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Microbial deracemization of α-substituted carboxylic acids: control of the reaction path

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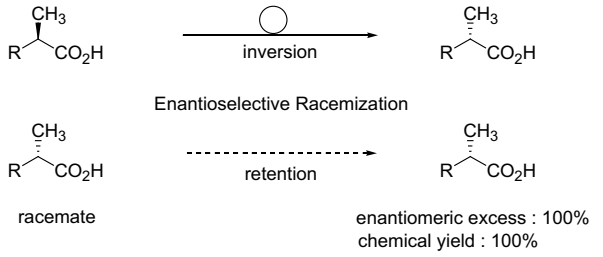
Abstract—A novel approach to preparing optically active α-substituted carboxylic acids using the whole cells of *Nocardia diaphanozonaria* JCM 3208 is described. When 2-phenylthiopropanoic acid and 2-methyl-3-phenylpropanoic acid were subjected to the reaction under aerobic conditions, the oxidation reaction proceeded preferentially rather than deracemization of these substrates. Herein, we report the design of reaction conditions to increase the deracemization activity in preference to oxidation reactions. In addition, we have successfully detected a metabolic intermediate in the reaction mixture of 2-methyl-3-phenylpropanoic acid, which indicates that the deracemization is a competitive reaction against the β-oxidation pathway of fatty acid metabolism.

1. Introduction

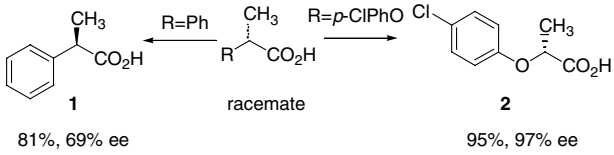
What is the best way for the preparation of optically active compounds starting from racemates? Although many scientists have made the effort to solve this problem, we still do not have a perfect answer at present. Recently, we and another group have introduced a new approach to preparing optically active α-substituted propanoic acids using a type of actinomycete.[1,2] This method is known as a deracemization reaction, which is a third option for obtaining optically active compounds after the following two techniques, that is, kinetic resolution and dynamic kinetic resolution.[3] Deracemization is a reaction, which inverts the configuration of either enantiomer of a racemate to the other antipode, resulting in an optically active compound starting from a racemic mixture (Scheme 1). Theoretically, this method is capable of giving the desired enantiomer in 100% yield and it means that the synthesis of racemates is almost equal to the synthesis of optically active compounds. This process can be realized by 'enantioselective racemization', because repetition of racemization of one enantiomer finally results in the accumulation of the other enantiomer.

We have already reported the deracemization reactions of 2-aryl- and 2-aryloxypropanoic acids using growing



Scheme 1.

cells of *Nocardia. diaphanozonaria* JCM 3208.[1] The enzyme system of *N. diaphanozonaria* catalyzes the inversion of the configuration of 2-phenylpropanoic acid **1** from (*S*)- to (*R*)-configuration (Scheme 2). In addition, the deracemization reaction also proceeds with 2-(4-chlorophenoxy)propanoic acid **2** to give the (*R*)-enantiomer in high enantiomeric excess. Surprisingly enough, the spacial arrangement of the ligands around the stereogenic center was opposite to that of **1**. Although the



Scheme 2.

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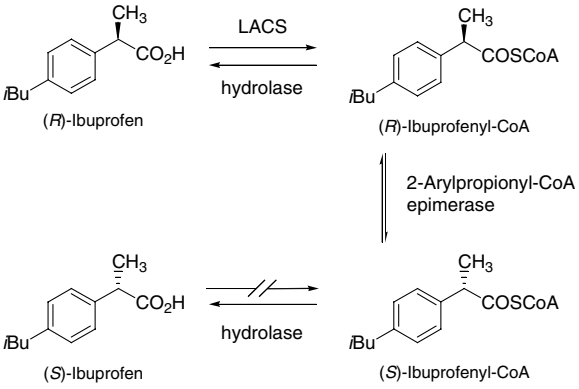
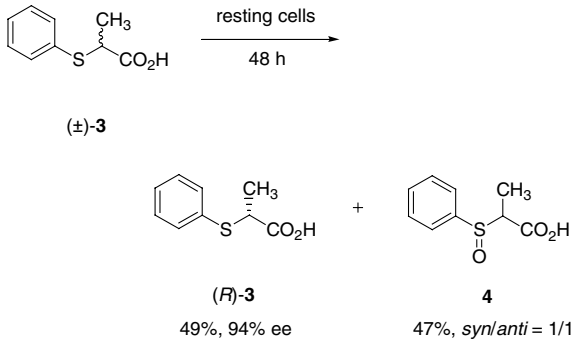


Scheme 3.



Scheme 4.

reason is not clear at present, it is very interesting to note that the insertion of only one atom brought about a dramatic change in enantioselectivity.

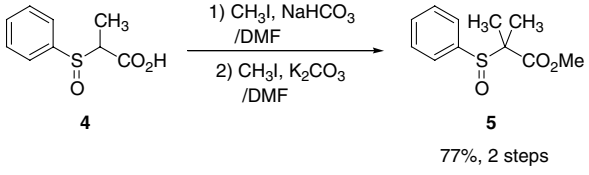
The deracemization reaction of 2-arylpropanoic acid has also been carried out using rat liver with the mechanistic studies already performed.[4] It was proposed that three enzymes related in this case and the (*R*)-2-arylpropanoic acid inverted to the (*S*)-form (Scheme 3). The initial step of this reaction is considered to involve the enantioselective formation of the coenzyme A (CoA) thioester of the (*R*)-acid with the aid of long-chain acyl-CoA synthetase (LACS). The thioester is subsequently epimerized and cleaved by a hydrolase to release the racemic acid. In these three steps, only the LACS-catalyzed reaction proceeded in an enantioselective manner. Thus, the enantiomeric ratio of the acid shifted to the (*S*)-form over the course of the reaction. These enzymes as well as the corresponding genes were purified and identified.

