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# Synthesis of high enantiomeric purity *gem*-dihalocyclopropane derivatives from biotransformations of nitriles and amides

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**Abstract**—Enantioselective biotransformations of geminally dihalogenated cyclopropanecarbonitriles and amides are described. Both the reaction rate and enantioselectivity of the nitrile hydratase and amidase involved in *Rhodococcus* sp. AJ270 microbial cells are strongly governed by the nature of *gem*-disubstituents on the cyclopropane ring; the amidase generally exhibits steric dependence on the substituents while both the steric and electronic factors of the substituents may affect the action of the nitrile hydratase. The match of steric bulkiness of the substituents at 2- with that at 3-positions on the cyclopropane ring benefits the efficient and highly enantioselective reaction. Coupled with facile chemical transformations, biocatalytic transformations of nitrile and amide supply an effective synthesis of optically active 2,2-disubstitued-3-phenylcyclopropanecarboxylic acid and amide in both enantiomeric forms.


## 1. Introduction

Biotransformations of nitriles, through either a direct nitrilase-catalyzed conversion to a carboxylic acid[1] or a nitrile hydratase-catalyzed hydration followed by the hydrolysis of amide to acid by the action of the amidase,[2] have been demonstrated by us[3] and others[4] as unique and environmentally benign methods for the synthesis of optically active carboxylic acids and their amide derivatives because of the excellent selectivity and very mild reaction conditions. We have previously shown that *Rhodococcus* sp. AJ270,[5] a microorganism isolated from a soil sample, is a robust and powerful nitrile hydratase/amidase-containing biocatalytic system capable of hydrolyzing a wide range of structurally diverse nitriles and dinitriles with excellent chemo-,[6] regio-[7] and enantioselectivities.[4] Very recently, we used a large number of differently substituted and configured cyclopropanecarbonitriles and amides (Chart 1) as probe molecules to systematically investigate *Rhodococcus* sp. AJ270 whole cell-catalyzed hydrolysis, and have concluded that a readily reachable reactive site is embedded within the spacious pocket of the nitrile hydratase while the amidase comprises a relatively deep-buried and size-limited enantioselective active site.[8]

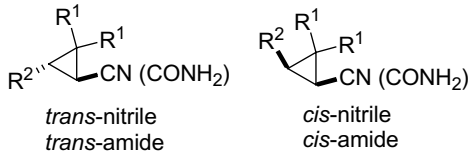


**Chart 1.**

Based on the reaction profile and the nature of the substituents and their substitution patterns, we can also propose a predictive model of the reaction efficiency and enantioselectivity. To racemic *trans* configured substrates, for example, the higher reaction rate with lower enantioselectivity is expected when both substituents $R^1$ and $R^2$ are small, whereas large substituents of $R^1$ and $R^2$ generally lead to a very slow reaction but with high enantioselection.[8–10] Good match of substituents $R^1$ and $R^2$ in steric bulkiness results in excellent enantiocontrol with satisfactory reaction velocity.[10] To gain deeper insight into the catalytic properties of the enzymes and also to validate further the proposed prediction model of the reaction, we undertook the current investigation of biotransformations of geminally dihalogenated cyclopropanecarbonitriles and amides.[11]

On the other hand, geminally dihalo-substituted cyclopropane derivatives are intriguing and versatile

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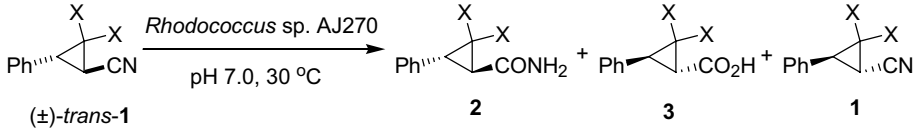
intermediates in organic synthesis.[12] The *gem*-difluorocyclopropane derivatives have been shown to possess interesting biological[13] and physiochemical properties.[14] In contrast to the tremendous development of research on chiral cyclopropane compounds, however, the chemistry of chiral dihalogenated cyclopropane derivatives remains largely unexplored probably due to the lack of efficient synthetic methods. To our knowledge, only few methods have been reported for the preparation of optically active *gem*-difluorocyclopropane compounds using lipase-mediated hydrolysis of esters[15] and esterification of alcohols,[14,16] respectively. We envisaged that biotransformations of nitriles and amides would provide a new approach to high enantiomeric purity dihalogenated cyclopropane compounds.

## 2. Results and discussion

### 2.1. Biotransformations of racemic nitriles

The biotransformations of geminally dihalogenated cyclopropanecarbonitriles **1a**–**c** were first studied under catalysis using *Rhodococcus* sp. AJ270 whole cells at 30 °C in aqueous phosphate buffer (pH 7.0) (Scheme 1). To examine the effect of the halogen substituent X on the reaction, biotransformations of 2-phenylcyclopropanecarbonitrile **1e** and of *gem*-dimethylcyclopropanecarbonitrile analogue **1f** were also included. The outcomes in Table 1 indicated clearly that the nature of the substituent X played an important role in determining both the reaction rate and reaction enantioselectivity. For example, *gem*-difluorocyclopropanecarbonitrile **1a** underwent rapid and efficient hydrolysis to afford good yields of (1*R*,3*R*)-**2a** and (1*S*,3*S*)-**3a** with enantiomeric excess (ee) of >99% and 53%, respectively (entry 1), a result comparable to that of biotransformation of the parent nitrile **1e** (entry 9). Prolonged incubation of (±)-**1a** led to the complete hydration of the nitrile and higher conversion of the resulting amide (entry 2). In contrast, the hydrolysis of *gem*-dibromocyclopropanecarbonitrile **1d** proceeded sluggishly; a week's interaction of **1d** with microbial cells gave almost 50% of the optically active nitrile (1*S*,3*S*)-**1d** (ee 54%) along with the isolation of 24% of the amide (1*R*,3*R*)-**2d** (ee 95%) and a trace amount of acid **3d** (entry 6). Under identical conditions, decrease of the concentration of substrate **1d** slightly increased the conversion (entry 7). Since both amide **2d** and acid **3d** were stable under the reaction conditions (vide infra), the loss of mass balance in this case is probably due to the decomposition of nitrile **1d**. The biocatalytic conversion of geminally dichlorinated cyclopropanecarbonitrile **1b** took place slowly to give acid (1*S*,3*S*)-**3b** in 35% yield with 61% ee, after a week, with more than 50% of recovered nitrile (1*S*,3*S*)-**1b** in 47% ee. It was surprising for us to note that no amide product **2b** was accumulated during the reaction (entries 3 and 4), indicating that the biohydrolysis of amide intermediate **2b** proceeded much faster than its generation from biohydration of the nitrile **1b**. Introduction of a methoxy group into the *ortho* position of the benzene ring led to a further slow down of the hydration of **1c**, more than 60% of starting nitrile **1c** remaining under the same conditions (entry 5).




