A novel approach to enantiopure cyclopropane compounds from biotransformation of nitriles†

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Rhodococcus sp. AJ270, a powerful and versatile nitrile hydratase/amidase containing microbial whole-cell system, catalyzed the enantioselective hydrolysis of both racemic *trans*- and *cis*-2-arylcyclopropanecarbonitriles to afford the corresponding amides and acids with enantiomeric excesses as high as $>99\%$. The reaction rate and enantioselectivity observed for both nitrile hydratase and amidase were also strongly dependent upon the nature of the substituent and substitution pattern on the benzene ring of the substrates. The application of and the advantages of biotransformation of nitriles were demonstrated by preparing (1*S*,2*R*)-2-phenylcyclopropylamine and (1*R*,2*R*)-2-phenylcyclopropylmethylamine through facile and straightforward chemical manipulations of (1*S*,2*S*)-2-phenylcyclopropanecarboxylic acid and (1*R*,2*R*)-2-phenylcyclopropanecarboxamide, respectively.

Although the past two decades have seen tremendous development of enzyme-catalyzed reactions,[1,2] nitrile-hydrolyzing enzymes in organic synthesis remained largely unexplored until recently,[3,4] despite the fact that nitriles are very important compounds[5] and the bioconversion of nitriles into the corresponding carboxylic acids has been known for decades.[6] Enzyme-catalyzed hydrolyses of nitriles have been shown to proceed by either direct transformation to carboxylic acid with a nitrilase[7] or by the nitrile-hydratase-catalyzed formation of amides that are further hydrolyzed to the acids by an amidase.[8] So far, a range of microorganisms containing a nitrilase or nitrile hydratase/amidase, or both, has been isolated[9] and some nitrilases, nitrile hydratases and amidases have also been purified.[10] Mechanisms for the enzymatic hydrolysis of nitriles using nitrilase and nitrile hydratase have been proposed, and for the latter the hydration step is believed to involve complexation of the nitrile function to a transition metal such as iron or cobalt.[11] It is particularly worth noting that the microbial hydration of acrylonitrile is currently being applied in Japan to produce tens of thousands of tonnes of acrylamide per year.[12]

In previous studies,[9,10] we have demonstrated that *Rhodococcus* sp. AJ270, a novel isolate from a soil sample,[13] is a powerful and robust nitrile hydratase/amidase containing microorganism. Compared with other microorganisms reported recently, it has a broad activity against almost all types of nitriles, and both amides and acids can be produced in high yields from appropriate nitriles.[9] Excellent regioselectivity has also been observed with this microorganism when it catalyzed the hydrolysis of aromatic dinitriles and a variety of aliphatic dinitriles.[10] Very recently, we have found that *Rhodococcus* sp. AJ270 can enantioselectively transform racemic[14–16] and prochiral nitriles.[17] The amidase involved in *Rhodococcus* sp. AJ270 exhibited a high *S*-enantiocontrol against α-substituted phenylacetamides while the nitrile hydratase displayed a low *R*-selectivity against nitriles.[16] To further explore its potential in organic synthesis, and also to gain a deeper insight into the mechanisms of enzymatic hydrolyses of nitrile and amide, we have undertaken a systematic study of *Rhodococcus* sp. AJ270 whole-cell catalyzed hydrolysis of nitriles and amides. We report herein the enantioselective biotransformation of 2-arylcyclopropanecarbonitriles and -carboxamides, a novel enzymatic method for the preparation of optically pure cyclopropane derivatives.[18]

Syntheses[19] of enantiopure cyclopropane derivatives have attracted much attention in recent years because such compounds occur widely in natural products and in synthetic pharmaceuticals and agrochemicals and their enantiomers often exhibit different biological activities. For example, (+)-(1*S*,2*R*)-2-phenylcyclopropylamine has been shown to display fivefold more potent monoamine oxidase inhibitor activity than its enantiomer,[20] whereas (−)-(1*R*,2*S*)-2-(2-hydroxyphenyl)-*N,N*-di-*n*-propylcyclopropylamine is a strong and selective 5-hydroxytryptamine receptor agonist.[21] Among the various studies constructing chiral cyclopropanes, the progress of catalytic asymmetric cyclopropanation has been most noticeable due to the pioneering works of Nozaki,[22] Aratani,[23] Pfaltz,[24] Masamune,[25] Evans[26] and Doyle.[19a,b] Though high enantiocontrol has been achieved for catalytic asymmetric intermolecular cyclopropanation reactions between diazoacetates and alkenes, it should be noted that few examples[19,27] have been reported to effect both high enantiocontrol and diastereocontrol. In other words, highly diastereoselectivity for either the *trans* or, particularly, *cis* isomer with high enantiomeric excess is difficult to accomplish. The lipase catalysis has been reported[28] to be able to catalyze a kinetic resolution of *trans*-2-phenylcyclopropanecarboxylic acid ester while, in Lonza, the amidase has been successfully utilized to resolve 2,2-dimethylcyclopropanecarboxamide.[29]

Results and discussion

In order to examine the scope and limitations of the biotransformations catalyzed by *Rhodococcus* sp. AJ270 and also to

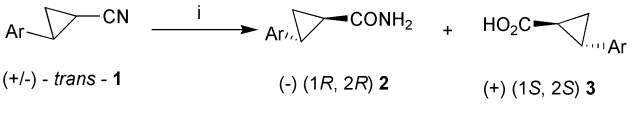
† Electronic supplementary information (ESI) available: preparation of racemic nitrile, amides and acids; spectroscopic data of racemic nitriles; biotransformation of racemic amides; chiral HPLC analyses of nitriles, amides, acids and amines. See http://www.rsc.org/suppdata/nj/b2/b200110a/

probe the active sites of the enzymes involved in the microbial cells, we prepared 2-arylcyclopropanecarbonitriles from the reaction between trimethylsulfoxonium iodide and substituted cinnamonitriles.[30] Both *trans* and *cis* isomers were readily separated using column chromatography.