We supposed that deracemization using *N. diaphanozonaria* was likely to proceed via essentially the same reaction mechanism as that of rat liver. Based on the deuterium exchange experiments using 2-deuterio-2-phenylpropanoic acid, we have proposed that enolate-type intermediate was formed during the deracemization reaction.[1] On the other hand, inhibitory effect experiments using the typical substrate of acyl-CoA synthetase, that is, benzoic acid and *n*-alkanoic acids with various chain length suggested that medium-chain acyl-CoA synthetase (MACS) took part in this chiral inversion process. This result is in marked contrast with the case of rat liver. Herein we report the improvement in reaction conditions to suppress the metabolic reactions and to lead the reaction to the direction of the deracemization using the resting cells of *N. diaphanozonaria*. In addition, we will show some supporting evidences that the deracemization process is a competitive reaction against the fatty acid metabolism and proceeds via a part of β-oxidation pathway.

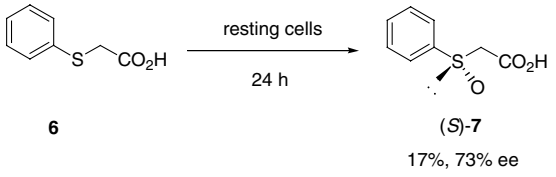
2. Results and discussion

Although the previously reported reaction conditions using the growing cells of *N. diaphanozonaria* are suitable for 2-aryl- and 2-aryloxypropanoic acid, degradation reactions proceeded preferentially in the case of other acids, which have sulfur, methylene, or amine at β-position.[1] In the case of 2-phenylthiopropanoic acid **3**, enantiomer-differentiating oxidation occurred on the sulfur atom (Scheme 4). While the (*R*)-enantiomer was recovered intact in good enantiomeric excess, the (*S*)-enantiomer was converted to 2-phenylsulfinylpropanoic acid **4**, which consisted of equal amounts of *syn*- and *anti*-isomers. To determine whether the oxidation reaction on the sulfur atom was enantioselective or not, the resulting 2-phenylsulfinylpropanoic acid **4** was converted to methyl 2-methyl-2-phenylsulfinylpropanoate **5** and analyzed by chiral phase HPLC (Scheme 5). It became clear that the sulfoxide was racemic thus indicating that the oxygenase did not differentiate the prochirality of the sulfide. This is surprising when compared to the fact that the reaction of 2-phenylthioacetic acid **6** proceeded in an enantioselective manner and (*S*)-**7** was obtained in 73% ee (Scheme 6). Although the yield was low, the rest of the recovered material is the starting compound, and the material balance is nearly quantitative.

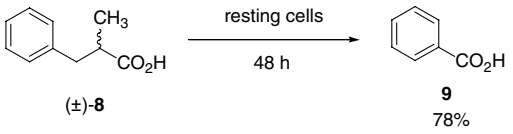
In the case of 2-methyl-3-phenylpropanoic acid **8**, a complete degradation reaction proceeded without enantioselectivity to give benzoic acid **9** (Scheme 7). The IR and NMR spectra, and melting point agreed with those



Scheme 5.



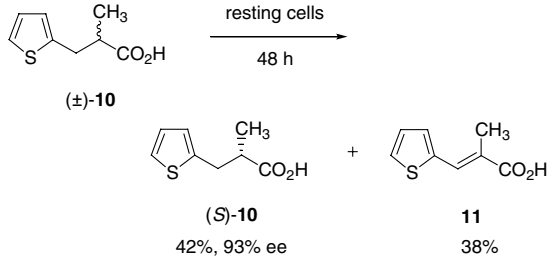
Scheme 6.



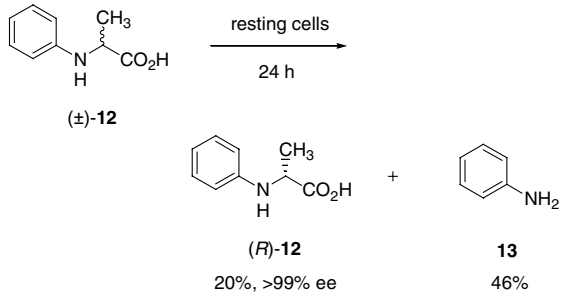
Scheme 7.

of an authentic sample. 2-Methyl-3-(2-thienyl)propanoic acid **10** was oxidized in an enantioselective manner to give α,β-unsaturated carboxylic acid **11** and the (*S*)-form enriched starting material (Scheme 8). Any other metabolic product, such as thiophenecarboxylic acid, could not be detected in the reaction mixture. 2-Aminophenylpropanoic acid **12** was converted to aniline **13**, also in an enantioselective manner, leaving behind the (*R*)-isomer in high ee (Scheme 9). These four substrates can be classified into two groups by the type of oxidation. The first group consists of 2-phenylthiopropanoic acid **3**, which is oxidized by oxygenase. The second group includes **8**, **10**, and **12**, which are thought to be oxidized by dehydrogenase in the first step.

At first, we tried to suppress the oxidation on the sulfur atom, because the oxidation was thought to be catalyzed by monooxygenase. This side reaction was suppressed by performing it under an inert gas. We also tested to see if the deracemization reaction itself proceeded under oxygen-free conditions using 2-(4-chlorophenoxy)propanoic acid **2**, which is one of the best substrates for this biocatalyst. When **2** was incubated with the resting cells of this bacterium under Ar, it took 96 h to complete the deracemization reaction (Table 1, entry 3), while only 24 h were needed to complete the reaction under the aerobic conditions. Thus, deracemization was confirmed to proceed in the absence of oxygen although the reaction

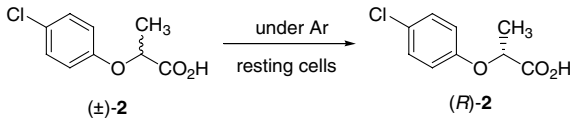


Scheme 8.



Scheme 9.