**Scheme 1.** Biotransformation of racemic nitriles **1**.

**Table 1.** Biotransformations of racemic nitriles **1**

| Entry | 1 | X | Conditions[a] | 2 (%)[b] | Ee (%)[c] | 3 (%)[b] | Ee (%)[c] | 1 (%)[b] | Ee (%)[c] |
|---|---|---|---|---|---|---|---|---|---|
| 1 | **1a** | F | 0.5 mmol, 1 h | 32 | >99 | 52 | 53 | 10 | 6[d] |
| 2 | **1a** | F | 0.5 mmol, 1.5 h | 22 | >99 | 74 | 28 | — | — |
| 3 | **1b** | Cl | 1 mmol, 7 d | — | — | 20 | 72 | 71 | 9 |
| 4 | **1b** | Cl | 0.5 mmol, 7 d | — | — | 35 | 61 | 58 | 47 |
| 5 | **1c**[g] | Cl | 0.5 mmol, 7 d | 13 | 60 | 12 | 93 | 64 | 2 |
| 6 | **1d** | Br | 0.5 mmol, 7 d | 24 | 95 | Trace | n.d.[e] | 48[f] | 54 |
| 7 | **1d** | Br | 0.25 mmol, 7 d | 35 | 97 | Trace | n.d.[e] | 28[f] | 72 |
| 8 | **1e** | H | 0.5 mmol, 20 min | 40 | >99 | 49 | 70 | 7 | <5 |
| 9 | **1e** | H | 0.5 mmol, 0.5 h | 34 | >99 | 62 | 57 | — | — |
| 10 | **1f** | Me | 0.5 mmol, 30 h | 48 | >99 | 46 | 99 | — | — |
| 11 | **1f** | Me | 1 mmol, 33 h | 29 | >99 | 50 | 99 | 16 | >99 |

[a] Reaction conditions were not optimized.
[b] Isolated yield.
[c] Determined with Chiral HPLC analysis.
[d] Configuration was determined as (1*R*,3*R*).
[e] Enantiomeric excess value was not determined.
[f] Decomposition of nitrile **1d** was observed.
[g] The phenyl group was replaced by 2-MeO–$C_6H_4$ group.

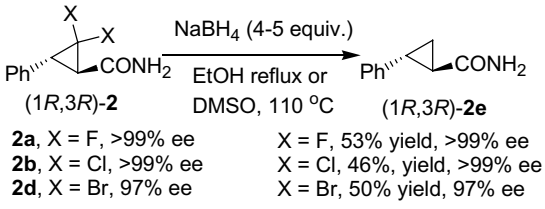
### 2.2. Biocatalytic kinetic resolution of racemic amides

To shed further light on the enantioselective biotransformations of nitriles **1**, the racemic amides (±)-**2** were subjected to *Rhodococcus* sp. AJ270 (Scheme 2) and the results were summarized in Table 2. *gem*-Difluorocyclopropanecarboxamide (±)-**2a** underwent very rapid kinetic resolution to afford the corresponding (1*R*,3*R*)-amide **2a** and (1*S*,3*S*)-acids **3a** in excellent and good enantiomeric excesses, respectively ($E = 74$) (entry 1). Efficient kinetic resolution of dichloro-substituted analogue **2b** was observed to produce highly enantiopure 1*R*,3*R*-amide **2b** and (1*S*,3*S*)-acids **3b** ($E = 125$) (entry 3). However, *gem*-dibromocyclopropanecarboxamide (±)-**2d** was resolved very slowly to give (1*R*,3*R*)-amide **2d** and (1*S*,3*S*)-acid **3d** with very low enantioselectivity ($E = 1.9$) (entry 7). To circumvent the problem of poor solubility of **2d**, the amount of the substrate was halved and co-solvent such as acetone and methanol was employed. These did slightly improve the reaction rate and enantioselectivity ($E = 4.7$–$7.3$) (entries 8 and 9). 2-Methoxyphenyl-substituted *gem*-dichlorocyclopropanecarboxamide (±)-**2c** underwent even slower hydrolysis, albeit in moderate enantioselectivity ($E = 30$) (entry 6).

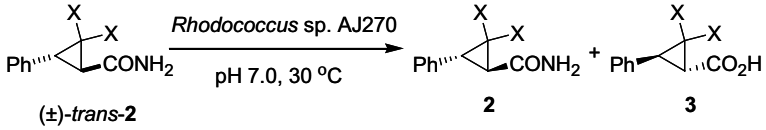
It is apparently advantageous to apply the biotransformation of amide rather than nitrile to prepare enantiomerically pure *gem*-difluoro- and -dichlorocyclopropane derivatives. To show the synthetic utility, the biocatalytic kinetic resolution of amide was then performed on a multi-gram scale. The biotransformation of (±)-**2b** (2.3 g, 10 mmol) led to the production of highly enantiopure (1*R*,3*R*)-**2b** (48% yield, >99% ee) and (1*S*,3*S*)-**3b** (48% yield, 95%).

### 2.3. Chemical transformations of chiral *gem*-halocyclopropane derivatives

There are no reports in the literature of the stereochemistry of dihalogenated cyclopropanecarboxylic acids and their derivatives that were obtained from this study. To determine the absolute configuration of the biotransformation products, and also to prepare the antipode, reductive dehalogenation reactions and functional group transformations were conducted. By refluxing *gem*-dichloro- and -dibromocyclopropanecarboxamides **2b–c** with $NaBH_4$ in ethanol, (1*R*,2*R*)-2-phenylcyclopropanecarboxamide **4** was produced in moderate yield. Only at elevated temperatures in dimethyl sulfoxide (DMSO), however, was reductive defluorination of **2a** effected (Scheme 3). The configuration of **4** was determined through measuring and comparing its specific rotation with that of an authentic sample.[8] Chemical hydrolysis of (1*R*,3*R*)-amide **2b** in refluxing hydrochloric acid (6 M) yielded (1*R*,3*R*)-acid **5**, while the dehydration of (1*R*,3*R*)-amides **2a**, **2b** and **2d** afforded (1*R*,3*R*)-nitriles **6** in good yield (Scheme 4). (1*S*,3*S*)-Dihalogenated cyclopropanecarboxamides **7**