Biotransformations of racemic *trans*-2-arylcyclopropanecarbonitriles

To begin our study, we first examined the hydrolysis of 2-phenylcyclopropanecarbonitrile, **1a**, catalyzed by *Rhodococcus* sp. AJ270 cells in aqueous phosphate buffer with pH 7.0 at 30 °C. As illustrated in Scheme 1, the reaction proceeded efficiently to produce optically active (1*R*,2*R*)-2-phenylcyclopropancarboxamide, **2a**, and (1*S*,2*S*)-2-phenylcyclopropanecarboxylic acid, **3a**. The results summarized in Table 1 indicates that, with the progression of the reaction, the enantiomeric excess of **2a** increased while the enantiomeric excess of **3a** decreased (entries 1–3), and optically inactive acid **3a** was obtained after the complete biocatalytic hydrolysis of nitrile **1a** and amide **2a** (entry 4). The nitrile **1a** recovered from the reaction (entry 1) was found to be almost optically inactive with an enantiomeric excess lower than 5%.

A similar change in enantiomeric excesses of both (1*R*,2*R*)-2-arylcyclopropancarboxamides **2b–g** and (1*S*,2*S*)-2-arylcyclopropanecarboxylic acids **3b–g** against the conversion of nitriles and amides was observed when the substrate was extended to a variety of 2-arylcyclopropanecarbonitrile analogs **1b–g**. The introduction of a substituent onto the benzene ring, however, led to an intriguing change in the reaction rate and enantiomeric selectivity. While the parent 2-phenylcyclopropanecarbonitrile **1a** and its *para*-substituted phenyl analogs **1b** and **1e–g** were efficient substrates and their conversion to the corresponding amides and acids finished in several hours, the reaction rate of the *ortho*- and particularly the *meta*-substituted substrates decreased remarkably. For example, the hydrolysis of 2-(4-chlorophenyl)cyclopropanecarbonitrile, **1b**, and 2-(2-chlorophenyl)cyclopropanecarbonitrile, **1d**, took place in hours and in several tens of hours, respectively, the effective conversion of 2-(3-chlorophenyl)cyclopropanecarbonitrile **1c**, was observed to take 6 days. Although the substitution pattern affected the hydrolysis rate, it did not drastically influence the enantioselectivity, as exemplified by the observation of comparable enantiomeric excesses obtained among amides **2b–d** and among acids **3b–d** from hydrolysis of chlorine-substituted phenylcyclopropanecarbonitriles **1b–d** (entries 5–10 in Table 1). Instead, it is the nature of the substituent that plays an important role in determining the enantiocontrol of the reaction. Good to excellent enantioselectivity was obtained from the biotransformations of 2-phenylcyclopropanecarbonitrile, **1a**, and its analogs **1b–f** bearing a methyl, chloro, or fluoro substituent, and the enantiomeric purity of (1*S*,2*S*)-2-arylcyclopropanecarboxylic acids **3** could be further improved by recrystallization. In contrast, the reaction of 2-(4-methoxyphenyl)cyclopropanecarbonitrile, **1g**, led to low enantioselectivity, giving the corresponding amide **2g** and acid **3g** in only 21–44% and 12–17% ee, respectively. It should be noted that the loss of mass balance from hydrolysis of nitriles is probably due to the degradation of organic molecules mediated by other enzymes present in *Rhodococcus* sp. AJ270 cells.



i. *Rhodococcus* sp. AJ270, phosphate buffer, pH 7.0, 30 °C

Scheme 1

Table 1 Biotransformations of racemic *trans*-2-arylcyclopropanecarbonitriles **1**

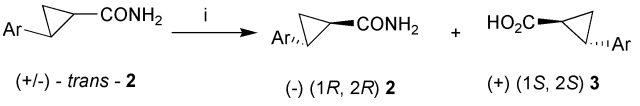
				2		**3**	
Entry	Nitrile **1**[a]	Ar	Time/h	Yield/%[b]	ee/%[c]	Yield/%[b]	ee/%[c]
1	**1a**	C_6H_5	0.5[d]	37	78	37	73 (81)[e]
2	**1a**	C_6H_5	1	50	>99	39	55
3	**1a**	C_6H_5	3	25	>99	61	19
4	**1a**	C_6H_5	7	–	–	85	0
5	**1b**	4-Cl–C_6H_4	4[d]	25	>99	43	52 (78)[e]
6	**1b**	4-Cl–C_6H_4	5.5	29	>99	62	41
7	**1b**	4-Cl–C_6H_4	12	16	>99	77	25
8	**1c**	3-Cl–C_6H_4	144	25	>99	70	31
9	**1d**	2-Cl–C_6H_4	30	48	72	45	72
10	**1d**	2-Cl–C_6H_4	43	21	>99	70	11
11	**1e**	4-F–C_6H_4	2	44	89	48	75 (91)[e]
12	**1e**	4-F–C_6H_4	5	33	>99	66	30
13	**1e**	4-F–C_6H_4	7	16	>99	73	25
14	**1f**	4-Me–C_6H_4	2[d]	46	69	32	75
15	**1f**	4-Me–C_6H_4	5	32	>99	53	71 (82)[e]
16	**1g**	4-MeO–C_6H_4	7[d]	31	44	36	17
17	**1g**	4-MeO–C_6H_4	10[d]	39	21	41	12

[a] Nitrile (1.73 mmol) was used. [b] Isolated yield. [c] Determined by chiral HPLC analysis. [d] Nitrile (*ca.* 20%, ee < 5%) was recovered. [e] Enantiomeric excess after recrystallization.

The outcomes obtained suggest that the formation of (1*R*,2*R*)-2-arylcyclopropancarboxamides **2** and (1*S*,2*S*)-2-arylcyclopropanecarboxylic acids **3** results from the combined effect of tandem kinetic resolutions of nitrile hydration and amide hydrolysis that are catalyzed, respectively, by the nitrile hydratase and the amidase in the cells. The nitrile hydratase involved in *Rhodococcus* sp. AJ270 shows very low stereoselectivity against the *trans*-2-arylcyclopropanecarbonitriles examined, which is exemplified by the fact that the recovered nitriles **1** had extremely low enantiomeric excesses (entries 1, 5, 14, 16 and 17 in Table 1). The amidase involved in the cell, however, exhibited 1*S*-selectivity against the *trans*-2-arylcyclopropanecarboxamides **2** generated from the hydration of the nitriles **1**. With the only exception of 4-methoxyphenyl-substituted compounds **2g** and **3g**, the formation of highly enantiomerically enriched amides **2** and acids **3**, irrespective of the nature of aryl group of the substrate, suggests that the amidase has a relatively broad spectrum against amides and its chiral recognition relies mainly on the primary structure of *trans*-2-phenylcyclopropanecarboxamide rather than on the substituent on the aromatic ring. It should be pointed out that the precise reason for the poor enantioselectivity of the biotransformation of *trans*-2-(4-methoxphenyl)cyclopropanecarbonitrile, **1g**, is not clear, although it is probably due to the electronic nature or/and most likely the steric effect of the methoxy group as other sterically smaller groups, either electron-withdrawing or electron-donating, caused no dramatic influence on the enantioselectivity of the reaction.

