, *J* = 2.8, 3.4, 5.8 Hz, 1H), 2.75 (dd, *J* = 4.0, 5.0 Hz, 1H), 2.47 (dd, *J* = 2.8, 5.0 Hz, 1H), 1.57–1.33 (m, 6H), 0.92 (t, *J* = 7.2 Hz, 3H); GC-EI-MS *m*/*z* (%) = 99 (1), 85 (5), 71 (80), 55 (48), 42 (100), 29 (83).

(*S*)-1,2-Epoxyheptane (4a): Colorless liquid, (84.0% *ee*); $[\alpha]_D^{24.8}$: −8.3 (*c* 1.01, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.93–2.89 (m, 1H), 2.75 (dd, *J* = 4.0, 5.0 Hz, 1H), 2.46 (dd, *J* = 2.8, 5.0 Hz, 1H), 1.56–1.28 (m, 8H), 0.90 (m, 3H);

(*S*)-1,2-Epoxyoctane (5a): Colorless liquid, (86.5% *ee*); $[\alpha]_D^{23.7}$: −8.9 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.93–2.89 (m, 1H), 2.75 (dd, *J* = 4.0, 5.0 Hz, 1H), 2.47 (dd, *J* = 2.8, 5.0 Hz, 1H), 1.56–1.27 (m, 10H), 0.89 (t, *J* = 6.9 Hz, 3H); GC-EI-MS *m*/*z* (%) = 127 (1), 113 (1), 99 (3), 71 (58), 55 (52), 41 (100), 29 (74).

(2*S*)-1,2-Epoxy-5-hexene (6a): Colorless liquid, (98.9% *ee*); $[\alpha]_D^{23.9}$: −6.5 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.85 (ddt, *J* = 6.7, 10.3, 17.0 Hz, 1H), 5.07 (dq, *J* = 1.7, 17.1 Hz, 1H), 5.00 (ddt, *J* = 1.3, 1.8, 10.2 Hz, 1H), 2.95 (m, 1H), 2.76 (dd, *J* = 4.1, 4.9 Hz, 1H), 2.50 (dd, *J* = 2.7, 5.0 Hz, 1H), 2.23 (m, 2H), 1.65 (m, 2H); GC-EI-MS: *m*/*z* (%) = 97 (2), 79 (4), 67 (36), 54 (48), 39 (83), 29 (100).

(2*S*,5*S*)-1,2,5,6-Diepoxyhexane (6b): Colorless liquid, (97.6% *ee*); $[\alpha]_D^{24.3}$: −26.1 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 3.00–2.97 (m, 2H), 2.78 (dd, *J* = 4.0, 4.9 Hz, 2H), 2.50 (dd, *J* = 2.7, 5.0 Hz, 2H), 1.85–1.59 (m, 4H); GC-EI-MS: *m*/*z* (%) = 114 (1), 95 (1), 83 (13), 69 (12), 55 (96), 41(90), 27 (100).

(2*S*)-1,2-Epoxy-7-octene (7a): Colorless liquid, (91.9% *ee*); $[\alpha]_D^{24.5}$: −10.4 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.81 (ddt, *J* = 6.7, 10.3, 17.0 Hz, 1H), 5.01 (ddd, *J* = 1.6, 1.9, 17.1 Hz, 1H), 4.95 (m, 1H), 2.91 (m, 1H), 2.75 (dd, *J* = 4.0, 5.0 Hz, 1H), 2.47 (dd, *J* = 2.8, 5.0 Hz, 1H), 2.08 (m, 2H), 1.49 (m, 6H); GC-EI-MS: *m*/*z* (%) = 107 (1), 93 (11), 79 (15), 67 (54), 54 (91), 41 (100), 29 (93).

(2*S*,3*S*)-*trans*-2,3-Epoxyheptane (8a): Colorless liquid, (> 99.9% *ee*); $[\alpha]_D^{24.5}$: −41.5 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.74 (dq, *J* = 2.3, 5.2 Hz, 1H), 2.63 (dt, *J* = 2.3, 5.6 Hz, 1H), 1.55–1.50 (m, 2H), 1.48–1.32 (m, 4H), 1.29 (d,

J=5.2 Hz, 3H), 0.91 (t, J=7.1 Hz, 3H); GC-EI-MS: m/z (%)=114 (1), 99 (7), 85 (34), 71 (20), 55 (58), 43 (100).

(2*R*,3*S*)- *cis*-2,3-Epoxyheptane (9a): Colorless liquid, (51.7% *ee*); $[\alpha]_D^{24.5}$: 0.41 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ=3.07–0.02 (m, 1H), 2.92–2.88 (m, 1H), 1.57–1.36 (m, 6H), 1.26 (d, J=5.6 Hz, 3H), 0.93 (t, J=6.9 Hz, 3H); GC-EI-MS: m/z (%)=114 (1), 99 (6), 85 (33), 71 (20), 55 (59), 43 (100).