**Scheme 3.** Reductive dehalogenation reaction of **2**.

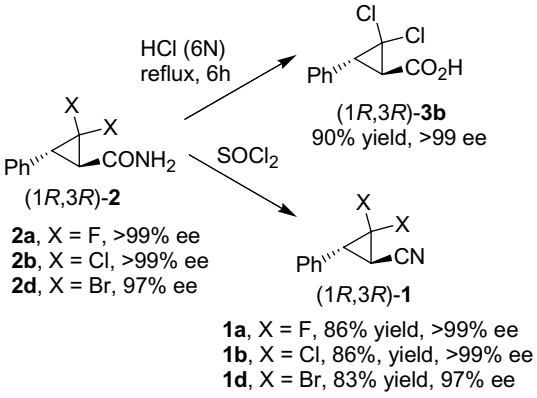



**Scheme 2.** Biocatalytic kinetic resolution of racemic amides **2**.

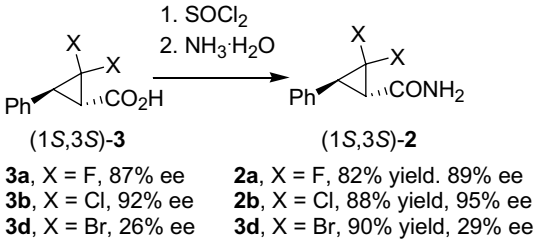
**Table 2.** Biocatalytic kinetic resolution of racemic amides **2**

| Entry | **2** | X | Conditions[a] | **2** (%)[b] | Ee (%)[c] | **3** (%)[b] | Ee (%)[c] | *E* |
|---|---|---|---|---|---|---|---|---|
| 1 | **2a** | F | 0.5 mmol, 15 min | 46 | >99 | 51 | 87 | 74 |
| 2 | **2a** | F | 0.5 mmol, 0.5 h | 40 | >99 | 57 | 67 | — |
| 3 | **2b** | Cl | 0.5 mmol, 8 h | 49 | >99 | 48 | 92 | 125 |
| 4 | **2b** | Cl | 0.5 mmol, 10 h | 45 | >99 | 48 | 91 | — |
| 5 | **2c**[f] | Cl | 0.5 mmol, 7 d | 71 | 14 | 16 | 89 | — |
| 6 | **2c**[f] | Cl | 0.25 mmol, 7 d | 68 | 36 | 27 | 91 | 30 |
| 7 | **2d** | Br | 0.5 mmol, 7 d | 49 | 11 | 43 | 26 | 1.9 |
| 8 | **2d** | Br | 0.25 mmol, 6 d[d] | 26 | 93 | 60 | 41 | 7.3 |
| 9 | **2d** | Br | 0.25 mmol, 87 h[e] | 34 | 71 | 59 | 40 | 4.7 |
| 10 | **2e** | H | 0.5 mmol, 20 min | 46 | 78 | 51 | 59 | 8.9 |
| 11 | **2e** | H | 0.5 mmol, 0.5 h | 31 | 86 | 67 | 39 | — |
| 12 | **2f** | Me | 0.5 mmol, 73 h | 46 | >99 | 52 | 92 | 125 |

[a] Reaction conditions were not optimized.
[b] Isolated yield.
[c] Determined with Chiral HPLC analysis.
[d] Acetone (2 mL) was added.
[e] Methanol (2 mL) was added.
[f] The phenyl group was replaced by 2-MeO–$C_6H_4$ group.




**Scheme 4.** Chemical hydrolysis and dehydration of amide (1*R*,3*R*)-**2**.




**Scheme 5.** Chemical preparation of optically active acid (1*S*,3*S*)-**2**.

were synthesized conveniently from (1*S*,3*S*)-acids **3** (Scheme 5). No racemerization was observed during these chemical transformations.

## 3. Discussion

The results aforementioned clearly indicated that it is the steric effect of the substituents on the cyclopropane ring that mainly control the reaction rate and enantioselectivity of amidase involved in *Rhodococcus* sp. AJ270 cells. For the amidase action, when the steric bulkiness of the substituents at the geminal position of the *trans*-3-phenylcyclopropanecarboxamides increase from H– to F–, Cl–, $CH_3$– and Br–, hydrolysis velocity decreases from minutes to hours and days. The enantiomeric selection (*E*), however, increases, with the exception of dibromo-substituted amide **2d**. Although it is hard to rule out the effect of hydrophobicity or hydrophilicity of the bromine substituent when interacting with the amidase, the solubility problem of the substrate accounts for one of the reasons of low enantioselectivity. The steric match of the substituent at 3-position with the substituents at the geminal position appears crucial for efficient kinetic resolution. This has been exemplified by the dramatic decrease of hydrolysis efficiency of 3-(2-methoxyphenyl)-substituted amide **2c** by comparison with 3-phenyl-substituted analogue **2b** (entries 3–6 in Table 2). Another example was the significant enhancement of enantioselection observed from 2,2-dimethylcyclopropanecarboxamide ($E = 11$) to 3-(2′,2′-dichlorovinyl)-2,2-dimethylcyclopropanecarboxamide ($E = 89$), 2,2-dimethyl-3-phenylcyclopropanecarboxamide ($E = 125$) and 2,2-dimethyl-3-(2′,2′-dimethylvinyl)cyclopropanecarboxamide ($E > 200$).[10] All the results of biocatalytic kinetic resolution of cyclopropanecarboxamide derivatives obtained so far fit well with the hypothesis of a relatively deep-buried and size-limited enantioselective active site of the amidase in *Rhodococcus* sp. AJ270.[8–10]

The nitrile hydratase-catalyzed hydration of geminally disubstituted cyclopropanecarbonitriles exhibits the dependence of reaction efficiency on both electronic and steric effects of the substituents on the cyclopropane ring. The hydration reaction proceeds in the order of H– > F– > Me– disubstitutions. The presence of dichloro and dibromo moieties has a detrimental effect on the hydration of nitrile to amide. Although the nitrile hydratase displays very low to only moderate enantioselectivity against geminally disubstituted cyclopropanecarbonitriles, the influence of the halogen substituent on enzymes enantiocontrol seems peculiar. It remains unclear at this stage whether the electronic or the steric nature determines the chiral recognition process of the enzyme, and more study is needed to clarify this issue.

## 4. Conclusions

On the basis of this and our previous studies,[8–10] we conclude that *Rhodococcus* sp. AJ270 cells are able to catalyze enantioselective biotransformations of *trans*-2,2-disubstituted-3-arylcyclopropancarbonitriles and amides. Both the reaction rate and enantioselectivity of the nitrile hydratase and amidase involved in microbial cells are strongly governed by the nature of *gem*-disubstituents on the cyclopropane ring; the amidase exhibits generally the steric dependence on the substituents while both the steric and electronic factors of the substituents may affect the action of the nitrile hydratase. We have shown again that the steric match of the substituents at 2- with that at 3-positions on the cyclopropane ring benefits the efficient and highly enantioselective reaction. This may help the design of *ideal* substrates. Coupled with facile chemical transformations, biocatalytic transformation of nitrile and amide supplies an effective syntheses of optically active 2,2-disubstitued-3-phenylcyclopropanecarboxylic acid and amide in both enantiomeric forms.

