Nitrile and Amide Biotransformations for Efficient Synthesis of Enantiopure *gem*-Dihalocyclopropane Derivatives

Mei-Xiang Wang,* Guo-Qiang Feng, Qi-Yu Zheng

Laboratory of Chemical Biology, Center for Molecular Science, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100080, P. R. China
Fax: (+86)-10-62569564, e-mail: mxwang@iccas.ac.cn

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Abstract: Catalyzed by *Rhodococcus* sp. AJ270 microbial cells, *trans*-2,2-dihalo-3-phenylcyclopropanecarbonitriles and -amides underwent enantioselective hydrolysis under very mild conditions. Both the efficiency and enantioselectivity of the nitrile hydratase and amidase involved in the cells were strongly determined by the nature of the halogen substituent. The synthetic utility of the biocatalytic process was illustrated by an efficient and multigram scale biotransformation and synthesis of enantiopure 2,2-dichloro-3-phenylcyclopropanecarboxylic acid and amide in both enantiomeric forms.

Keywords: amidase; biotransformation; 2,2-dihalo-3-phenylcyclopropanecarboxylic acid derivatives; enantioselectivity; hydrolysis; kinetic resolution; nitrile hydratase; *Rhodococcus* sp. AJ270

The geminally dihalogenated cyclopropane derivatives, easily available from the addition reaction of dihalocarbenes to carbon-carbon double bonds, are intriguing and versatile synthetic intermediates in organic chemistry. The ring opening reactions of *gem*-dihalocyclopropane derivatives under different conditions, for example, have been reported to yield various interesting products including aromatics,[1–3] mono-substituted acetylenes,[4] and carbocyclic[5–7] and heterocyclic compounds.[8] *gem*-Dihalocyclopropane derivatives have also been utilized as unique reactants to synthesize cyclopropane-containing compounds through selective dehalogenation,[9] regioselective radical cyclization,[10] and stereoselective carbon-carbon bond-forming reaction.[11] On the other hand, *gem*-dihalo-, especially, *gem*-difluorocyclopropane derivatives have been shown to possess interesting biological[12] and physiochemical[13] properties.

Despite their easy availability, versatile chemical transformations and potential biological applications, the chemistry of geminally dihalogenated cyclopropane derivatives remains largely unexplored. This is particularly true for chiral *gem*-dihalocyclopropane compounds although some of the products derived from their reactions are stereogenic,[3,5,6,9–11] and chiral *gem*-dihalocyclopropane molecules show remarkably different bioactivity.[12] This is most probably due to the lack of efficient methods for the generation of chiral *gem*-dihalocyclopropane structures. To our knowledge, only few methods have been reported for the preparation of optically active *gem*-difluorocyclopropane compounds using lipase-mediated hydrolysis and esterification of esters[14] and alcohols,[13,15] respectively.

Biotransformations of nitriles,[16] through either a direct nitrilase-catalyzed conversion of a nitrile to a carboxylic acid[17] or a nitrile hydratase-catalyzed hydration of a nitrile followed by the hydrolysis of amide to acid by the action of the amidase,[18] have been demonstrated as being unique and environmentally benign methods for the synthesis of chiral carboxylic acids and their amide derivatives because of the excellent selectivity and very mild reaction conditions. Our earlier work has shown that *Rhodococcus* sp. AJ270, a robust nitrile hydratase/amidase-containing microorganism,[19] was able to hydrolyze a wide range of structurally diverse nitriles,[20] dinitriles[21] and racemic nitriles[22] or prochiral dinitriles[23] with excellent chemo-, regio- and enantioselectivieties. Very recently, we have found that *Rhodococcus* sp. AJ270 could efficiently and enantioselectively catalyze the hydrolysis of a number of differently substituted and configurated racemic cyclopropanecarbonitriles and amides to produce enantiopure carboxylic acids and/or amides in high yields. It has been also proposed that a readily reachable reactive site be embedded within the spacious pocket of the enantioselective nitrile hydratase while the amidase comprises a relatively deep-buried and size-limited enantioselective active site.[24,25] Our continuous interest in understanding the reaction scope and applications of both nitrile hydratase and amidase involved in *Rhodococcus* sp. AJ270, and in synthesizing enantiopure cyclopropane derivatives led us to undertake the current study on the biotransformation of racemic 2,2-dihalo-3-phenylcyclopropanecarbonitriles and -amides. It is also hoped that the use of *gem*-dihalocyclopropanecarbonitriles and amides as the substrates would allow us to further define the steric