Table 1. Deracemization reaction of **2** under the inert gas conditions[a]

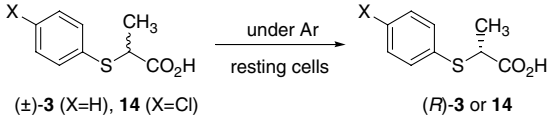


(±)-**2** (*R*)-**2**

Entry	Reaction time (h)	Yield (%)[b]	Ee (%)[c]
1	24	91	65
2	48	86	79
3	96	87	>99

[a] The starting compounds were incubated with the resting cells of *N. diaphanozonaria* under Ar at 30 °C.
[b] Isolated yield after conversion to the corresponding methyl ester.
[c] Ee of the product was determined by HPLC analysis after conversion to the corresponding methyl ester.

Table 2. Suppression of the oxidation reaction on the sulfur atom[a]



(±)-**3** (X=H), **14** (X=Cl) (*R*)-**3** or **14**

Entry	X	Reaction time (h)	Yield (%)[b]	Ee (%)[c]
1	H	24	68	28
2	H	48	79	42
3	H	96	68	88
4	H	240	9	92
5	Cl	48	83	21
6	Cl	96	77	31
7	Cl	168	88	54
8	Cl	240	88	90
9	Cl	336	77	91

[a] The starting compounds were incubated with the resting cells of *N. diaphanozonaria* under Ar at 30 °C.
[b] Isolated yield after conversion to the corresponding methyl ester.
[c] Ee of the product was determined by HPLC analysis after conversion to the corresponding methyl ester.

rate was greatly reduced. Also in the case of 2-phenylthiopropanoic acid **3** and 2-(4-chlorophenylthio)propanoic acid **14**, the enzyme system of *N. diaphanozonaria* showed the deracemization activity under Ar as expected (Table 2). The chiral inversion proceeded smoothly without the formation of other metabolic products. After 96-h incubation, the yield and the ee of **3** were 68% and 88% (*R*), respectively (Table 2, entry 3). In the case of **14**, the product was recovered in high enantiomeric excess (90% ee) after a 240-h incubation (Table 2, entry 8).

We then proceeded to the next stage to suppress the metabolic process of the second group. The key for directing the reaction toward deracemization was to take into consideration the possibility that the reaction proceeded via a common intermediate, an α,β-unsaturated carboxylic acid (Fig. 1). If we supposed that the enzyme

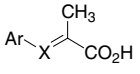
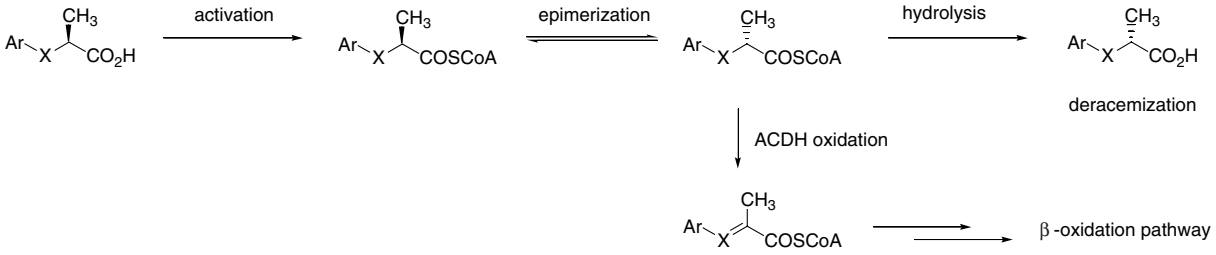


Figure 1. The common metabolic intermediate for **8**, **10**, and **12**.



Scheme 10.

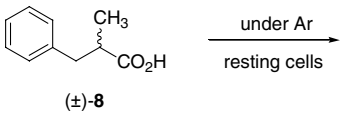
system of *N. diaphanozonaria* acted via the same reaction mechanism as in the case of rat liver, the first step of the deracemization reaction would be the activation of the carboxylic acid by the formation of a thioester linkage with CoA (Scheme 3). The acyl-CoA would be dehydrogenated by acyl-CoA dehydrogenase (ACDH) and further metabolized via β-oxidation pathway. It is not surprising that this undesired reaction took precedence over the deracemization reaction under aerobic conditions. In other words, the deracemization process may be a competitive reaction against the fatty acid metabolism (Scheme 10).

Thus, we focused our attention on an ACDH-catalyzed oxidation as it would be the initial step towards the β-oxidation pathway. If this oxidation step can be inhibited by some method, the following downstream will be directed to deracemization process. ACDH is a flavoenzyme and utilizes molecular oxygen for the coenzyme regeneration. Accordingly, the blocking of this coenzyme regeneration system was considered to be one of the effective options.

Thus, as the first trial, the reaction of 2-methyl-3-phenylpopanoic acid **8** with the resting cells of *N. diaphanozonaria* was carried out under Ar (Table 3). The ee of the starting material gradually increased and finally enantiomerically pure (*S*)-**8** and α-methylcinnamic acid **15** were recovered in the ratio of 50:50 (Table 3, entry 3). However, these two compounds could not be separated by column chromatography or chiral phase HPLC. Thus, the ee of the product was determined after selective removal of **15** as a benzaldehyde via the oxidative cleavage of the double bond with the aid of osmium tetroxide–sodium metaperiodite system. As **15** is considered as the metabolic intermediate of the β-oxidation pathway, formation of this compound indicates that the deracemization process is a competitive reaction against β-oxidation pathway.