Kinetic resolution of racemic *trans*-2-arylcyclopropanecarboxamides

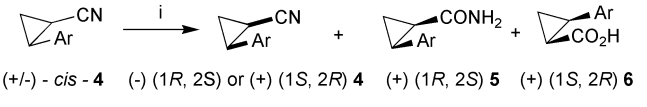
To shed further light on the stereoselective biotransformations of nitriles, some racemic *trans*-2-arylcyclopropanecarboxamides **2** were synthesized[31] and subjected to the biohydrolysis. Under identical conditions, *Rhodococcus* sp. AJ270 cells catalyzed the hydrolysis of amides effectively (Scheme 2), and half of the amides was transformed within several hours into (−)-(1*R*,2*R*)-2-arylcyclopropanecarboxamides **2a**, **2b** and **2f** and (+)-(1*S*,2*S*)-2-arylcyclopropanecarboxylic acids **3a**, **3b** and **3f** (Table 2). Although the enantioselectivity of this kinetic resolution was only moderate ($E = 2.1–6.8$), it confirmed the preferential interaction of the amidase towards (1*S*,2*S*)-2-arylcyclopropanecarboxamide isomers and the combined effect of



(+/-) - *trans* - **2** (-) (1*R*, 2*R*) **2** (+) (1*S*, 2*S*) **3**

i. *Rhodococcus* sp. AJ270, phosphate buffer, pH 7.0, 30 °C

Scheme 2



(+/-) - *cis* - **4** (-) (1*R*, 2S) or (+) (1*S*, 2*R*) **4** (+) (1*R*, 2S) **5** (+) (1*S*, 2*R*) **6**

i. *Rhodococcus* sp. AJ270, phosphate buffer, pH 7.0, 30 °C

Scheme 3

stereoselective amidase and nitrile hydratase towards the hydrolysis of nitriles. The lower enantioselectivity observed in these kinetic resolutions is probably attributed to the low solubility of amides **2** in aqueous buffer, which leads to only a limited amount of both enantiomers of amide in solution available to the amidase. We did find a similar phenomenon that when a very small amount of nitrile or amide was fed to the organism under the same conditions; the conversion to acid was rapid and usually went to completion with the formation of racemic acid.[16] In the case of *trans*-2-(4-methoxyphenyl)cyclopropanecarboxamide, **2g**, the same low enantioselectivity ($E = 1.3$) as that of nitrile hydrolysis was obtained (entry 6 in Table 2), suggesting again that the methoxy group causes the low efficiency of enantio-differentiation of the amidase. In practical synthesis, it is apparently advantageous to apply the biotransformation of nitriles rather than amides to prepare enantiopure (1*R*,2*R*)-2-arylcyclopropanecarboxamides **2** and optically active (1*S*,2*S*)-2-arylcyclopropanecarboxylic acids **3**.

Biotransformations of racemic *cis*-2-arylcyclopropanecarbonitriles

An extraordinarily high enantioselectivity and a sluggish reaction were observed when racemic *cis*-2-arylcyclopropanecarbonitrile substrates **4**[30] were incubated with *Rhodococcus* sp. AJ270 (Table 3). Compared to the rapid and efficient bioconversion of *trans* isomer **1a**, 7 days' interaction of *cis* isomer **4a** with biocatalyst under the identical conditions did not even furnish complete hydration, and more than half the starting nitrile were recovered (entry 1 in Table 3). The use of a co-solvent such as acetone (entry 3 in Table 3) and the employment of a biphasic system of aqueous phosphate buffer and *n*-hexane (entry 4 in Table 3) did not improve the conversion of nitriles. Only when the substrate concentration decreased to 4 mM, did the complete transformation of nitrile **1a** take place in a week to afford enantiopure (1*R*,2*S*)-2-phenylcyclopropanecarboxamide, **5a**, and (1*S*,2*R*)-2-phenylcyclopropanecarboxylic acid, **6a**, in excellent yields (entry 6 in Table 3 and Scheme 3). It is also noteworthy that the reaction outcome was strongly governed by both the nature of the substituent and the substitution pattern of the benzene ring as well. The presence of a substituent at the *para* position of the aromatic ring of **4** inhibited the hydration of the nitrile and, more greatly, the hydrolysis of the amide. The sterically bulkier the substituent, the slower the reaction. For instance, *cis*-2-(4-fluorophenyl)cyclopropanecarbonitrile, **4e**, gave the corresponding optically pure amide **5e** and acid **6e** in 38% and 46% yields, respectively, whereas the methyl and methoxy substituted nitrile analogs **4f** and **4g** produced only small amounts of the amide (< 20% yield) and no acid at all was formed, even with the use of twice the amount of biomass or β-cyclodextrin (β-CD) as an additive in the latter case (entries 9–14 in Table 3). Changing of the chloro substitution from the *para* (**4b**) to *ortho* (**4d**) positions of the benzene ring seemed to slightly facilitate biotransformations of nitrile and amide, which led to higher conversion of **4d** and to higher yields of enantiopure 2-(2-chlorophenyl)cyclopropanecarboxylic acid, **6d** (entries 7 and 8).

Table 2 Kinetic resolution of racemic *trans*-2-arylcyclopropanecarboxamides **2**

				2		**3**		
Entry	(+/−)-**2**[a]	Ar	Time/ h	Yield/ %[b]	ee/ %[c]	Yield/ %[b]	ee/ %[c]	*E*
1	**2a**	C_6H_5	2	47	51	53	33	3.2
2	**2a**	C_6H_5	4	22	77	77	8	2.1
3	**2b**	4-Cl–C_6H_4	4	52	58	47	44	4.5
4	**2b**	4-Cl–C_6H_4	6	37	68	59	20	2.7
5	**2f**	4-Me–C_6H_4	6	54	57	42	59	6.8
6	**2g**	4-MeO–C_6H_4	8	55	2	32	13	1.3

[a] Amide (1.73 mmol) was used. [b] Isolated yield. [c] Determined by chiral HPLC analysis.