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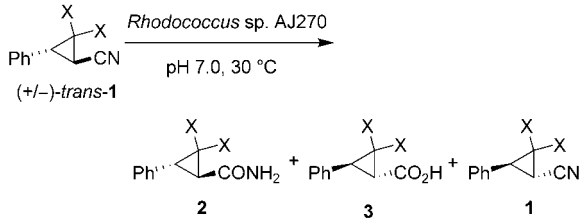
Table 1. Biotransformations of racemic nitriles **1**.

Entry	**1**	X	Conditions[a]	**2** [%]	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	0.5 mmol, 7 d	–	–	35	61	58	47
4	**1c**	Br	0.5 mmol, 7 d	24	95	trace	n.d.[e]	48	54
5	**1d**	H	0.5 mmol, 20 min	40	>99	49	70	7	>5
6	**1d**	H	1.73 mmol, 30 min	37	78	37	73	20	<5
7	**1e**	Me	0.5 mmol, 30 h	48	>99	46	99	–	–
8	**1e**	Me	1 mmol, 12 h	32	59	43	>99	19	>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.



Scheme 1. Biotransformations of racemic nitriles **1**.

feature of the active site of the nitrile hydratase and amidase.

We first tested the biotransformation of *gem*-dihalocyclopropanecarbonitriles **1a – c** catalyzed by *Rhodococcus* sp. AJ270 whole cells at 30 °C in aqueous phosphate buffer (pH 7.0) (Scheme 1, Table 1). To examine the effect of halogen substituent X on the reaction, biotransformations of 2-phenylcyclopropanecarbonitrile **1d** and of the geminally dimethylated nitrile analogue **1e** were also included.[24,25] The outcomes demonstrated in Table 1 indicated clearly that the substituent X played an important role in determining both the reaction rate and reaction enantioselectivity. Geminally difluoro-substituted cyclopropanecarbonitrile **1a**, for example, underwent rapid and efficient hydrolysis to afford good yield 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 **1d**[24] (Entry 5). Prolonged incubation of (+/–)-**1a** led to the complete hydration of nitrile and higher conversion of the resulting amide (Entry 2). In contrast, the hydrolysis of *gem*-dibromocyclopropanecarbonitrile **1c** proceeded sluggishly; a week's interaction of **1c** with microbial cells gave almost 50% of the optically active nitrile 1*S*, 3*S*-**1a** (ee 54%) along with the isolation of 24% of the amide 1*R*,3*R*-**2c** (ee 95%) and a trace amount of acid **3c** (Entry 4). Since both amide **2c** and acid **3c** were stable under the reaction conditions (*vide infra*), the loss of mass balance in this case is probably ascribed to the decomposition of nitrile **1c**, which was evidenced by the formation of a mixture of inseparable by-products. The biocatalytic conversion of *gem*-dichlorocyclopropanecarbonitrile **1b** took place slowly to give, after a week, more than 50% of recovered nitrile 1*S*,3*S*-**1b** in 47% ee and acid 1*S*,3*S*-**3b** in 35% yield with 61% ee. Noticeably, no amide product **2b** was accumulated during the progress of reaction (Entry 3), indicating that the biohydrolysis of amide intermediate **2b** proceeded much fast than its generation from biohydration of the nitrile **1b**.

To shed further light on the enantioselective biotransformation of nitriles **1**, the racemic amides (+/–)-**2** were subjected to *Rhodococcus* sp. AJ270 (Scheme 2) and the results are summarized in Table 2. Both fluoro- and chloro-substituted *gem*-dihalocyclopropanecarboxamides (+/–)-**2a** and (+/–)-**2b** underwent rapid and efficient kinetic resolution to afford the corresponding 1*R*,3*R*-amides **2** and 1*S*,3*S*-acids **3** in high enantiomeric purity (Entries 1–3). However, the *gem*-dibromocyclopropanecarboxamide (+/–)-**2c** was resolved very slowly to give 1*R*,3*R*-amide **2c** and 1*S*,3*S*-acid **3c** with very low enantioselectivity (E = 1.9). The presence of co-solvent such as acetone and methanol slightly improved the reaction rate and enantioselectivity (E = 5.5–7 3) (Entries 4–6). The results in Table 2 showed that the reaction rate of amide hydrolysis decreased with the increase of steric bulkiness of the substituent X. On the other hand, the bulkier the substituent X, the higher is the enantioselectivity with exception of bromo-substituted substrate **2c**.