Thus to inhibit the β-oxidation path more efficiently, the reaction of **8** was carried out in the presence of an ACDH inhibitor, such as 2-bromohexanoic acid and 2-bromooctanoic acid.[5] However, when compound **8** was incubated with the resting cells under Ar in the presence of 3.0 mM of these inhibitors, deracemization activity was lost completely, and only the racemic starting material was recovered. After several trials, we found that the deracemization reaction proceeded preferentially in the presence of an appropriate amount of inhibitor under aerobic conditions. 2-Methyl-3-phenylpropanoic acid **8** was recovered in its optically active form with a minimum amount of by-product (Table 4). The best result was obtained under the conditions in entry 1. In the presence of 1.2 mM of 2-bromohexanoic acid, **8** was actually deracemized and recovered in its (*S*)-form in 88% ee after 48 h incubation. As this result could not be realized via simple enantioselective degradation, it clearly shows the presence of chiral inversion process. In this case, only **15** was formed as by-product while **9** could not be detected in the reaction mixture. It is interesting that the deracemization activity was affected dramatically by the chain length of the inhibitors and their concentration (entry 1 vs 4, 6 vs 8).

Table 3. Reaction of **8** under Ar[a]



(±)-**8**

(*S*)-**8** **15**

Entry	Reaction time (h)	(*S*)-**8**		**15**
		Yield (%)[b]	Ee (%)[c]	Yield (%)[d]
1	48	60	33	20
2	96	53	91	40
3	240	42	>99	49

[a] The starting compounds were added to the resting cells of *N. diaphanozonaria* under Ar at 30 °C.
[b] Isolated yield after conversion to the corresponding methyl ester and selective removal of α-cinnamic acid under the condition of double bond cleavage oxidation.
[c] Ee of the product was determined by HPLC analysis after conversion to the corresponding methyl ester.
[d] Yields are calculated in comparison with the value of integration in ^{1}H NMR spectrum of the mixture of **8** and **15**.

In the case of 2-methyl-3-(2-thienyl)propanoic acid **10**, however, 2-bromohexanoic acid could not suppress the unsaturation reaction by ACDH (Table 5). α,β-Unsaturated product **11** was formed regardless of the presence or absence of 2-bromohexanoic acid (2-BH) while the concentration of the inhibitor had no effect. In the case of 2-aminophenylpropanoic acid **12**, the deracemization reaction did not become the major path even under an

Table 4. Suppression of the dehydrogenase reaction of **8** ACDH inhibitor[a]

Entry	Inhibitor[b]	Concn (mM)	(*S*)-**8**		**15**
			Yield (%)[c]	Ee (%)[d]	Yield (%)[e]
1	2-BH	1.2	71	88	26
2	2-BH	3.0	74	82	22
3	2-BH	6.1	78	65	19
4	2-BH	12.2	77	34	4
5	2-BO	0.63	73	35	12
6	2-BO	1.2	75	42	12
7	2-BO	3.0	71	8	3
8	2-BO	4.1	71	3	1

[a] The starting compounds were added to the resting cells of *N. diaphanozonaria* after the incubation with appropriate amount of ACDH inhibitor for 10 min at 30 °C.
[b] 2-BH: 2-bromohexanoic acid, 2-BO: 2-bromooctanoic acid.
[c] Isolated yield after conversion to the corresponding methyl ester and selective removal of α-cinnamic acid under the condition of double bond cleavage oxidation.
[d] Ee of the product was determined by HPLC analysis after conversion to the corresponding methyl ester.
[e] Yields are calculated in comparison with the value of integration in ^{1}H NMR spectrum of the mixture of **8** and **15**.

Table 5. Effect of ACDH inhibitor on the reaction of **10**[a]

Entry	Concn of 2-BH (mM)	Reaction time (h)	(*S*)-**10**		**11**
			Yield (%)[b]	Ee (%)[c]	Yield (%)[b]
1	0	3	ND	ND	ND
2	0	6	70	51	10
3	0	12	74	93	24
4	0	24	73	93	25
5	0	48	42	93	38
6	1.2	3	75	18	5
7	1.2	6	66	30	7
8	1.2	12	ND	ND	ND
9	1.2	24	70	93	27
10	1.2	48	48	93	34
11	3.0	48	52	93	36
12	6.0	48	79	54	16

[a] The starting compounds were added to the resting cells of *N. diaphanozonaria* after the incubation with 2-bromohexanoic acid (2-BH) for 10 min at 30 °C and incubated under aerobic conditions at 30 °C.
[b] Yields are calculated in comparison with the value of integration in ^{1}H NMR spectrum.
[c] Ee of the product was determined by HPLC analysis after conversion to the corresponding methyl ester.

atmosphere of an inert gas or in the presence of ACDH inhibitor (Table 6). When 2-BH was present, however, aniline **13** could not be detected in the reaction mixture although the ee of recovered **12** was 0%. This result indicates that under aerobic conditions, the metabolic reaction of **12** catalyzed by ACDH would give an imine intermediate, followed by hydrolysis to produce **13**.

Although, we could not find the reaction conditions to make the deracemization reaction major path for **10** and **12**, the investigation gave us some precious hints for the elucidation of reaction mechanism. First, the metabolic reaction will proceed via a common intermediate, an α,β-unsaturated thioester. Second, ACDH will catalyze the metabolic reaction after the chiral inversion step because of the following reason: it has already been reported that ACDH recognizes the chirality of α-methylacyl-CoA,[6] and works on the (*S*)-form. As the (*R*)-enantiomer of **8** is degraded or converted to the (*S*)-enantiomer, it is reasonable to suppose that the oxidative metabolism occurs after the racemization step, which is considered to be also playing an important role in the deracemization reaction.

Table 6. Metabolic reaction of **12** under the various conditions

Entry	Condition	Reaction time (h)	(*S*)-**12**		**13**
			Yield (%)[a]	Ee (%)[b]	Yield (%)[c]
1	Aerobic[d]	24	20	>99	46
2	Ar[e]	48	37	65	ND
3	2-BH[f]	48	69	0	0
4	2-BO[g]	48	42	19	ND

[a] Isolated yield after conversion to the corresponding methyl ester.
[b] Ee of the product was determined by HPLC analysis after conversion to the corresponding methyl ester.
[c] Isolated yield after conversion to acetanilide.
[d] The starting compounds were incubated with resting cells of *N. diaphanozonaria* under aerobic conditions at 30 °C.
[e] The starting compounds were incubated with resting cells of *N. diaphanozonaria* under Ar at 30 °C.
[f] The starting compounds were added to the resting cells of *N. diaphanozonaria* after the incubation with 1.2 mM 2-bromohexanoic acid for 10 min at 30 °C.
[g] The starting compounds were added to the resting cells of *N. diaphanozonaria* after the incubation with 1.2 mM 2-bromooctanoic acid for 10 min at 30 °C.