It has been generally believed that the nitrile hydratase in various microbial organisms exhibits only low to moderate enantioselectivity against a variety of racemic nitriles.[5,16,32] This is also true for the hydration of *trans*-2-arylcyclopropanecarbonitriles **1** as aforementioned. In the case of hydration of some *cis*-2-arylcyclopropanecarbonitriles such as **4a** and **4e**, however, the nitrile hydratase of *Rhodococcus* sp. AJ270 displays good 1*S*-enantioselectivity, as the nitrile recovered after more than 50% conversion showed an enantiomeric excess value as high as 99%. More surprisingly, enantioselection of the nitrile hydratase inverted from 1*S* to 1*R* when the substituent on the benzene ring varied from others to the *para*-methoxy group. As indicated by the formation of enantiopure (1*S*,2*R*)-2-arylcyclopropanecarboxylic acids **6a**, **6b**, **6d** and **6e**, the amidase, which is very sensitive to the presence of the substituent on the benzene ring, has a very strict 1*S*-enentiocontrol against amides.

It has been proposed that the nitrile hydratase is an iron or cobalt-containing metelloenzyme while amidase is a normal hydrolytic enzyme with nucleophilic group such as esterases and proteases. To account for the enantioselectivity of biotransformations of *trans*- and *cis*-2-arylcyclopropanenitriles, we propose that a readily reachable reactive site be embedded within the larger pocket of the 1*S*-enantioselective nitrile hydratase while the amidase comprises a relatively deep-buried and size-limited 1*S*-enantioselective active site. The efficiency of catalysis and enantioselection, however, results from effective and selective binding of the substrates to the surface of the active site of the enzymes, which in turn is strongly governed by the structure of the substrates. In other words, easy and rapid binding of nitrile or amide substrates to the active sites of nitrile hydratase or amidase, respectively, in the absence of a steric interaction between the substrate and enzyme during the chiral recognition process, would result in efficient reaction but with low enantioselectivity. As a consequence, a sterically less crowded cyano functional group such as in both enantiomers of *trans*-2-arylcyclopropanecarbonitrile **1** could fit comfortably and efficiently in the spacious active site of nitrile hydratase, leading to rapid hydration of nitrile **1** with almost no enantioselectivity. In contrast, bearing an adjacent *cis*-aryl group, the heavily sterically hindered cyano group of **4** could not be bound freely and non-selectively by the nitrile hydratase. Only slow and selective hydration can occur in a lengthy incubation with 1*S*,2*R*-2-phenylcyclopropanecarbonitrile, **4a**, and its 2-(4-fluorophenyl)-substituted analog **4e**, yielding recovered (1*R*,2*S*)-2-arylcyclopropanecarbonitriles in excellent enantiomeric excesses. Although it remains a mystery, the inversion of the enantioselectivity observed for *cis*-2-(4-methoxyphenyl)cyclopropanecarbonitrile, **4g**, could also probably

Table 3 Biotransformations of racemic *cis*-2-arylcyclopropanecarbonitriles **4**

					Recovered **4**		(1*R*,2*S*)-**5**			(1*S*,2*R*)-**6**	
Entry	Nitrile **4**	Ar	Conc/mmol	Time/days	Yield/%[a]	ee/%[b]	Config.	Yield/%[a]	ee/%[b]	Yield/%[a]	ee/%[b]
1	**4a**	C_6H_5	1.73	7	52	90	1*R*,2*S*	7	79	30	>99
2	**4a**	C_6H_5	1	7	15	>99	1*R*,2*S*	32	>99	49	>99
3	**4a**	C_6H_5	1[c]	7	27	>99	1*R*,2*S*	24	>99	43	>99
4	**4a**	C_6H_5	1[d]	7	90	9	1*R*,2*S*	–	–	9	90
5	**4a**	C_6H_5	0.5	2	49	>99	1*R*,2*S*	6	66	43	>99
6	**4a**	C_6H_5	0.2	7	–	–	–	41	>99	48	>99
7	**4b**	4-Cl–C_6H_4	0.25	7	67	6	Nd[e]	18	>99	8	>99
8	**4d**	2-Cl–C_6H_4	0.6	7	45	49	1*R*,2*S*	15	>99	33	>99
9	**4e**	4-F–C_6H_4	0.6	7	11	>99	1*R*,2*S*	38	>99	46	>99
10	**4f**	4-Me–C_6H_4	0.6	7	85	2	Nd[e]	4	>99	–	–
11	**4f**	4-Me–C_6H_4	0.25	7	80	8	Nd[e]	11	>99	–	–
12	**4g**	4-MeO–C_6H_4	0.25	7	77	17	1*S*,2*R*	18	>99	–	–
13	**4g**	4-MeO–C_6H_4	0.25[f]	7	71	22	1*S*,2*R*	17	>99	–	–
14	**4g**	4-MeO–C_6H_4	0.25[g]	7	62	27	1*S*,2*R*	15	>99	–	–

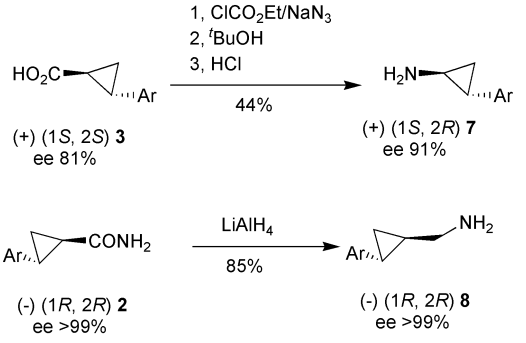
[a] Isolated yield. [b] Determined by chiral HPLC analysis. [c] A biphasic system of phosphate buffer (25 ml) and *n*-hexane (25 ml) was used. [d] Acetone (3 ml) was added as a co-solvent. [e] Configuration was not determined. [f] Four grams of wet cell were used. [g] β-CD (100 mg) was added.

be due to the steric effect. Such an ever increased aryl group as 4-methoxyphenyl might cause a mismatch in the recognition between the 1*S*,2*R*-enantiomer of **4g** and the chiral nitrile hydratase, and instead, the 1*R*,2*S*-enantiospecific but inefficient catalysis took place, which gave enantiopure (1*S*,2*R*)-2-(4-methoxyphenyl)cyclopropanecarboxamide, **5g**, in 18% yield.