## 5. Experimental

### 5.1. General

Melting points were determined using a Reichert Kofler hot-stage apparatus. Both melting points and boiling points are uncorrected. NMR spectra were recorded in $CDCl_3$ solution on a Bruker AM 300 spectrometer. Chemical shifts are reported in ppm and coupling constants are given in hertz. IR spectra were obtained on a

Bruker IMX20 instrument as liquid films or KBr discs. Mass spectra were measured on an AEI MS-50 mass spectrometer and microanalyses were carried out by the Analytical Laboratory of the Institute. Polarimetry was carried out using an Optical Activity AA-10R polarimeter and the measurements were made at the sodium D-line with a 0.5 dm pathlength cell. Concentrations (*c*) are given in gram per 100 mL. All HPLC were run using a Shimadzu SCL-10AVP HPLC system with a UV detector set at 204 nm. The enantiomeric excess (ee) values of all products were obtained by means of Chiral HPLC analyses using authentic racemic samples as references.

### 5.2. Preparation of racemic nitriles and amides

Nitriles **1a**–**d** and amides **2a**–**d** were prepared via conventional chemical transformation from *trans-gem*-dihalocyclopropanecarboxylic acids **3a**–**d**. Racemic acid **3a** was prepared following a literature method,[17] while racemic acids **3b**–**d** were prepared starting from the reaction of dichloro or dibromo carbene with *trans*-cinnamaldehyde diethyl acetal,[18] followed by deprotection of diethyl acetal group and by the oxidation reaction of the resulting *gem*-dihalocyclopropanecarboxaldehydes.

**5.2.1. Racemic; *trans*-2,2-difluoro-3-phenylcyclopropanecarbonitrile 1a.** Oil; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.23–7.26 (m, 2H, Ar-H), 7.38–7.43 (m, 3H, Ar-H), 3.34 (m, 1H, CH), 2.48 (m, 1H, CH); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 129.9, 128.7, 127.8, 113.9 (CN), 108.5 (t, 1C, $J = 1170$, $CF_2$), 34.1 (dd, 1C, $J = 42$, 42, CH), 16.2 (dd, 1C, $J = 42$, 66, CH); IR (KBr) 2251 cm$^{-1}$ (CN); MS (EI) *m*/*z* 180 ($M^+$+1, 12), 179 ($M^+$, 100%), 178 ($M^+$−1, 21), 159 (13), 152 (99), 140 (9), 129 (58), 128 (26), 127 (48), 102 (20). Anal. Calcd for $C_{10}H_7F_2N$: C, 67.04; H, 3.94; N, 7.82. Found: C, 67.02; H, 4.04; N, 7.78.

**5.2.2. Racemic; *trans*-2,2-dichloro-3-phenylcyclopropanecarbonitrile 1b.** Oil; $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 7.40 (m, 5H, Ar-H), 3.38 (d, 1H, $J = 7.8$, CH), 2.64 (d, 1H, $J = 7.8$, CH); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 130.8, 129.0, 128.9, 128.5, 115.6, 60.4, 42.1, 23.0; IR (KBr) 2248 cm$^{-1}$ (CN); MS (EI) *m*/*z* 213 ($M^+$+2, 4), 211 ($M^+$, 7%), 178 (17), 176 (54), 149 (17), 141 (25), 140 (100). Anal. Calcd for $C_{10}H_7Cl_2N$: C, 56.63; H, 3.33; N, 6.60. Found: C, 56.81; H, 3.32; N, 6.25.

**5.2.3. Racemic; *trans*-2,2-dichloro-3-(2-methoxylphenyl)-cyclopropanecarbonitrile 1c.** White solid; mp 81–82 °C; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.37–7.43 (m, 1H, Ar-H), 6.97–7.06 (m, 3H, Ar-H), 3.95 (s, 3H, $OCH_3$), 3.39 (d, 1H, $J = 8.1$, CH), 2.60 (d, 1H, $J = 8.0$, CH); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 158.5, 130.1, 128.1, 120.2, 119.7 (CN), 115.7, 110.4, 60.5, 55.6, 38.6, 22.4; IR (KBr) 2245 cm$^{-1}$ (CN); MS (EI) *m*/*z* 241 ($M^+$, 9%), 208 (34), 206 (100), 170 (73), 140 (41). Anal. Calcd for $C_{11}H_9Cl_2NO$: C, 54.26; H, 3.62; N, 5.38. Found: C, 54.57; H, 3.75; N, 5.79.

**5.2.4. Racemic; *trans*-2,2-dibromo-3-phenylcyclopropanecarbonitrile 1d.** Oil; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.25–7.43 (m, 5H, Ar-H), 3.38 (d, 1H, $J = 7.8$, CH), 2.69 (d, 1H, $J = 7.8$, CH); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 132.1, 128.7 (3C), 128.3 (2C), 116.4 (CN), 42.7, 26.2, 23.3; IR (KBr) 2248 cm$^{-1}$ (CN); MS (EI) *m*/*z* 222 [($M^+$−Br)+2, 18%], 220 ($M^+$−Br, 19), 141 ($M^+$−2Br, 100), 140 (78). Anal. Calcd for $C_{10}H_7Br_2N$: C, 39.91; H, 2.34; N, 4.65. Found: C, 40.08; H, 2.25; N, 4.55.

### 5.3. General procedure for the biotransformation of nitriles and amides

To an Erlenmeyer flask (250 mL) with a screw cap was added *Rhodococcus* sp. AJ270 cells (2 g wet weight) and potassium phosphate buffer (0.1 M, pH 7.0, 50 mL) and the resting cells were activated at 30 °C for 0.5 h with orbital shaking. Nitriles or amides (see Tables 1 and 2) were added in one portion to the flask and the mixture was incubated at 30 °C using an orbital shaker (200 rpm). The reaction, monitored by TLC, was quenched after a period of time (see Tables 1 and 2) by removing the biomass through Celite pad filtration. The resulting aqueous solution was basified to pH 12 with aqueous NaOH (2 M). Extraction with ethyl acetate gave, after drying ($MgSO_4$) and concentration, the amide and unconverted nitrile. Separation of amide and nitrile was effected by column chromatography. The aqueous solution was then acidified using aqueous HCl (2 M) to pH 2 and extracted with ethyl acetate. Acid was obtained after removal of the solvent.