3. Conclusion

We have succeeded in establishing the reaction conditions necessary to deracemize 2-phenylthiopropanoic acid **3** and 2-methyl-3-phenylpropanoic acid **8**, and thus expand the applicability of the biotransformation system of *N. diaphanozonaria*. While, the degradation reactions proceeded preferentially under aerobic conditions for these substrates, when **3** and its derivative **14** were subjected to the resting cells of *N. diaphanozonaria* under Ar, the deracemization reaction became the major pathway. In the case of **8**, the enzyme system of the microorganism showed the deracemization activity in the presence of an ACDH inhibitor. In addition, a key intermediate of the fatty acid metabolism, α-methylcinnamic acid **15**, was also detected in the reaction mixture, which suggests that the deracemization process is a competitive reaction against the β-oxidation pathway. Further investigation on the reaction path and enzyme purification is now underway.

4. Experimental

4.1. General

Starting materials and reagents were purchased and used without purification unless otherwise noted. Analytical thin layer chromatography (TLC) was developed

on E. Merck Silica Gel 60 F256 plates (0.25 mm thickness). Column chromatography was performed on Katayama Chemical Co., Inc. Silica Gel 60 K070-WH (70–230 mesh). NMR spectra were obtained on JEOL AL-300, GX-400 spectrometers (^{1}H at 300, 400 MHz and ^{13}C at 100 MHz) at ambient temperature. ^{1}H chemical shifts were referenced with $CDCl_3$ at 7.26 ppm and ^{13}C chemical shifts with $CHCl_3$ at 77.0 ppm or acetone-d_6 at 29.8 ppm. IR spectra were measured as films for oils and as KBr discs for solids with a JASCO FT/IR-410 instrument. Optical rotations were measured on a JASCO DIP 360 polarimeter. The strain, *N. diaphanozonaria* JCM3208, used in this experiment is available from the Japan Collection of Microorganism: The Institute of Physical and Chemical Research (Riken), 2-1 Hirosawa, Wako 351-0106, Japan.

4.2. Synthesis of substrates

2-(4-Chlorophenoxy)propanoic acid **2**, 2-phenylthiopropanoic acid **3**, 2-methyl-3-phenylpropanoic acid **8**, and 2-(4-chlorophenylthio)propanoic acid **14** were synthesized according to the same procedure described.[1]

4.2.1. 2-Methyl-3-(2-thienyl)propanoic acid 10. Sodium ethoxide (1.10 g, 16.2 mmol) was dissolved in EtOH (6 mL) at 0 °C. After stirring for 5 min, the mixture was allowed to warm to room temperature. A solution of diethyl methylmalonate (2.00 g, 11.5 mmol) in EtOH (2 mL) was added dropwise over a period of 5 min and the mixture stirred for 30 min at the same temperature. Then, an ethanol solution (2 mL) of 2-chloromethylthiophene (3.30 g, 24.8 mmol), which was freshly prepared from 2-thiophenemethanol and thionyl chloride, was added. The mixture was heated under reflux for 15 h. The reaction mixture was cooled in an ice bath, acidified with 2 M hydrochloric acid and extracted with EtOAc. The organic layer was concentrated in vacuo. To a solution of KOH (2.6 g, 9.5 mmol) in water (15 mL), was added this oily residue dissolved in EtOH (15 mL). The mixture was stirred at room temperature for 11 h. The reaction mixture was cooled in an ice-water bath, acidified with 2 M hydrochloric acid and extracted with EtOAc. The organic layer was concentrated in vacuo. The resulting solid was dissolved in a mixture of water (50 mL) and concentrated sulfuric acid (5 mL), and heated under reflux for 30 h. The mixture was then cooled to room temperature and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. The residue was distilled (140 °C/200 Pa) to give 2-methyl-3-(2-thienyl)propanoic acid[7] (1.54 g, 81% yield) as colorless oil; IR (film) 2978, 2659, 1707, 1462, 1292, 1234, 937, 850, 698 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.15 (dd, J = 1.3, 5.1 Hz, 1H), 6.93 (dd, J = 3.5, 5.1 Hz, 1H), 6.83 (d, J = 3.5 Hz, 1H), 3.26 (dd, J = 6.6, 14.7 Hz, 1H), 2.94 (dd, J = 7.3, 14.7 Hz, 1H), 2.80 (m, 1H), 1.24 (d, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$): δ 182.0 (CO), 141.2 (C), 126.8 (CH), 125.7 (CH), 123.9 (CH), 41.6 (CH_2), 33.2 (CH), 16.5 (CH_3).

4.2.2. 2-Phenylaminopropanoic acid 12. A solution of aniline (9.3 g, 0.10 mol), anhydrous sodium acetate (8.3 g, 0.10 mol), and ethyl 2-bromopropanoate (18 g, 0.10 mol) in EtOH (3 mL) was heated under reflux for 25 h. After being cooled to room temperature, the mixture was diluted with water (40 mL) and extracted with ether. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. The oily residue was then heated under reflux in 10% aqueous NaOH (70 mL) for 2 h. The mixture was then cooled to room temperature and washed with ether. The aqueous layer was cooled in an ice-water bath and the solution acidified to pH 2 with concentrated HCl. The resulting slurry suspension was allowed to stand in an ice-water bath for 12 h, filtered, washed sequentially with water (200 mL) and hexane (200 mL), and dried under high vacuum. This crude precipitate was recrystallized from chloroform/acetone to give 2-phenylaminopropanoic acid[8] (11.8 g, 71% yield) as a colorless crystal: IR (KBr disc) 2979, 2775, 1572, 1495, 1394, 1358, 1090, 958, 850, 756, 696, 553 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.09 (m, 2H), 6.61 (m, 3H), 4.09 (q, J = 6.8 Hz, 1H), 1.45 (d, J = 6.8 Hz, 3H); ^{13}C NMR (acetone): δ 175.7 (CO), 148.4 (C), 129.6 (CH × 2), 117.8 (CH), 113.6 (CH × 2), 52.2 (CH), 18.8 (CH_3).