Being different from the nitrile hydratase, 1*S*-enantioselective amidase appears to be much more sensitive to the structure of substrates and has always showed differentiation between a pair of enantiomers of both *trans*- and *cis*-2-arylcyclopropanecarboxamides, albeit in different degrees. In the case of less sterically hindered *trans*-2-arylcyclopropanecarboxamides **2**, an efficient and stereoselective interaction between the amidase and substrate is allowed, which gives rise to the rapid formation of (1*S*,2*S*)-2-arylcyclopropanecarboxylic acids **3** in moderate to good enantiomeric excesses. When *cis*-2-arylcyclopropanecarboxamides **5a–e**, which were generated from the hydration of nitriles **4a–e**, were available to amidase, enantiospecific hydrolytic reaction proceeded slowly to give (1*S*,2*R*)-2-arylcyclopropanecarboxylic acids **6a–e** as the sole product in good yield. The proposed steric-sensitive amidase model is also supported by the fact that the migration of chlorine from the 4- to 2-position of the phenyl accelerated the conversion of amide into the acid, as the accessibility of the amide group to the surface of amidase increases from **5b** to **5d**. The observation of no biotransformation of amides **5f** and **5g**, which bear even larger substituents such as methyl and methoxy, respectively, further supports the model of size-limited amidase.

Preparation of chiral amines containing the cyclopropane moiety

Both optically active 2-arylcyclopropanecarboxylic acids and their amide derivatives obtained from this study are useful precursors of cyclopropane-containing amines of therapeutic significance. Antidepressant tranylcypromine, (+)-(1*S*,2*R*)-2-phenylcyclopropylamine, **7**, for example, was readily prepared from (1*S*,2*S*)-2-phenylcyclopropanecarboxylic acid, **3a**, through a modified Curtius rearrangement.[28,33] In addition, a convenient and straightforward reduction of (1*R*,2*R*)-2-phenylcyclopropanecarboxamide, **2a**, using $LiAlH_4$ afforded high yields of enantiopure (1*R*,2*R*)-2-phenylcyclopropylmethylamine, a potential candidate as an antihypertensive agent[34] (Scheme 4). These chemo-enzymatic examples reflect one of the extra advantages of biotransformations of nitriles over that



Scheme 4

of esters, that is not only the optically active carboxylic acid but also the optically active amides, important organic nitrogen compounds, are readily produced.

Conclusion

Rhodococcus sp. AJ270 is a powerful and versatile nitrile hydratase/amidase containing biocatalytic system able to catalyze the enantioselective hydrolysis of both racemic *trans*- and *cis*-2-arylcyclopropanecarbonitriles to afford the optically active *trans*- and *cis*-2-arylcyclopropanecarboxylic acids and their derivatives with high enantiomeric excesses. High enantioselectivity of the biotransformations of nitriles results from the combined effect of the nitrile hydratase and amidase. The reaction rate and enantioselectivity of both nitrile hydratase and amidase present in *Rhodococcus* sp. AJ270 microbial cells are strongly dependent upon the configuration of the substrates, and the nature of the substituents and their substitution pattern on the benzene ring of the substrates. On the basis of the outcomes of this study, it is proposed that a readily reachable reactive site be embedded within the spacious pocket of the 1*S*-enantioselective nitrile hydratase while the amidase might comprise a relatively deep-buried and size-limited 1*S*-enantioselective active site. We have also demonstrated the advantages of biotransformation of nitriles over that of esters by converting (1*S*,2*S*)-2-phenylcyclopropanecarboxylic acid, **3a**, and (1*R*,2*R*)-2-phenylcyclopropanecarboxamide, **2a**, into (1*S*,2*R*)-2-phenylcyclopropylamine, **7**, and (1*R*,2*R*)-2-phenylcyclopropylmethylamine, **8**, respectively, through facile and straightforward chemical manipulations.

Experimental

General

Both melting points, which were determined using a Reichert Kofler hot-stage apparatus, 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 spectrameter 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 g 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.

All regents were obtained from commercial suppliers and used without further purification. Column chromatography was performed using 200–300 mesh silica gel. Racemic *trans*- and *cis*-2-arylcyclopropanecarbonitriles **1** and **4** were prepared following the literature method[30] and their configurations were determined by the ^{1}H NMR spectrum, which gave distinct coupling patterns of 2-H (Fig. 1).[35] Racemic amides[31] **2** and **5** and racemic acids[36] **3** and **6** were obtained from chemical hydrolysis of nitriles **1** and **4** according to the literature.

General procedure for the biotransformations 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 2–4) 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 2–4) by removing the biomass through Celite pad filtration. The resulting aqueous solution was basified to pH 12 with aqueous NaOH (2 M). Extraction with CH_2Cl_2 or 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 CH_2Cl_2 or ethyl acetate. Acid was obtained after removal of the solvent. All products were characterized by their spectral data and comparison of the melting points and optical rotary power with the known compounds, which are listed below, or by full characterization.

Enzymatic hydrolysis of (±)-*trans*-2-phenylcyclopropanecarbonitrile (1a). *(−)-(1R,2R)-2-Phenylcyclopropanecarboxamide (**2a**).* 1 h (50%); ee > 99% (chiral HPLC); mp 138–140 °C [lit.[37] 189–190 °C (DL)]; $[\alpha]_D^{25} = -312$ ($c = 1.2$, CH_3OH) [lit.[37] $[\alpha]_D^{25} = -312$ ($c = 1.35$, $CHCl_3$)]; ^{1}H NMR

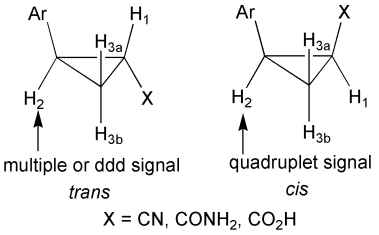


Fig. 1

δ 7.12–7.34 (m, 5H, Ar-H), 6.25 (br s, 2H, NH_2), 2.56–2.63 (m, 1H, H_2), 1.67–1.77 (m, 2H, H_1 and H_{3b}), 1.37–1.44 (m, 1H, H_{3a}); IR (KBr) 3353, 3174 (NH_2), 1659, 1628; MS (EI) m/z 161 (M^+, 23%), 144 (18), 117 (100), 115 (59). Anal. calcd. for $C_{10}H_{11}NO$: C, 74.51; H, 6.88; N, 8.69. Found: C, 74.17; H, 6.70; N, 8.59.