(3*S*,4*S*)-*trans*-3,4-Epoxyheptane (10a): Colorless liquid, (37.5% *ee*); $[\alpha]_D^{24.3}$: −24.49 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ=2.70–2.63 (m, 2H), 1.58–1.40 (m, 6H), 0.98 (t, J=7.5 Hz, 3H), 0.96 (t, J=7.1 Hz, 3H); GC-EI-MS: m/z (%)=114 (1), 99 (2), 85 (8), 72 (27), 57 (58), 41 (100).

(*S*)-2-Methyl-1,2-epoxyhexane (11a): Colorless liquid, (90.2% *ee*); $[\alpha]_D^{24.3}$: 7.88 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ=2.61 (dd, J=0.3, 4.8 Hz, 1H), 2.57 (dd, J=0.7, 4.9 Hz, 1H), 1.63–1.27 (m, 6H), 1.31 (s, 3H), 0.91 (t, J=7.1 Hz, 3H); GC-EI-MS: m/z (%)=114 (1), 99 (5), 85 (75), 72 (36), 55 (100), 41 (97).

2,2-Dimethyl-3-propyloxirane (12a): Colorless liquid, (90.4% *ee*); ^{1}H NMR ($CDCl_3$, 400 MHz): δ=2.73 (t, J=5.9 Hz, 1H), 1.57–1.41 (m, 4H), 1.32 (s, 3H), 1.27 (s, 3H), 0.97 (t, J=7.2 Hz, 3H); GC-EI-MS m/z (%)=99 (4), 85 (32), 72 (9), 59 (76), 41 (100), 27 (59).

(*S*)-6-Chloro-1,2-epoxyhexane (13a): Colorless liquid, (95.8% *ee*); $[\alpha]_D^{24.3}$: −8.1 (c 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ=3.56 (t, J=6.6 Hz, 2H), 2.94–2.90 (m, 1H), 2.76 (dd, J=4.2, 4.9 Hz, 1H), 2.48 (dd, J=2.7, 5.0 Hz, 1H), 1.84 (m, 2H), 1.68–1.47 (m, 4H); GC-EI-MS: m/z (%)=102 (1), 85 (8), 71 (100), 55 (45), 41 (85), 27 (66).

(*S*)-6-Bromo-1,2-epoxyhexane (14a): Colorless liquid, (98.8% *ee*); $[\alpha]_D^{24.3}$: −7.00 (*c* 1.00, $CHCl_3$): ^{1}H NMR ($CDCl_3$, 400 MHz): δ=3.43 (t, J=6.7 Hz, 2H), 2.95–2.90 (m, 1H), 2.77 (t, J=4.5 Hz, 1H), 2.49 (dd, J=2.7, 5.0 Hz, 1H), 1.97–1.90 (m, 2H), 1.68–1.46 (m, 4H); 107(2), 81(8), 71(80), 55(21), 41(100), 27(62).

(5*S*)-5,6-Epoxy-1-hexanol (15a): Colorless liquid, (> 99.9% *ee*); $[\alpha]_D^{24.7}$: −12.8 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ=3.67 (t, J=6.3 Hz, 2H), 2.93 (m, 1H), 2.76 (dd, J=4.0, 5.0 Hz, 1H), 2.49 (dd, J=2.8, 5.0 Hz, 1H), 1.68–1.48 (m, 6H); GC-EI-MS: m/z (%)=97 (7), 83 (12), 67 (29), 57 (59), 41 (75), 31 (100).

(*S*)-4-Oxiran-2-ylbutanenitrile (16a): Light brown liquid, (99.8% *ee*); $[\alpha]_D^{24.8}$: −20.7 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ=2.96–2.92 (m, 1H), 2.78 (dd, J=2.6, 4.9 Hz, 1H), 2.52 (dd, J=2.6, 4.9 Hz, 1H), 2.47–2.43 (m, 2H), 1.92–1.81 (m, 3H), 1.60–1.50 (m, 1H); GC-EI-MS: m/z (%)=110 (3), 82 (7), 71 (5), 55 (58), 41 (100), 27 (51).

1-Methylcarbonyloxy-5,6-epoxyhexane (17a): Colorless liquid; ^{1}H NMR ($CDCl_3$, 400 MHz): δ=4.08 (t, J=6.6 Hz, 2H), 2.94–2.90 (m, 1H), 2.77 (dd, J=4.0, 5.0 Hz, 1H), 2.48 (dd, J=2.7, 5.0 Hz, 1H), 2.06 (s, 3H), 1.73–1.47 (m, 6H); GC-EI-MS: m/z (%)=114 (1), 97 (7), 85 (12), 67 (14), 55 (12), 43 (100), 29 (20).