4.3. General procedure for the deracemization reaction of α-substituted carboxylic acids by the aid of *N. diaphanozonaria* JCM3208

4.3.1. General procedure for the deracemization reaction under aerobic conditions. The ingredients of the medium were as follows: glycerol (10 g/L), peptone (2 g/L), beef extract (3 g/L), yeast extract (3 g/L), KH_2PO_4 (1 g/L), K_2HPO_4 (1 g/L), $MgSO_4{\cdot}7H_2O$ (0.3 g/L), pH 7.0. To 90 mL of a nutrient medium, was added a suspension of 48-h incubated cells of *N. diaphanozonaria* in 10 mL of the broth and the incubation carried out at 30 °C for 24 h. The wet cells were harvested by centrifugation (5000 rpm, 10 min) and washed with phosphate buffer (0.1 M, pH 7.0). The wet cells, together with the substrate (100 mg, 0.1% w/v), were re-suspended in 100 mL of a phosphate buffer (0.1 M, pH 7.0) in a 500-mL shaking culture (Sakaguchi) flask. The flask was shaken at 30 °C on a reciprocal shaker for the appropriate time as indicated in Tables 1–6. The reaction mixture was filtered through a pad of Celite to remove the cells. The Celite was washed with EtOAc and the washing combined with the filtrate. After being acidified by 2 M hydrochloric acid, the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. The residue was converted to the corresponding methyl ester with diazomethane, and purified by silica gel column chromatography to give the ester as a colorless oil.

4.3.2. General procedure for the deracemization reaction under inert gas condition. The wet cells harvested as described in Section 4.3.1 and the substrate (100 mg, 0.1% w/v), re-suspended in 100 mL of a phosphate buffer (0.1 M, pH 7.0) in a 500-mL shaking culture (Sakaguchi) flask. The flask was purged with argon, equipped with a balloon filled with argon, and shaken at 30 °C on a

reciprocal shaker for the appropriate time as indicated in Tables 1–6. The work-up procedure was the same as Section 4.3.1.

4.3.3. General procedure for the deracemization reaction in the presence of acyl-CoA dehydrogenase inhibitor. The wet cells harvested as described above and the appropriate amount of acyl-CoA dehydrogenase inhibitor were suspended in 100 mL of a phosphate buffer (0.1 M, pH 7.0) in a 500-mL shaking culture (Sakaguchi) flask. The flask was shaken for 10 min. To this cell suspension, the substrate (100 mg, 0.1% w/v) was added and shaken at 30 °C on a reciprocal shaker for the appropriate time as indicated in Tables 1–6. The work-up procedure was the same as Section 4.3.1.

4.4. Recovery and analysis of the products

4.4.1. Methyl (*R*)-(+)-2-(4-chlorophenoxy)propanoate, methyl ester of 2. The extract of the reaction mixture was treated with diazomethane to give the methyl ester as a colorless oil: IR (film) 2954, 1758, 1596, 1492, 1283, 1240, 1135, 1090, 979, 825, 667 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.25 (dd, $J = 3.4$, 9.9 Hz, 2H), 6.83 (dd, $J = 3.4$, 9.9 Hz, 2H), 5.50 (q, $J = 6.8$ Hz, 1H), 3.78 (s, 3H), 1.64 (d, $J = 6.8$ Hz, 3H); ^{13}C NMR ($CDCl_3$): δ 172.2 (CO), 156.0 (C), 129.4 (CH × 2), 126.5 (C), 116.3 (CH × 2), 72.9 (CH), 52.4 (CH_3), 18.6 (CH_3). Ee 97% (Daicel Chiralcel OJ column (9/1 hexane/2-propanol; 0.5 mL/min; 254 nm): (*R*)-(major) $t_R = 18.5$ min, (*S*)-(minor) $t_R = 25.0$ min), $[\alpha]_D^{20} = +44.7$ (*c* 0.94, EtOH), lit.,[9] (*S*)-form, 96% ee, $[\alpha]_D = -41.1$ (*c* 50, EtOH).

4.4.2. Methyl (*R*)-(+)-2-phenylthiopropanoate, methyl ester of 3. The extract of the reaction mixture was treated with diazomethane to give the methyl ester as a colorless oil: IR (film) 3059, 2989, 2952, 1733, 1437, 1330, 1260, 1227, 1191, 1161, 1067, 854, 749, 691 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.38 (m, 2H), 7.23 (m, 2H), 3.72 (q, $J = 6.8$ Hz, 1H), 3.60 (s, 3H), 1.41 (d, $J = 6.8$ Hz, 3H); ^{13}C NMR ($CDCl_3$): δ 172.9 (CO), 136.6 (C), 133.0 (CH × 2), 128.8 (CH × 2), 128.0 (C), 52.3 (CH), 45.2 (CH_3), 17.5 (CH_3). Ee 93% (Daicel Chiralcel OJ column (9/1 hexane/2-propanol; 0.5 mL/min; 254 nm): (*R*)-(major) $t_R = 30.1$ min, (*S*)-(minor) $t_R = 39.7$ min), $[\alpha]_D^{23} = +145.7$ (*c* 1.02, EtOH), $[\alpha]_D^{22} = +141.2$ (*c* 0.68, acetone), lit.,[10] (*R*)-form, $[\alpha]_D = +65.5$ (*c* 1.0, acetone).