*(+)-(1S,2S)-2-Phenylcyclopropanecarboxylic acid (**3a**).* 0.5 h (37%); ee 73% (ee 81% after recrystallization, chiral HPLC); mp 57–59 °C (ee 81%) [lit.[28] 46–47 °C, (+)-(1*S*, 2*S*) enantiomer, ee > 99%]; $[\alpha]_D^{25} = +253.3$ ($c = 4.7$, $CHCl_3$) [lit.[28] $[\alpha]_D^{20} = +377.1$ ($c = 1.04$, $CHCl_3$), ee > 99%]; ^{1}H NMR δ 7.70 (br s, 1H, COOH), 7.10–7.32 (m, 5H, Ar–H), 2.58–2.64 (m, 1H, H_2), 1.87–1.94 (m, 1H, H_1), 1.64–1.70 (m, 1H, H_{3b}), 1.39–1.45 (m, 1H, H_{3a}); IR (KBr) 2530–3031 (COOH), 1697 (C=O), 1603, 1453; MS (EI) m/z 162 (M^+, 34%), 144 (17), 117 (100), 116 (30). Anal. calcd for $C_{10}H_{10}O_2$: C, 74.06; H, 6.21. Found: C, 74.11; H 6.24.

*(+)-(1S,2S)-2-Phenylcyclopropanecarbonitrile (**1a**).* 0.5 h (24%); ee 3% (chiral HPLC); mp 50–52 °C (DL) [lit. 51–52 °C (DL)[37]; 78–80 °C (+) (1*S*, 2*S*) enantiomer[42]]; $[\alpha]_D^{25} = +8.75$ ($c = 1.6$, $CHCl_3$) [lit.[42] $[\alpha]_D^{25} = +337.5$ ($c = 0.2400$, EtOH)]; ^{1}H NMR δ 7.11–7.35 (m, 5H, Ar–H), 2.61–2.66 (m, 1H, H_2), 1.57–1.64 (m, 1H, H_1), 1.51–1.56 (m, 1H, H_{3b}) 1.42–1.47 (m, 1H, H_{3a}); IR(KBr), 2223 (CN); MS (EI) m/z 143 (M^+, 100%), 116 (72), 115 (76).

Enzymatic hydrolysis of (±)-*trans*-2-(4-chlorophenyl)cyclopropanecarbonitrile (1b). *(−)-(1R,2R)-2-(4-Chlorophenyl)-cyclopropanecarboxamide (**2b**).* 5.5 h (29%); ee > 99% (chiral HPLC); mp 170–172 °C [lit.[20a] 183–184.5 °C (DL)]; $[\alpha]_D^{25} = -302$ ($c = 0.9$, $CHCl_3$); ^{1}H NMR δ 7.25 (d, J 8.4, 2H, Ar–H), 7.04 (d, J 8.4, 2H, Ar–H), 6.40 (br s, 1H, N*H*H), 5.99 (br s, 1H, NH*H*), 2.52–2.58 (m, 1H, H_2), 1.64–1.72 (m, 2H, H_1 and H_{3b}), 1.30–1.38 (m, 1H, H_{3a}); IR (KBr) 3366, 3202 (NH_2), 1649, 1625; MS (EI) m/z 197 ($M^+ + 2$, 12), 195 (M^+, 40%), 180 (10), 178 (27), 153 (15), 151 (44), 117 (49), 116 (51), 115 (100). Anal. calcd for $C_{10}H_{10}ClNO$: C, 61.39; H, 5.15; N, 7.16. Found: C, 61.26; H 5.21; N, 7.12.

*(+)-(1S,2S)-2-(4-Chlorophenyl)cyclopropanecarboxlic acid (**3b**).* 4 h (43%); ee 52% (ee 78% after recrystallization, chiral HPLC); mp 94–95 °C (ee 78%) [lit.[38] 115–116 °C (DL)]; $[\alpha]_D^{25} = +178.8$ ($c = 3.2$, $CHCl_3$); ^{1}H NMR δ 7.95 (br s, 1H, COOH), 7.24 (d, J 8.3, 2H, Ar–H), 7.03 (d, J 8.3, 2H, Ar–H), 2.54–2.60 (m, 1H, H_2), 1.84–1.90 (m, 1H, H_1), 1.63–1.70 (m, 1H, H_{3b}), 1.34–1.41 (m, 1H, H_{3a}); IR (KBr) 2533–3025 (COOH), 1691 (C=O), 1434, 1242; MS (EI) m/z 198 ($M^+ + 2$, 10), 196 (M^+, 33%), 153 (18), 151 (55), 141 (17), 116 (54), 115 (100). Anal. calcd for $C_{10}H_9ClO_2$: C, 61.08; H, 4.61. Found: C, 61.01; H, 4.62.

*(+)-(1S,2S)-2-(4-Chlorophenyl)cyclopropanecarbonitrile (**1b**).* 4 h (29%); ee 3% (chiral HPLC); mp 84–85 °C (DL); $[\alpha]_D^{25} = +17$ ($c = 3.75$, $CHCl_3$); ^{1}H NMR δ 7.28 (d, J 8.4, 2H, Ar–H), 7.05 (d, J 8.4, 2H, Ar–H), 2.60–2.63 (m, 1H, H_2), 1.61–1.68 (m, 1H, H_1), 1.50–1.56 (m, 1H, H_{3b}), 1.39–1.46 (m, 1H, H_{3a}); IR (KBr) 2232 (CN); MS (EI) m/z 179 ($M^+ + 2$, 12), 177 (M^+, 38%), 142 (71), 115 (100). Anal. calcd for $C_{10}H_8ClN$: C, 67.64; H, 4.51; N, 7.89. Found: C, 67.91; H 4.64; N, 8.04.