**5.3.1. Enzymatic hydrolysis of racemic *trans*-2,2-difluoro-3-phenylcyclopropanecarbonitrile 1a.** *(−)-(1R,3R)-2,2-Difluoro-3-phenylcyclopropanecarboxamide* ***2a***: Mp 131–132 °C; $[\alpha]_D^{25} = -112$ (*c* 1.0, $CHCl_3$); ee >99% (Chiral HPLC); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.24–7.35 (m, 5H, Ar-H), 6.36 (br s, 1H, N*H*H), 6.09 (br s, 1H, NH*H*), 3.52 (m, 1H, CH), 2.63 (m, 1H, CH); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 166.6 ($CONH_2$), 131.3, 128.6, 128.0, 127.7, 110.7 (t, 1C, $J = 1170$, $CF_2$), 33.1 (t, 1C, $J = 39$, CH), 32.4 (t, 1C, $J = 42$, CH); IR (KBr) 3340, 3194 ($NH_2$), 1658 cm$^{-1}$; MS (EI) *m*/*z* 197 ($M^+$, 3%), 179 (61), 154 (46), 153 (100), 152 (65), 134 (86), 133 (82), 103 (35), 77 (59). Anal. Calcd for $C_{10}H_9F_2NO$: C, 60.91; H, 4.60; N, 7.10. Found: C, 60.92; H, 4.53; N, 7.00. HPLC analysis: Chiralpak AD, hexane–isopropanol 9:1, flow rate 0.8 mL/min, $t_{(+)} = 9.684$ min, $t_{(-)} = 16.733$ min.

*(+)-(1S,3S)-2,2-Difluoro-3-phenylcyclopropanecarboxylic acid* ***3a***: Mp 86–88 °C; $[\alpha]_D^{25} = +66.7$ (*c* 1.5, $CHCl_3$); ee 53% (Chiral HPLC); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 9.80 (br s, 1H, COOH), 7.23–7.39 (m, 5H, Ar-H), 3.52 (m, 1H, CH), 2.74 (m, 1H, CH); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 172.6 (COOH), 130.4, 128.8 (3C), 128.0 (2C), 110.6 (dd, 1C, $J = 1140$, 1170, $CF_2$), 33.8 (t, 1C, $J = 39$, CH), 32.0 (t, 1C, $J = 48$, CH); IR (KBr) 2240–3600 (br,

COOH), 1692 $cm^{-1}$; MS (EI) $m/z$ 198 ($M^+$, 4%), 178 (100), 153 (39), 133 (66), 105 (53), 77 (38). Anal. Calcd for $C_{10}H_8F_2O_2$: C, 60.61; H, 4.07. Found: C, 60.74; H, 4.08. HPLC analysis: Chiralpak AD, hexane–isopropanol 9:1, flow rate 0.8 mL/min, $t_{(+)} = 6.975$ min, $t_{(-)} = 8.557$ min.

*(−)-(1R,3R)-2,2-Difluoro-3-phenylcyclopropanecarbonitrile* ***1a***: Oil; $[\alpha]_D^{25} = -12$ ($c$ 0.675, $CHCl_3$); ee 6% (Chiral HPLC); identical spectra data as that of racemic **1a** were obtained. HPLC analysis: Chiralpak AD, hexane–isopropanol 9:1, flow rate 0.4 mL/min, $t_{(-)} = 13.572$ min, $t_{(+)} = 14.946$ min.

**5.3.2. Enzymatic hydrolysis of racemic *trans*-2,2-dichloro-3-phenylcyclopropanecarbonitrile 1b.** *(+)-(1R,3R)-2,2-Dichloro-3-phenylcyclopropanecarboxylic acid* ***3b***: $[\alpha]_D^{25} = +52$ ($c$ 1.25, $CHCl_3$); ee 72% (Chiral HPLC); $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 10.15 (br s, 1H, COOH), 7.28–7.45 (m, 5H, Ar-H), 3.54 (d, 1H, $J = 8.4$, CH), 2.93 (d, 1H, $J = 8.3$, CH); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 172.8 (COOH), 132.1, 128.7, 128.6, 128.3, 62.2, 40.9, 37.0; IR (KBr) 2400–3500 (br, COOH), 1715 $cm^{-1}$; MS (EI) $m/z$ 230 ($M^+$, <2%), 194 (23%), 185 (8), 160 (34), 149 (18), 131 (22), 116 (37), 115 (100), 105 (29). Anal. Calcd for $C_{10}H_8Cl_2O_2$: C, 51.98; H, 3.49. Found: C, 51.83; H, 3.33. HPLC analysis: Chiralpak AD, hexane–isopropanol 9:1, flow rate 0.8 mL/min, $t_{(+)} = 14.433$ min, $t_{(-)} = 7.942$ min.

*(−)-(1S,3S)-2,2-Dichloro-3-phenylcyclopropanecarbonitrile* ***1b***: Oil; $[\alpha]_D^{25} = -3.4$ ($c$ 3.8, $CHCl_3$); ee 29% (Chiral HPLC); identical spectra data as that of racemic **1b** were obtained. HPLC analysis: Chiralcel OJ, hexane–isopropanol 9:1, flow rate 0.8 mL/min, $t_{(+)} = 18.262$ min, $t_{(-)} = 12.841$ min.

**5.3.3. Enzymatic hydrolysis of racemic *trans*-2,2-dichloro-3-(2-methoxylphenyl)cyclopropanecarbonitrile 1c.** *(+)-(1R,3R)-2,2-Dichloro-3-(2-methoxylphenyl)cyclopropanecarboxamide* ***2c***: Mp 137–139 °C; $[\alpha]_D^{25} = +23.5$ ($c$ 0.85, $CHCl_3$); ee 60% (Chiral HPLC); $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 6.85–7.25 (m, 5H, Ar-H), 6.10 (br s, 2H, $NH_2$), 3.83 (s, 3H, $OCH_3$), 3.43 (d, 1H, $J = 8.5$, CH), 2.61 (d, 1H, $J = 8.6$, CH); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 167.2 ($CONH_2$), 158.9, 129.3, 128.3, 121.8, 120.0, 110.4, 62.1, 55.6, 37.7, 35.5; IR (KBr) 3437, 3156 ($NH_2$), 1685 $cm^{-1}$; MS (EI) $m/z$ 261 ($M^+$+2, 1), 259 ($M^+$, 1%), 241 (1), 221 (14), 206 (11), 181 (35), 135 (100), 77 (50). Anal. Calcd for $C_{11}H_{11}Cl_2NO_2$: C, 50.79; H, 4.26; N, 5.38. Found: C, 50.87; H, 4.16; N, 5.20. HPLC analysis: Chiralcel OJ, hexane–isopropanol 9:1, flow rate 0.8 mL/min, $t_{(+)} = 18.684$ min, $t_{(-)} = 24.548$ min.