4.4.3. Methyl 2-(phenylsulfinyl)propanoate, methyl ester of 4. The extract of the reaction mixture was treated with diazomethane to give the methyl ester[11] as a colorless oil: IR (film) 3058, 2982, 2939, 2359, 1731, 1318, 1253, 1206, 1167, 1050, 858, 693 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.59 (m, 5H), 3.84 (q, $J = 7.1$ Hz, 0.5H, diastereomer), 3.51 (q, $J = 7.1$ Hz, 0.5H, diastereomer), 3.67 (s, 1.5H, diastereomer), 3.66 (s, 1.5H, diastereomer), 1.47 (d, $J = 7.1$ Hz, 1.5H, diastereomer), 1.32 (d, $J = 7.1$ Hz, 1.5H, diastereomer).

4.4.4. Methyl 2-methyl-2-(phenylsulfinyl)propanoate 5. To the extract from 100 mL reaction mixture dissolved in DMF (1.5 mL) was added sodium hydrogen carbonate (0.3 g, 3.57 mmol) and methyl iodide (200 μL, 3.60 mmol). The mixture was stirred at room temperature for 3 h. The saturated ammonium chloride (10 mL) was then added and extracted with hexane. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/EtOAc = 9/1 then 1/1) to give methyl 2-(phenylsulfinyl)propanoate as a colorless oil.

A solution of methyl 2-(phenylsulfinyl)propanoate, potassium carbonate (0.11 g, 0.80 mmol), and methyl iodide (60 μL, 0.93 mmol) in DMF (4 mL) was stirred at room temperature. After 16 h, saturated ammonium chloride (10 mL) was added and extracted with hexane. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/EtOAc = 1/1) to give methyl 2-methyl-2-(phenylsulfinyl)propanoate (47.5 mg, 77% yield) as a colorless oil: IR (film) 3059, 2925, 1734, 1577, 1475, 1441, 1327, 1456, 1076, 744, 687, 594 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.50 (m, 5H), 3.65 (s, 3H), 1.58 (s, 3H), 1.28 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 171.0 (CO), 139.8 (C), 131.6 (C), 128.6 (CH × 2), 125.4 (CH × 2), 66.3 (C), 52.5 (CH_3), 20.5 (CH_3), 16.0 (CH_3). Ee racemate (Daicel Chiralcel OD column (9/1 hexane/2-propanol; 0.5 mL/min; 254 nm, 37 °C): $t_R = 22.2$ min, 25.4 min.

4.4.5. Methyl (*S*)-phenylsulfinylacetate, methyl ester of 7. The extract of the reaction mixture was treated with diazomethane to give the methyl ester as a colorless oil: IR (film) 2981, 1736, 1583, 1481, 1441, 1367, 1282, 1151, 1028, 742, 690 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.69 (m, 2H), 7.55 (m, 3H), 3.85 (d, $J = 13.7$ Hz, 1H), 3.71 (s, 3H), 3.67 (d, $J = 13.7$ Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 169.5 (CO), 134.9 (C), 129.9 (CH × 2), 128.9 (CH × 2), 126.8 (C), 61.5 (CH_2), 45.2 (CH_3). Ee 73% (Daicel Chiralcel OB column (9/1 hexane/2-propanol; 0.5 mL/min; 254 nm, 37 °C): (*S*)-(major) $t_R = 73.1$ min, (*R*)-(minor) $t_R = 82.6$ min). The absolute configuration was determined by comparison of the retention time with that described in a literature.[12]

4.4.6. Methyl (*S*)-(+)-2-methyl-3-phenylpropanoate, methyl ester of 8. The reaction mixture was treated with diazomethane. To a mixture of the methyl esters of **8** and **15**, sodium metaperiodate (0.3 g, 1.40 mmol), water (3 mL), and diethyl ether (3 mL) were added 20 mM osumium tetroxide in *tert*-BuOH (1.5 mL, 0.03 mmol). The mixture was stirred at room temperature for 12 h. Saturated sodium thiosulfate (2 mL) was then added and extracted with diethyl ether. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/EtOAc = 19/1) to give methyl 2-methyl-3-phenylpropanoate as colorless oil: IR (film) 3028, 2975, 2951, 1739, 1496, 1455, 1166, 986, 832, 745, 701 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.33 (m, 5H), 3.73 (s, 3H), 3.12 (m, 1H), 2.79 (m, 2H), 1.25 (d, $J = 6.8$ Hz, 3H); ^{13}C NMR ($CDCl_3$): δ 176.5 (CO), 139.3 (C), 128.9 (CH × 2), 128.3 (CH × 2), 126.3 (CH), 51.2 (CH_3), 41.4 (CH_2),

39.7 (CH), 16.7 (CH_3). Ee 88% (Daicel Chiralcel OJ column (9/1 hexane/2-propanol; 0.5 mL/min; 254 nm): (*R*)-(minor) t_R = 24.0 min, (*S*)-(major) t_R = 28.6 min), $[\alpha]_D^{25}$ = +39.3 (*c* 0.95, MeOH), lit.,[13] (*S*)-form, $[\alpha]_D^{20}$ = +49.8 (*c* 2.042, MeOH).

4.4.7. Benzoic acid 9. The product was analyzed by HPLC with a Shenshu Pak PEGASIL Silica 60-5 (98%/2%/0.05% hexane/2-propanol/TFA; 1.0 mL/min; 254 nm): t_R = 5.84 min.

4.4.8. Methyl 2-methyl-3-(2-thienyl)propanoate, methyl ester of 10. The extract of the reaction mixture was treated with diazomethane to give the methyl ester as a colorless oil: IR (film) 2956, 2860, 1738, 1460, 1275, 1072, 850, 696 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.11 (dd, *J* = 1.0, 5.1 Hz, 1H), 6.91 (dd, *J* = 3.3, 5.1 Hz, 1H), 6.80 (dd, *J* = 1.0, 3.3 Hz, 1H), 3.22 (dd, *J* = 7.0, 14.7 Hz, 1H), 2.93 (dd, *J* = 7.2, 14.7 Hz, 1H), 2.77 (m, 1H), 1.20 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$): δ 165.5 (CO), 146.0 (C), 126.8 (CH), 125.6 (CH), 123.8 (CH), 51.7 (CH_3), 41.8 (CH_2), 33.6 (CH), 16.8 (CH_3). Ee 93% (Daicel Chiralcel OJ column (9/1 hexane/2-propanol; 0.5 mL/min; 254 nm): (*R*)-(minor) t_R = 9.90 min, (*S*)-(major) t_R = 10.9 min).