***n*-Butyl glycidyl ether (18a):** Colorless liquid, (87.4% *ee*); $[\alpha]_D^{24.8}$: −12.4 (*c* 1.00, EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz): δ=3.71 (dd, J=3.1, 11.5 Hz, 1H), 3.50 (ddt, J=6.6, 9.2, 19.8 Hz, 2H), 3.39 (dd, J=5.8, 11.5 Hz, 1H), 3.17–3.13 (m, 1H), 2.80 (dd, J=4.2, 5.0 Hz, 1H), 2.61 (dd, J=2.7, 5.0 Hz, 1H), 1.61–1.54 (m, 2H), 1.43–1.33 (m, 2H), 0.92 (t, J=7.4 Hz, 3H); GC-EI-MS: m/z (%)=99 (1), 87 (1), 73 (5), 57 (46), 41 (37), 29 (100).

(*S*)-Allyl glycidyl ether (19a): Colorless liquid, (91.0% *ee*); $[\alpha]_D^{24.7}$: −10.5 (*c* 1.00, EtOH), ^{1}H NMR ($CDCl_3$, 400 MHz): δ=5.92 (ddt, J=5.7, 10.4, 17.2 Hz, 1H), 5.30 (dq, J=1.6, 17.2 Hz, 1H), 5.20 (ddt, J=1.3, 1.6, 10.4 Hz, 1H), 4.05 (dddt, J=1.4, 5.8, 12.8, 20.1 Hz, 2H), 3.73 (dd, J=3.1, 11.4 Hz, 1H), 3.41 (dd, J=5.8, 11.4 Hz, 1H), 3.17 (dquin, J=2.9, 4.2 Hz, 1H), 2.81 (dd, J=4.2, 5.0 Hz, 1H), 2.63 (dd, J=2.7, 5.0 Hz, 1H); GC-EI-MS: m/z (%)=83 (1), 71 (2), 57 (61), 41 (93), 29 (100).

(*S*)-Amyl methyl sulfoxide (20a): Yellow liquid, (21.0% *ee*); $[\alpha]_D^{24.9}$: +22.6 (*c* 1.00, EtOH); ^{1}H NMR ($CDCl_3$, 400 MHz): δ=2.79–2.62 (m, 2H), 2.57 (s, 3H), 1.81–1.73 (m, 2H), 1.53–1.33 (m, 4H), 0.93 (t, J=7.1 Hz, 3H); GC-EI-MS: m/z (%)=117 (10), 78 (1), 64 (20), 55 (10), 43 (100), 29 (35).

References

[1] S. Chang, J. M. Galvin, E. N. Jacobsen, *J. Am. Chem. Soc.* **1994**, *116*, 6937–6938.
[2] T. Katsuki, K. B. Sharpless, *J. Am. Chem. Soc.* **1980**, *102*, 5974–5976.
[3] Y. Tu, Z.-X. Wang, Y. Shi, *J. Am. Chem. Soc.* **1996**, *118*, 9806–9807.
[4] R. Irie, K. Noda, Y. Ito, T. Katsuki, *Tetrahedron Lett.* **1991**, *32*, 1055–1058.
[5] R. M. Hanson, K. B. Sharpless, *J. Org. Chem.* **1986**, *51*, 1922–1925.
[6] L. Shu, Y. Shi, *Tetrahedron* **2001**, *57*, 5213–5218.
[7] S. E. Schaus, B. D. Brandes, J. F. Larrow, M. Tokunaga, K. B. Hansen, A. E. Gould, M. E. Furrow, E. N. Jacobsen, *J. Am. Chem. Soc.* **2002**, *124*, 1307–1315.
[8] H. Toda, N. Itoh, *J. Biosci. Bioeng.* **2012**, *113*, 12–19.
[9] S. Hartmans, M. J. van der Werf, J. A. de Bont, *Appl. Environ. Microbiol.* **1990**, *56*, 1347–1351.
[10] A. M. Marconi, F. Beltrametti, G. Bestetti, F. Solinas, M. Ruzzi, E. Galli, E. Zennaro, *Appl. Environ. Microbiol.* **1996**, *62*, 121–127.
[11] E. W. van Hellemond, D. B. Janssen, M. W. Fraaije, *Appl. Environ. Microbiol.* **2007**, *73*, 5832–5839.
[12] F. Beltrametti, A. M. Marconi, G. Bestetti, C. Colombo, E. Galli, M. Ruzzi, E. Zennaro, *Appl. Environ. Microbiol.* **1997**, *63*, 2232–2239.
[13] P. Di Gennaro, A. Colmegna, E. Galli, G. Sello, F. Pelizzoni, G. Bestetti, *Appl. Environ. Microbiol.* **1999**, *65*, 2794–2797.
[14] A. Schmid, K. Hofstetter, H. J. Feiten, F. Hollmann, B. Witholt, *Adv. Synth. Catal.* **2001**, *343*, 732–737.
[15] D. Tischler, D. Eulberg, S. Lakner, S. R. Kaschabek, W. J. H. van Berkel, M. Schlomann, *J. Bacteriol.* **2009**, *191*, 4996–5009.
[16] D. Kuhn, B. Buhler, A. Schmid, *J. Ind. Microbiol. Biotechnol.* **2012**, *39*, 1125–1133.
[17] M. K. Julsing, D. Kuhn, A. Schmid, B. Buhler, *Biotechnol. Bioeng.* **2012**, *109*, 1109–1119.
[18] B. Halan, A. Schmid, K. Buehler, *Biotechnol. Bioeng.* **2010**, *106*, 516–527.
[19] N. Itoh, K. Yoshida, K. Okada, *Biosci. Biotechnol. Biochem.* **1996**, *60*, 1826–1830.