*(−)-(1S,3S)-2,2-Dichloro-3-(2-methoxylphenyl)cyclopropanecarboxylic acid* ***3c***: Mp 108–110 °C; $[\alpha]_D^{25} = -30$ ($c$ 0.6, $CHCl_3$); ee 93% (Chiral HPLC); $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 10.10 (br s, 1H, COOH), 6.99–7.41 (m, 5H, Ar-H), 3.99 (s, 3H, $CH_3$), 3.59 (d, 1H, $J = 8.7$, CH), 2.90 (d, 1H, $J = 8.4$, CH); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 172.6 (COOH), 158.7, 129.4, 128.2, 121.1, 120.0, 110.3, 62.4, 55.5, 37.2, 36.3; IR (KBr) 2200–3240 (br, COOH), 1712 $cm^{-1}$; MS (EI) $m/z$ 262 ($M^+$+2, 1), 260 ($M^+$, 1%), 227 (10), 226 (34), 225 (27), 224 (100), 209 (49), 189 (21), 181 (22), 179 (25), 135 (83), 115 (57), 77 (51). Anal. Calcd for $C_{11}H_{10}Cl_2O_3$: C, 50.60; H, 3.86. Found: C, 50.78; H, 4.12. HPLC analysis: Chiralpak AD, hexane–isopropanol 9:1, flow rate 0.8 mL/min, $t_{(-)} = 7.263$ min, $t_{(+)} = 8.754$ min.

*(±)-trans-2,2-Dichloro-3-(2-methoxylphenyl)cyclopropanecarbonitrile* ***1c***: Mp 81–82 °C; $[\alpha]_D^{25} = 0$; identical spectra data as that of racemic **1c** were obtained. HPLC analysis: Chiralcel OD, hexane–isopropanol 9:1, flow rate 0.8 mL/min, 14.242 min, 22.364 min.

**5.3.4. Enzymatic hydrolysis of racemic *trans*-2,2-dibromo-3-phenylcyclopropanecarbonitrile 1d.** *(+)-(1R,3R)-2,2-Dibromo-3-phenylcyclopropanecarboxamide* ***2d***: Mp 59–161 °C; $[\alpha]_D^{25} = +68.4$ ($c$ 0.95, $CHCl_3$); ee 95% (Chiral HPLC); $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 7.19–7.34 (m, 5H, Ar-H), 6.06 (br s, 2H, $NH_2$), 3.51 (d, 1H, $J = 7.8$, CH), 2.79 (d, 1H, $J = 8.1$, CH); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 167.4 ($CONH_2$), 134.6, 129.1, 128.8, 128.4, 40.0, 38.8, 29.2; IR (KBr) 3420, 3187 ($NH_2$), 1660 $cm^{-1}$; MS (EI) $m/z$ 276 ($M^+$−41, 2%), 237 ($M^+$−HBr, 3%), 235 (2), 105 (100), 77 (68). Anal. Calcd for $C_{10}H_9Br_2NO$: C, 37.65; H, 2.84; N, 4.39. Found: C, 37.77; H, 2.77; N, 4.19. HPLC analysis: Chiralcel OD, hexane–isopropanol 9:1, flow rate 0.8 mL/min, $t_{(+)} = 21.585$ min, $t_{(-)} = 19.214$ min.

*(−)-(1S,3S)-2,2-Dibromo-3-phenylcyclopropanecarbonitrile* ***1d***: Oil; $[\alpha]_D^{25} = -27$ ($c$ 1.0, $CHCl_3$); ee 72% (Chiral HPLC); identical spectra data as that of racemic **1d** were obtained. HPLC analysis: Chiralcel OJ, hexane–isopropanol 9:1, flow rate 0.8 mL/min, $t_{(-)} = 18.931$ min, $t_{(+)} = 32.626$ min.

**5.3.5. Enzymatic hydrolysis of racemic *trans*-2,2-difluoro-3-phenylcyclopropanecarboxamide 2a.** *(−)-(1R,3R)-2,2-Difluoro-3-phenylcyclopropanecarboxamide* ***2a***: 15 min (46% yield), mp 131–132 °C; $[\alpha]_D^{25} = -112$ ($c$ 1.0, $CHCl_3$); ee > 99% (Chiral HPLC).

*(+)-(1S,3S)-2,2-Difluoro-3-phenylcyclopropanecarboxylic acid* ***3a***: 15 min (51% yield), mp 105–107 °C; $[\alpha]_D^{25} = +110$ ($c$ 1.5, $CHCl_3$); ee 87% (Chiral HPLC).

**5.3.6. Enzymatic hydrolysis of racemic *trans*-2,2-dichloro-3-phenylcyclopropanecarboxamide 2b.** *(+)-(1R,3R)-2,2-Dichloro-3-phenylcyclopropanecarboxamide* ***2b***: 8 h (49% yield), mp 152–153 °C; $[\alpha]_D^{25} = +73$ ($c$ 1.0, $CHCl_3$); ee > 99% (Chiral HPLC); $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 7.26–7.40 (m, 5H, Ar-H), 6.08 (br s, 2H, $NH_2$), 3.52 (d, 1H, $J = 8.2$, CH), 2.74 (d, 1H, $J = 8.2$, CH); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 166.7 ($CONH_2$), 132.7, 128.7, 128.4, 128.0, 61.7, 39.1, 38.2; IR (KBr) 3476, 3206 ($NH_2$), 1642 $cm^{-1}$; MS (EI) $m/z$ 230 ($M^+$, <2%), 212 (<1%), 188 (32), 187 (19), 186 (50), 153 (27), 151 (100), 149 (50), 115 (95), 105 (24). Anal. Calcd for $C_{10}H_9Cl_2NO$: C, 52.20;

H, 3.94; N, 6.09. Found: C, 52.21; H, 3.90; N, 5.86. HPLC analysis: Chiralcel OJ, hexane–isopropanol 9:1, flow rate 0.8 mL/min, $t_{(+)} = 19.809$ min, $t_{(-)} = 17.700$ min.

*(−)-(1S,3S)-2,2-Dichloro-3-phenylcyclopropanecarboxylic acid* ***3b***: 8 h (48% yield), mp 89–91 °C; $[\alpha]_D^{25} = -64.4$ (*c* 0.8, $CHCl_3$); ee 92% (Chiral HPLC).

The reaction was performed in a multi-gram scale to produce highly enantiopure (1*R*,3*R*)-**2b** (48% yield, >99% ee) and (1*S*,3*S*)-**3b** (48% yield, 95%).[11]

**5.3.7. Enzymatic hydrolysis of racemic *trans*-2,2-difluoro-3-(2-methoxylphenyl)cyclopropanecarboxamide 2c.** *(+)-(1R,3R)-2,2-Dichloro-3-(2-methoxylphenyl)cyclopropanecarboxamide* ***2c***: 7 d (71% yield), mp 143–145 °C; $[\alpha]_D^{25} = +5.7$ (*c* 1.4, $CHCl_3$); ee 14% (Chiral HPLC).

*(−)-(1S,3S)-2,2-Dichloro-3-(2-methoxylphenyl)cyclopropanecarboxylic acid* ***3c***: 7 d (16% yield), mp 108–110 °C; $[\alpha]_D^{25} = -29.5$ (*c* 0.95, $CHCl_3$); ee 89% (Chiral HPLC).