Enzymatic hydrolysis of (±)-*trans*-2-(3-chlorophenyl)cyclopropanecarbonitrile (1c). *(−)-(1R,2R)-2-(3-Chlorophenyl)-cyclopropanecarboxamide (**2c**).* 144 h (25%); ee > 99% (chiral HPLC); mp 133–134 °C; $[\alpha]_D^{25} = -250$ ($c = 0.7$, $CHCl_3$); ^{1}H NMR δ 7.02–7.38 (m, 4H, Ar–H), 6.71 (br s, 1H, N*H*H), 4.23 (br s, 1H, NH*H*), 2.59–2.65 (m, 1H, H_2), 1.74–1.83 (m, 1H, H_1), 1.67–1.73 (m, 1H, H_{3b}), 1.41–1.49 (m, 1H, H_{3a}); IR (KBr) 3354, 3173 (NH_2), 1665, 1629, 1611, 1434, 1283, 1043; MS (EI) m/z 197 ($M^+ + 2$, 30), 195 (M^+, 88%), 153 (25), 151 (76), 117 (83), 116 (51), 115 (100). Anal. calcd for

$C_{10}H_{10}ClNO$: C, 61.39; H, 5.15; N, 7.16. Found: C, 61.29; H 5.15; N, 7.16.

*(+)-(1S,2S)-2-(3-Chlorophenyl)cyclopropanecarboxlic acid (**3c**).* 144 h (70%); ee 31% (chiral HPLC); mp 56–58 °C [lit.[38] 108.0–108.5 °C]; $[\alpha]_D^{25} = +122.9$ ($c = 3.05$, $CHCl_3$); ^1H NMR δ 7.95 (br s, 1H, COOH), 7.01–7.26 (m, 4H, Ar–H), 2.54–2.60 (m, 1H, H_2), 1.84–1.90 (m, 1H, H_1), 1.63–1.70 (m, 1H, H_{3b}), 1.34–1.41 (m, 1H, H_{3a}); IR (KBr) 2535–3053 (COOH), 1691 (C=O), 1434, 1242; MS (EI) m/z 198 ($M^+ + 2$, 10), 196 (M^+, 33%), 153 (22), 151 (55), 141 (17), 117 (23), 116 (54), 115 (100). Anal. calcd for $C_{10}H_9ClO_2$: C, 61.08; H, 4.61. Found: C, 60.90; H, 4.46.

Enzymatic hydrolysis of (±)-*trans*-2-(2-chlorophenyl)cyclopropanecarbonitrile (1d). *(−)-(1R,2R)-2-(2-Chlorophenyl)-cyclopropanecarboxamide (**2d**).* 43 h (21%); ee > 99% (chiral HPLC); mp 101–102 °C; $[\alpha]_D^{25} = -94.4\%$ ($c = 1.25$, $CHCl_3$); ^1H NMR δ 6.99–7.40 (m, 4H, Ar–H), 6.56 (br s, 1H, N*H*H), 5.20 (br s, 1H, NH*H*), 2.72–2.82 (m, 1H, H_2), 1.83–1.88 (m, 1H, H_1), 1.68–1.77 (m, 1H, H_{3b}), 1.44–1.50 (m, 1H, H_{3a}); IR (KBr) 3345, 3184 (NH_2), 1654, 1624, 1436; MS (EI) m/z 197 ($M^+ + 2$, 6), 195 (M^+, 18%), 160 (100), 153 (10), 151 (30), 117 (71), 116 (50), 115 (78). Anal. calcd for $C_{10}H_{10}ClNO$: C, 61.39; H, 5.15; N, 7.16. Found: C, 61.76; H 5.22; N, 7.03.

*(+)-(1S,2S)-2-(2-Chlorophenyl)cyclopropanecarboxlic acid (**3d**).* 30 h (45%); ee 72% (chiral HPLC); mp 64–65 °C; $[\alpha]_D^{25} = +112.6$ ($c = 5.4$, $CHCl_3$); ^1H NMR δ 9.20 (br s, 1H, COOH), 7.02–7.40 (m, 4H, Ar–H), 2.80–2.85 (m, 1H, H_2), 1.80–1.85 (m, 1H, H_1), 1.67–1.73 (m, 1H, H_{3b}), 1.40–1.43 (m, 1H, H_{3a}); IR (KBr) 2536–3018 (COOH), 1693 (C=O), 1450, 1240; MS (EI) m/z 198 ($M^+ + 2$, 12), 196 (M^+, 37%), 161 (22), 153 (15), 151 (47), 141 (26), 117 (22), 116 (53), 115 (100). Anal. calcd for $C_{10}H_9ClO_2$: C, 61.08; H, 4.61. Found: C, 61.19; H, 4.57.

Enzymatic hydrolysis of (±)-*trans*-2-(4-fluorophenyl)cyclopropanecarbonitrile (1e). *(−)-(1R,2R)-2-(4-Fluorophenyl)-cyclopropanecarboxamide (**2e**).* 5 h (33%); ee > 99% (chiral HPLC); mp 153–155 °C; $[\alpha]_D^{25} = -292.3$ ($c = 0.65$, $CHCl_3$); ^1H NMR δ 6.96–7.12 (m, 4H, Ar–H), 5.74 (br s, 2H, NH_2), 2.53–2.59 (m, 1H, H_2), 1.61–1.69 (m, 2H, H_1 and H_{3b}), 1.24–1.33 (m, 1H, H_{3a}); IR (KBr) 3398, 3212 (NH_2), 1652, 1616; MS (EI) m/z 179 (M^+, 54%), 162 (38), 136 (27), 135 (100), 134 (39), 133 (69). Anal. calcd for $C_{10}H_{10}FNO$: C, 67.03; H, 5.62; N, 7.82. Found: C, 66.79; H, 5.67; N, 7.70.

*(+)-(1S,2S)-2-(4-Fluorophenyl)cyclopropanecarboxylic acid (**3e**).* 2 h (48%); ee 75% (ee 91% after recrystallization, chiral HPLC); mp 74–76 °C (ee 91%) [lit.[33b] 110–112 °C (DL)], $[\alpha]_D^{25} = +261$ ($c = 1.7$, $CHCl_3$)]; ^1H NMR δ 6.94–7.29 (m, 4H, Ar–H), 6.88 (br s, 1H, COOH), 2.56–2.63 (m, 1H, H_2), 1.83–1.88 (m, 1H, H_1), 1.62–1.69 (m, 1H, H_{3b}), 1.34–1.41 (m, 1H, H_{3a}); IR (KBr) 2535–3025 (COOH), 1702 (C=O), 1227; MS (EI) m/z 180 (M^+, 37%), 162 (19), 135 (100), 134 (27), 133 (49). Anal. calcd for $C_{10}H_9FO_2$: C, 66.66; H, 5.03. Found: C, 66.51; H. 5.05.