It has been revealed that both the nitrile hydratase and the amidase involved in *Rhodococcus* sp. AJ270 are sensitive to the steric effect of the substituents on the cyclopropane ring. While the amidase is generally enantioselective, the nitrile hydratase shows either excellent or almost no enantioselectivity against *trans*-2-arylcyclopropanecarbonitriles depending on whether a pair of geminal methyl substituents is present or not.[24,25] Our current study has further demonstrated that the introduction of geminal halogen substituents

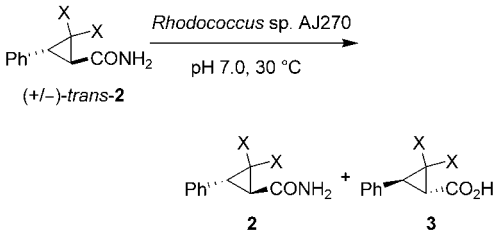
Table 2. Biocatalytic kinetic resolution of racemic amides **2**.

Entry	**2**	X	Conditions[a]	**2** [%][b]	ee [%][c]	**3** [%][b]	3e [%][c]	E
1	**2a**	F	0.5 mmol, 15 min	46	>99	51	87	74
2	**2a**	F	0.5 mmol, 2 h	10	>99	81	11	–
3	**2b**	Cl	0.5 mmol, 8 h	49	>99	48	92	125
4	**2c**	Br	0.5 mmol, 7 d	49	11	43	26	1.9
5	**2c**	Br	0.25 mmol, 6 d[d]	26	93	60	41	7.3
6	**2c**	Br	0.25 mmol, 7 d[e]	16	>99	78	18	5.5
7	**2d**	H	0.5 mmol, 20 min	46	78	51	59	8.9
8	**2e**	Me	0.5 mmol, 73 h	46	>99	52	92	125

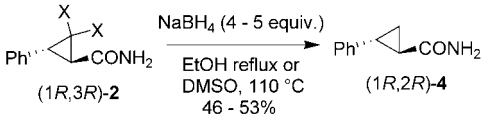
[a] Reaction conditions were not optimized.
[b] Isolated yield.
[c] Determined with chiral HPLC analysis.
[d] Acetone (2 mL) was added.
[e] Methanol (2 mL) was added.

also increases the enantioselection of the amidase. When the X in amide **2** was getting as large as a bromine substituent, however, the amidase action became dramatically inefficient with poor enantioselectivity (Table 2). Although the hydration reaction slowed down, the presence of two geminal chlorine or bromine substituents appeared beneficial for the enhancement of enantioselectivity of the nitrile hydratase, albeit in a moderate degree. The overall efficiency and enantioselectivity of biotransformations of nitriles originated from the combined effects of nitrile hydratase and amidase involved in microbial cells (Table 1). It is apparently advantageous to apply biotransformations of amide rather than nitrile to prepare enantiomerically pure *gem*-difluoro- and *gem*-dichlorocyclopropane derivatives. To show the synthetic utility, the biocatalytic kinetic resolution of amide (+/−)-**2** was then performed on a multi-gram scale. A one-pot biotransformation of (+/−)-**2b** (2.3 g, 10 mmol) therefore 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% ee).