**5.3.8. Enzymatic hydrolysis of racemic *trans*-2,2-dibromo-3-phenylcyclopropanecarboxamide 2d.** *(+)-(1R,3R)-2,2-Dibromo-3-phenylcyclopropanecarboxamide* ***2d***: 180 h (16% yield), mp 167–168 °C; $[\alpha]_D^{25} = +71.7$ (*c* 0.6, $CHCl_3$); ee > 99% (Chiral HPLC).

*(−)-(1S,3S)-2,2-Dibromo-3-phenylcyclopropanecarboxylic acid* ***3d***: 6 d (60% yield), mp 113–115 °C; $[\alpha]_D^{25} = -40$ (*c* 2.0, $CHCl_3$); ee 41% (Chiral HPLC); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 9.55 (br s, 1H, COOH), 7.20–7.32 (m, 5H, Ar-H), 3.41 (d, 1H, $J = 8.2$, CH), 2.88 (d, 1H, $J = 8.4$, CH); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 173.2 (COOH), 133.6, 128.6 (3C), 128.3 (2C), 41.4, 37.0, 28.3; IR (KBr) 2150–3400 (br, COOH), 1707 cm$^{-1}$; MS (EI) *m/z* 320 ($M^+$+2, <2%), 277 ($M^+$−41, 2%), 275 ($M^+$−43, 5%), 273 (2), 240 (23), 239 (13), 238 (21), 195 (14), 193 (14), 159 (39), 116 (24), 115 (100). Anal. Calcd for $C_{10}H_8Br_2O_2$: C, 37.54; H, 2.52. Found: C, 37.59; H, 2.43. HPLC analysis: Chiralpak AD, hexane–isopropanol 9:1, flow rate 0.8 mL/min, $t_{(+)} = 18.000$ min, $t_{(-)} = 8.420$ min.

### 5.4. Chemical transformations of optically active amides and acids, and determination of the configurations of biotransformation products

**5.4.1. Determination of the configuration of (−)-(1*R*,3*R*)-2,2-difluoro-3-phenylcyclopropanecarboxamides 2a.** Dehalogenation of (−)-2,2-difluoro-3-phenylcyclopropanecarboxamides **2a** (ee >99% Chiral; HPLC) to (−)-*trans*-(1*R*,3*R*)-2-phenylcyclopropanecarboxamide **2e** was accomplished in 53% yield from the reaction with $NaBH_4$ (5 equiv) in DMSO at 110 °C. (−)-(1*R*,3*R*)-**2e**: $[\alpha]_D^{25} = -210.7$ (*c* 0.75, $CHCl_3$); ee 74% (Chiral HPLC). Identical spectra as for (−)-**2e**[8] obtained directly from biotransformation were obtained.

**5.4.2. Determination of the configuration of (+)-(1*R*,3*R*)-2,2-dichloro-3-phenylcyclopropanecarboxamides 2b.** Dehalogenation of (+)-2,2-dichloro-3-phenylcyclopropanecarboxamides **2b** (ee > 99%; Chiral HPLC) to (−)-*trans*-(1*R*,3*R*)-2-phenylcyclopropanecarboxamide **2e** was accomplished in 46% yield by the reaction with $NaBH_4$ (4 equiv) in ethanol under refluxing conditions. (−)-(1*R*,3*R*)-**2e**: $[\alpha]_D^{25} = -308$ (*c* 0.5, $CHCl_3$); ee >99% (Chiral HPLC). Identical spectra as for (−)-**2e**[8] obtained directly from biotransformation were obtained.

**5.4.3. Determination of the configuration of (+)-(1*R*,3*R*)-2,2-dibromo-3-phenylcyclopropanecarboxamides 2d.** Dehalogenation of (+)-2,2-dichloro-3-phenylcyclopropanecarboxamides **2d** (ee > 99%; Chiral HPLC) to (−)-*trans*-(1*R*,3*R*)-2-phenylcyclopropanecarboxamide **2e** was accomplished in 50% yield by the reaction with $NaBH_4$ (5 equiv) in ethanol under refluxing conditions. (−)-(1*R*,3*R*)-**2e**: $[\alpha]_D^{25} = -300$ (*c* 0.5, $CHCl_3$); ee 97% (Chiral HPLC). Identical spectra as for (−)-**2e**[8] obtained directly from biotransformation were obtained.

**5.4.4. Preparation of (1*S*,3*S*)-2,2-dihalo-3-phenylcyclopropanecarboxamides 2a, 2b and 2d.** (+)-(1*S*,3*S*)-2,2-Difluoro-3-phenylcyclopropanecarboxylic acid **3a** (ee 87%), (−)-(1*S*,3*S*)-2,2-dichloro-3-phenyldichlorocyclopropanecarboxylic acid **3b** (ee 92%) and (−)-(1*S*,3*S*)-2,2-dibromo-3-phenylcyclopropanecarboxylic acid **3d** (ee 26%) reacted, respectively, with freshly distilled $SOCl_2$ at room temperature to afford the corresponding acid chloride, and the excess $SOCl_2$ was then removed under reduced pressure. To the resulting solution was added cold aqueous ammonia solution while stirring at 0 °C, and after workup, (+)-(1*S*,3*S*)-2,2-difluoro-3-phenylcyclopropanecarboxamide **2a**, (−)-(1*S*,3*S*)-2,2-dichloro-3-phenylcyclopropanecarboxamide **2b** and (−)-(1*S*,3*S*)-2,2-dibromo-3-phenylcyclopropanecarboxamide **2d** were yielded in 84%, 88% and 90% yields, respectively. (+)-(1*S*,3*S*)-**2a**: $[\alpha]_D^{25} = +96$ (*c* 0.8, $CHCl_3$); ee 89% (Chiral HPLC). (−)-(1*S*,3*S*)-**2b**: $[\alpha]_D^{25} = -41$ (*c* 1.0, $CHCl_3$); ee 95% (Chiral HPLC). (−)-(1*S*,3*S*)-**2d**: $[\alpha]_D^{25} = -24$ (*c* 1.0, $CHCl_3$); ee 29% (Chiral HPLC). Identical spectra data as for (−)-**2a**, (+)-**2b** and (+)-**2d** obtained directly from biotransformation were obtained.

**5.4.5. Synthesis and determination of the configurations of optically active *gem*-dihalocyclopropanecarbonitriles 1a, 1b and 1d.** Dehydration of (−)-(1*R*,3*R*)-amide **2a** (ee >99%), (+)-(1*R*,3*R*)-amide **2b** and (+)-(1*R*,3*R*)-amide **2d** to (−)-*trans*-(1*R*,3*R*)-2,2-difluoro-3-phenylcyclopropanecarbonitrile **1a**, (+)-(1*R*,3*R*)-2,2-dichloro-3-phenylcyclopropanecarbonitrile **1b** and (+)-(1*R*,3*R*)-2,2-dibromo-3-phenylcyclopropanecarbonitrile **1d** was accomplished in 86%, 86% and 83% yields, respectively, from the treatment with $SOCl_2$ in benzene and DMF at room temperature. (−)-**1a**: $[\alpha]_D^{25} = -130.7$ (*c* 0.75, $CHCl_3$); ee >99% (Chiral HPLC). (+)-**1b**: $[\alpha]_D^{25} = +11.8$ (*c* 0.85, $CHCl_3$); ee >99% (Chiral HPLC). (+)-**1d**: $[\alpha]_D^{25} = +44.3$ (*c* 0.3, $CHCl_3$); ee 97% (Chiral HPLC).