4.4.9. Methyl 2-methyl-3-(2-thienyl)propenoate, methyl ester of 11. The extract of the reaction mixture was treated with diazomethane to give the methyl ester as a colorless oil: IR (film) 3020, 1716, 1653, 1522, 1473, 1421, 1215, 928, 762, 699 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.86 (s, 1H), 7.49 (d, *J* = 5.1 Hz, 1H), 7.28 (d, *J* = 3.3 Hz, 1H), 7.12 (dd, *J* = 3.7, 5.1 Hz, 1H), 3.81 (s, 3H), 2.22 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 169.0 (CO), 139.2 (C), 131.7 (C), 131.7 (CH), 129.1 (CH), 127.3 (CH), 124.7 (CH), 52.1 (CH_3), 14.3 (CH_3). The product was analyzed by HPLC with a Daicel Chiralcel OJ column (9/1 hexane/2-propanol; 0.5 mL/min; 254 nm): t_R = 12.8 min.

4.4.10. Methyl (*R*)-(+)-2-phenylaminopropanoate, methyl ester of 12. To the extract of the reaction mixture from 100 mL medium in methanol (6 mL) was added trimethylsilyl chloride (600 μL, 4.72 mmol). The mixture was stirred at room temperature for 24 h. Water (5 mL) and an aqueous solution of saturated sodium hydrogen carbonate (5 mL) were then added and extracted with EtOAc. The organic layer was washed with an aqueous solution of saturated sodium hydrogen carbonate and brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/EtOAc = 5/1) to give methyl 2-phenylaminopropanoate as a colorless oil: IR (film) 3392, 2952, 1739, 1604, 1508, 1315, 1207, 1163, 1055, 750, 694 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.19 (m, 2H), 6.76 (m, 1H), 6.63 (m, 2H), 4.16 (q, *J* = 6.8 Hz, 1H), 3.73 (s, 3H), 1.49 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$): δ 174.8 (CO), 146.0 (C), 129.3 (CH × 2), 118.8 (CH), 113.8 (CH × 2), 52.2 (CH), 52.2 (CH_3), 18.8 (CH_3). Ee >99% (Daicel Chiralcel OJ column (9/1 hexane/2-propanol; 0.5 mL/min; 254 nm): (*R*)-(major) t_R = 28.2 min, (*S*)-(minor) t_R = 44.1 min), Only the (*R*)-enantiomer could be detected, $[\alpha]_D^{22}$ = +84.6 (*c* 0.53, MeOH).

4.4.11. Acetoanilide, *N*-acetyl protection of 13. To the extract of the reaction mixture from 100 mL medium in CH_2Cl_2 (1 mL) were added pyridine (200 μL, 2.47 mmol) and acetic anhydride (114 μL, 1.22 mmol). The mixture was stirred at room temperature for 2 h. Water (5 mL) was then added and acidified by 2 M hydrochloric acid. The mixture was extracted with EtOAc. The organic layer was washed with 0.2 M hydrochloric acid, an aqueous solution of saturated sodium hydrogen carbonate, and brine, and then dried over anhydrous sodium sulfate, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/EtOAc = 1/1) to give acetoanilide as colorless needles, mp 113 °C: IR (KBr disc) 3294, 3136, 1664, 1599, 1556, 1434, 1323, 1263, 1041, 756, 694, 509 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.49 (d, *J* = 7.9 Hz, 2H), 7.31 (t, *J* = 7.9 Hz, 2H), 7.10 (d, *J* = 7.2 Hz, 1H), 2.17 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 168.4 (CO), 137.8 (C), 129.0 (CH × 2), 124.3 (CH), 119.9 (CH × 2), 24.6 (CH_3).

4.4.12. Methyl (*R*)-(+)-2-(4-chlorophenylthio)propanoate, methyl ester of 14. The extract of the reaction mixture was treated with diazomethane to give methyl ester as a colorless oil: IR (film) 2952, 1738, 1574, 1477, 1329, 1263, 1163, 1095, 1012, 823, 733, 499 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.31 (m, 2H), 7.21 (m, 2H), 3.69 (q, *J* = 7.3 Hz, 1H), 3.61 (s, 3H), 1.40 (d, *J* = 7.3 Hz, 3H); ^{13}C NMR ($CDCl_3$): δ 172.7 (CO), 134.4 (C), 134.4 (CH × 2), 131.4 (C), 129.0 (CH × 2), 52.4 (CH), 45.3 (CH_3), 17.4 (CH_3). Ee 90% (Daicel Chiralcel OJ column (9/1 hexane/2-propanol; 0.5 mL/min; 254 nm): (*R*)-(major) t_R = 15.4 min, (*S*)-(minor) t_R = 17.3 min), $[\alpha]_D^{21}$ = +140.2 (*c* 1.0, MeOH), lit.,[9] (*S*)-form, $[\alpha]_D$ = −144.6 (*c* 1.0, MeOH).

4.4.13. Methyl α-methylcinnamate, methyl ester of 15. The extract of the reaction mixture was treated with diazomethane to give the methyl ester as a colorless oil: IR (film) 2991, 1709, 1259, 1120, 933, 768, 710, 511 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.39 (s, 1H), 7.25 (m, 5H), 3.82 (s, 3H), 2.13 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 169.2 (CO), 138.9 (C), 135.9 (CH), 129.6 (CH × 3), 128.4 (CH), 128.3 (C), 52.1 (CH_3), 14.1 (CH_3). The product was analyzed by HPLC with a Daicel Chiralcel OJ column (9/1 hexane/2-propanol; 0.5 mL/min; 254 nm): t_R = 29.7 min.

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