[20] H. Toda, R. Imae, T. Komio, N. Itoh, *Appl. Microbiol. Biotechnol.* **2012**, 96, 407–418.
[21] N. Itoh, K. Isotani, M. Nakamura, K. Inoue, Y. Isogai, Y. Makino, *Appl. Microbiol. Biotechnol.* **2012**, *93*, 1075–1085.
[22] H. Toda, R. Imae, N. Itoh, *Tetrahedron: Asymmetry* **2012**, *23*, 1542–1549.
[23] S. W. May, M. S. Steltenkamp, R. D. Schwartz, C. J. McCoy, *J. Am. Chem. Soc.* **1976**, *98*, 7856–7858.
[24] K. Furuhashi, A. Taoka, S. Uchida, I. Karube, S. Suzuki, *Eur. J. Appl. Microbiol. Biotechnol.* **1981**, *12*, 39–45.
[25] K. McClay, B. G. Fox, R. J. Steffan, *Appl. Environ. Microbiol.* **2000**, *66*, 1877–1882.
[26] T. J. Smith, J. S. Lloyd, S. C. Gallagher, W. L. J. Fosdike, J. C. Murrell, H. Dalton, *Eur. J. Biochem.* **1999**, *260*, 446–452.
[27] K. Otto, K. Hofstetter, M. Rothlisberger, B. Witholt, A. Schmid, *J. Bacteriol.* **2004**, *186*, 5292–5302.
[28] V. Massey, *J. Biol. Chem.* **1994**, *269*, 22459–22462.
[29] R. Ruinatscha, C. Dusny, K. Buehler, A. Schmid, *Adv. Synth. Catal.* **2009**, *351*, 2505–2515.
[30] C. Chiappe, A. Cordoni, G. Lo Moro, C. D. Palese, *Tetrahedron: Asymmetry* **1998**, *9*, 341–350.
[31] T. Ema, D. Tanida, T. Sakai, *Org. Lett.* **2006**, *8*, 3773–3775.
[32] Y. Sawada, K. Matsumoto, T. Katsuki, *Angew. Chem.* **2007**, *119*, 4643–4645; *Angew. Chem. Int. Ed.* **2007**, *46*, 4559–4561.
[33] T. Hamada, R. Irie, T. Katsuki, *Synlett* **1994**, 479–481.
[34] P. C. B. Page, B. R. Buckley, H. Heaney, A. J. Blacker, *Org. Lett.* **2005**, *7*, 375–377.
[35] E. J. Allain, L. P. Hager, L. Deng, E. N. Jacobsen, *J. Am. Chem. Soc.* **1993**, *115*, 4415–4416.
[36] W. Kroutil, M. Mischitz, K. Faber, *J. Chem. Soc. Perkin Trans. 1* **1997**, 3629–3636.
[37] A. Zaks, D. R. Dodds, *J. Am. Chem. Soc.* **1995**, *117*, 10419–10424.
[38] B. A. Kulkarni, G. P. Roth, E. Lobkovsky, J. A. Porco, *J. Comb. Chem.* **2002**, *4*, 56–72.
[39] R. Sharma, P. G. Bulger, M. McNevin, P. G. Dormer, R. G. Ball, E. Streckfuss, J. F. Cuff, J. Yin, C.-Y. Chen, *Org. Lett.* **2009**, *11*, 3194–3197.