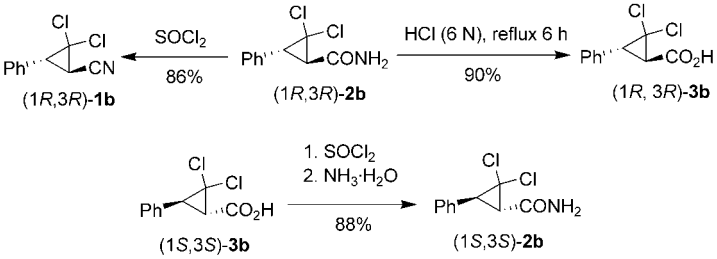
To determine the absolute configuration of and also to prepare the antipode of the biotransformation products, a reductive dehalogenation reaction and functional group transformations were conducted. By refluxing *gem*-dichloro- and *gem*-dibromocyclopropanecarboxamides **2b, c** with $NaBH_4$ in ethanol, 1*R*,2*R*-2-phenylcyclopropanecarboxamide **4** was obtained in moderate yield. The configuration of **4** was determined through measuring and comparing its optical rotation with that of an authentic sample.[24] Only at elevated temperatures was reductive defluorination of **2a** effected efficiently (Scheme 3). The 1*R*,3*R*-amide **2b** underwent ready chemical hydrolysis and dehydration reactions to furnish 1*R*,3*R*-acid **3b** and 1*R*,2*R*-nitrile **1b**, respectively, in good yield, while the 1*S*,3*S*-amide **2b** was easily prepared from 1*S*,3*S*-**3b** *via* an acid chloride intermediate (Scheme 4). No racemerization was observed during these chemical transformations.



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



Scheme 3. Reductive dehalogenation of (1*R*,2*R*)-**2**.



Scheme 4. Preparation of optically active compounds.

In conclusion, we have shown that *Rhodococcus* sp. AJ270 cells are able to catalyze enantioselective biotransformations of *trans*-2,2-dihalo-3-phenylcyclopropancarbonitriles and -amides. Both the reaction rate and enantioselectivity of the nitrile hydratase and amidase involved in the microbial cells are strongly governed by the nature of halogen substituent. Coupled with facile chemical transformations, this biocatalytic process has provided effective syntheses of optically active 2,2-dichloro-3-phenylcyclopropanecarboxylic acid and amide in both enantiomeric forms.

Experimental Section

Typical Procedure for the Multi-Gram Scale Biotransformation of Racemic 2,2-Dichloro-3-phenylcyclopropanecarboxamide (2b)

To a suspension of *Rhodococcus* sp. AJ270 cells (4 g wet weight) in aqueous potassium phosphate buffer (0.1 M, pH 7.0, 80 mL) was added racemic amide **2b** (1.2 g) and the mixture was then incubated at 30 °C using an orbital shaker (200 rpm). Another portion of racemic amide **2b** (1.1 g) and the suspension of cells (4 g wet weight in 20 mL of buffer) were added at 20- and 84-h intervals, respectively. After continuous incubation for a total of 130 h, the biomass was removed through Celite pad filtration. The resulting aqueous solution was basified to pH 12 with aqueous NaOH (2 M). Extraction with ethyl acetate or CH_2Cl_2 gave, after drying (anhydrous $MgSO_4$) and concentration, (+)-(1*R*,3*R*)-2,2-dichloro-3-phenylcyclopropanecarboxamide: yield: 48%; mp 152–153 °C; $[\alpha]_D^{25}$: +76 (*c* 1.0, $CHCl_3$); ee >99% (chiral HPLC); ^{1}H 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, $J = 8.2$ Hz, CH), 2.74 (d, 1H, $J = 8.2$ Hz, 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): $\nu = 3476, 3206$ (NH_2), 1642 cm^{-1}; MS (EI): *m/z* (%) = 188 (32), 187 (19), 186 (50), 185 ($M^+ - 44$, 22), 153 (27), 151 (100), 149 (51), 115 (95); 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, $t_{(+)} =$ 19.81 min, $t_{(-)} = 17.70$ min.

The aqueous solution was then acidified using aqueous HCl (2 M) to pH 2 and extracted with ethyl acetate or CH_2Cl_2. After removal of solvent, (−)-(1*S*,3*S*)-2,2-dichloro-3-phenylcyclopropanecarboxylic acid was obtained as a solid: yield: 48%; mp 89–91 °C; $[\alpha]_D^{25}$: −51.2 (*c* 1.0, $CHCl_3$); ee 95% (chiral HPLC); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 10.15$ (br s, 1H, COOH), 7.28–7.45 (m, 5H, Ar-H), 3.54 (d, 1H, $J = 8.4$ Hz, CH), 2.93 (d, 1H, $J = 8.3$ Hz, 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; MS (EI): *m/z* (%) = 196 (8), 194 ($M^+ - 36$, 23), 160 (34), 151 (8), 149 (18), 115 (100); 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, $t_{(+)} = 14.43$ min, $t_{(-)} = 7.94$ min.

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