Identical spectra as for racemic **1a**, **1b** and **1d** were obtained.

**5.4.6. Chemical hydrolysis of (+)-*trans*-(1*R*,3*R*)-2,2-dichloro-3-phenylcyclopropanecarboxamide 2b.** (+)-*trans*-(1*R*,3*R*)-2,2-Dichloro-3-phenylcyclopropanecarboxamide **2b** (20 mg, ee >99%) was refluxed in HCl (6 N, 4 mL) for 6 h to give, after the workup, 90% yield of (+)-*trans*-(1*R*,3*R*)-2,2-dichloro-3-phenylcyclopropanecarboxylic acid **3b**: mp 93–95 °C; $[\alpha]_D^{25} = +64$ (*c* 0.75, $CHCl_3$); ee > 99% (Chiral HPLC). Identical spectra as for (−)-**3b** were obtained.

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## References and notes

1. (a) Harper, D. B. *Biochem. Soc. Trans.* **1976**, *4*, 502; (b) Harper, D. B. *Biochem. J.* **1977**, *165*, 309; (c) Kobayashi, M.; Shimizu, S. *FEMS Microbiol. Lett.* **1994**, *120*, 217.
2. (a) Asano, Y.; Tani, Y.; Yamada, H. *Agric. Biol. Chem.* **1980**, *44*, 2251; (b) Asano, Y.; Fujishiro, K.; Tani, Y.; Yamada, H. *Agric. Biol. Chem.* **1982**, *46*, 1165; (c) Asano, Y.; Tachibana, M.; Tani, Y.; Yamada, H. *Agric. Biol. Chem.* **1982**, *46*, 1175.
3. (a) Wang, M.-X.; Lu, G.; Ji, G.-J.; Huang, Z.-T.; Meth-Cohn, O.; Colby, J. *Tetrahedron: Asymmetry* **2000**, *11*, 1123; (b) Wang, M.-X.; Li, J.-J.; Ji, G.-J.; Li, J.-S. *J. Mol. Catal. B: Enzym.* **2001**, *14*, 77; (c) Wang, M.-X.; Zhao, S.-M. *Tetrahedron Lett.* **2002**, *43*, 6617; (d) Wang, M.-X.; Zhao, S.-M. *Tetrahedron: Asymmetry* **2002**, *13*, 169; (e) Wang, M.-X.; Lin, S.-J. *Tetrahedron Lett.* **2001**, *42*, 6925; (f) Wang, M.-X.; Lin, S.-J. *J. Org. Chem.* **2002**, *67*, 6542; (g) Wang, M.-X.; Wu, Y. *Org. Biomol. Chem.* **2003**, *1*, 535; (h) Wang, M.-X.; Lin, S.-J.; Liu, C.-S.; Zheng, Q.-Y.; Li, J.-S. *J. Org. Chem.* **2003**, *68*, 4570; (i) Wang, M.-X.; Liu, C.-S.; Li, J.-S. *Tetrahedron Lett.* **2000**, *41*, 8549; (j) Wang, M.-X.; Liu, C.-S.; Li, J.-S. *Tetrahedron: Asymmetry* **2001**, *12*, 3367.
4. For a recent review, see: (a) Sugai, T.; Yamazaki, T.; Yokoyama, M.; Ohta, H. *Biosci. Biotechnol. Biochem.* **1997**, *61*, 1419, and references cited therein; (b) For recent examples of enantioselective biotransformation of nitriles, see: Wegman, M. A.; Heinemann, U.; Stolzs, A.; van Rantwijk, F.; Sheldon, R. A. *J. Mol. Catal. Enzym.* **2001**, *11*, 249; (c) Bauer, R.; Knackmuss, H. J.; Stolzs, A. *Appl. Microbiol. Biotechnol.* **1998**, *49*, 89; (d) Matoishi, K.; Sano, A.; Yamazaki, T.; Yokoyama, M.; Sugai, T.; Ohta, H. *Tetrahedron: Asymmetry* **1998**, *9*, 1097; (e) Wu, Z.-L.; Li, Z.-Y. *Tetrahedron: Asymmetry* **2001**, *12*, 3305.
5. Blakey, A. J.; Colby, J.; Williams, E.; O'Reilly, C. *FEMS Microbiol. Lett.* **1995**, *129*, 57.
6. (a) Meth-Cohn, O.; Wang, M.-X. *Tetrahedron Lett.* **1995**, *36*, 9561; (b) Meth-Cohn, O.; Wang, M.-X. *J. Chem. Soc., Perkin Trans. 1* **1997**, 1099.
7. (a) Meth-Cohn, O.; Wang, M.-X. *Chem. Commun.* **1997**, 1041; (b) Meth-Cohn, O.; Wang, M.-X. *J. Chem. Soc., Perkin Trans. 1* **1997**, 3197.
8. Wang, M.-X.; Feng, G.-Q. *New J. Chem.* **2002**, *26*, 1575.
9. Wang, M.-X.; Feng, G.-Q. *J. Mol. Catal. B: Enzym.* **2002**, *18*, 267.
10. Wang, M.-X.; Feng, G.-Q. *J. Org. Chem.* **2003**, *68*, 621.
11. Part of this work has appeared as a preliminary communications: Wang, M.-X.; Feng, G.-Q.; Zheng, Q.-Y. *Adv. Synth. Catal.* **2003**, *345*, 695.
12. For a very recent comprehensive review, see: Fedorynski, M. *Chem. Rev.* **2003**, *103*, 1099.
13. Shibuya, A.; Sato, A.; Taguchi, T. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1979.
14. Miyazawa, K.; Yufit, D. S.; Howard, J. A. K.; Meijere, A. *Eur. J. Org. Chem.* **2000**, 4109.
15. Itoh, T.; Mitsukura, K.; Furutani, M. *Chem. Lett.* **1998**, 903.
16. Mitsukura, K.; Korekiyo, S.; Itoh, Y. *Tetrahedron Lett.* **1999**, *40*, 5739.
17. Kobayashi, Y.; Taguchi, T.; Morikawa, T.; Takase, H.; Takanashi, H. *J. Org. Chem.* **1982**, *47*, 3232.
18. Dehmlow, E. V. *Angew. Chem., Int. Ed. Engl.* **1974**, *13*, 170.