Enzymatic hydrolysis of (±)-*trans*-2-(4-methylphenyl)cyclopropanecarbonitrile (1f). *(−)-(1R,2R)-2-(4-Methylphenyl)-cyclopropanecarboxamide (**2f**).* 5 h (32%); ee > 99% (chiral HPLC); mp 185–187 °C [lit.[39] 208 °C (DL)]; $[\alpha]_D^{25} = -333.3$ ($c = 0.75$, $CHCl_3$); ^1H NMR δ 7.10 (d, J 7.79, 2H, Ar–H), 7.01 (d, J 7.81, 2H, Ar–H), 6.05 (br s, 2H, NH_2), 2.51–2.57 (m, 1H, H_2), 2.33 (s, 3H, CH_3), 1.61–1.71 (m, 2H, H_1 and H_{3b}), 1.29–1.35 (m, 1H, H_{3a}); IR (KBr) 3383, 3210 (NH_2), 1627, 1616; MS (EI) m/z 175 (M^+, 56%), 158 (38), 132 (20), 131 (100), 117 (35), 116 (33), 115 (52). Anal. calcd for $C_{11}H_{13}NO$: C, 75.40; H, 7.48; N, 7.99. Found: C, 74.98; H, 7.29; N, 7.92.

*(+)-(1S,2S)-2-(4-Methylphenyl)cyclopropanecarboxylic acid (**3f**).* 5 h (53%); ee 71% (ee 82% after recrystallization, chiral HPLC); mp 93–95 °C (ee 82%) [lit.[38] 119–120 °C (DL)]; $[\alpha]_D^{25} = +258.2$ ($c = 2.2$, $CHCl_3$); ^1H NMR δ 7.12 (d, J 7.9, 2H, Ar–H), 7.03 (d, J 7.9, 2H, Ar–H), 6.50 (br s, 1H, COOH), 2.57–2.64 (m, 1H, H_2), 2.35 (s, 3H, CH_3), 1.87–1.93 (m, 1H, H_1), 1.64–1.70 (m, 1H, H_{3b}) 1.38–1.45 (m, 1H, H_{3a}); IR (KBr) 2505–3000 (COOH), 1692 (C=O), 1427, 1287; MS (EI) m/z 176 (M^+, 38%), 158 (18), 131 (100). Anal. calcd. for $C_{11}H_{12}O_2$: C, 74.98; H, 6.86. Found C, 74.90; H, 6.74.

*(+)-(1S,2S)-2-(4-Methylphenyl)cyclopropanecarbonitrile (**1f**).* 2 h (22%); ee 4% (chiral HPLC); mp 85–86 °C (DL); $[\alpha]_D^{25} = +16$ ($c = 2.4$, $CHCl_3$); ^1H NMR δ 7.13 (d, J 8.0, 2H, Ar–H), 7.01 (d, J 8.0, 2H, Ar–H), 2.58–2.65 (m, 1H, H_2), 2.34 (s, 3H, CH_3), 1.58–1.64 (m, 1H, H_1), 1.49–1.55 (m, 1H, H_{3b}), 1.40–1.17 (m, 1H, H_{3a}); IR (KBr) 2229 (CN); MS (EI) m/z 157 (M^+, 100%), 156 (89), 142 (68), 129 (39), 115 (73). Anal. calcd for $C_{11}H_{11}N$: C, 84.08; H 7.01; N, 8.91. Found: C, 84.08; H, 6.96; N, 9.10.

Enzymatic hydrolysis of (±)-*trans*-2-(4-methoxylphenyl)-cyclopropanecarbonitrile (1g). *(−)-(1R,2R)-2-(4-Methoxyl-phenyl)cyclopropanecarboxamide (**2g**).* 7 h (31%); ee 44% (chiral HPLC); mp 155–157 °C; $[\alpha]_D^{25} = -92$ ($c = 0.5$, CH_3OH); ^1H NMR δ 7.04 (d, J 8.6, 2H, Ar–H), 6.85 (d, J 8.6, 2H, Ar–H), 5.90 (br s, 1H, N*H*H), 3.80 (s, 3H, CH_3O), 3.20 (br s, 1H, NH*H*), 2.51–2.57 (m, 1H, H_2), 1.58–1.66 (m, 2H, H_1 and H_{3b}), 1.25–1.32 (m, 1H, H_{3a}); IR (KBr) 3353, 3178 (NH_2), 1663, 1624, 1246, 1023; MS (EI) m/z 191 (M^+, 75%), 174 (75), 159 (20), 147 (100), 131 (37), 115 (36), 103 (30). Anal. calcd for $C_{11}H_{13}NO_2$: C, 69.09; H, 6.85; N, 7.32. Found: C, 68.93; H, 6.79; N 7.30.

*(+)-(1S,2S)-2-(4-Methoxylphenyl)cyclopropanecarboxylic acid (**3g**).* 7 h (36%), ee 17% (chiral HPLC); mp 104–106 °C [lit.[38] 112–113 °C (DL)]; $[\alpha]_D^{25} = +49.4$ ($c = 3.4$, CH_3OH); ^1H NMR δ 10.75 (br, 1H, COOH), 7.05 (d, J 7.9, 2H, Ar–H), 6.84 (d, J 7.9, 2H, Ar–H), 3.82 (s, 3H, CH_3O), 2.56–2.63 (m, 1H, H_2), 1.82–1.88 (m, 1H, H_1), 1.61–1.68 (m, 1H, H_{3b}), 1.35–1.41 (m, 1H, H_{3a}); IR (KBr) 2508–3003 (COOH), 1702 (C=O), 1612, 1242, 1027; MS (EI) m/z 192 (M^+, 39%), 147 (100). Anal. calcd for $C_{11}H_{12}O_3$: C, 68.74; H, 6.29. Found: C, 68.60; H, 6.19.

*trans-2-(4-Methoylphenyl)cyclopropanecarbonitrile (**1g**).* 7 h (20%); mp 76–78 °C (DL); $[\alpha]_D^{25} = 0$; ^1H NMR δ 7.06 (d, J 8.6, 2H, Ar–H), 6.87 (d, J 8.6, 2H, Ar–H), 3.82 (s, 3H, CH_3O), 2.59–2.65 (m, 1H, H_2), 1.57–1.63 (m, 1H, H_1), 1.45–1.54 (m, 1H, H_{3b}), 1.39–1.48 (m, 1H, H_{3a}); IR (KBr) 2241 (CN); MS (EI) m/z 173 (M^+, 100%), 158 (23), 130 (36). Anal. calcd for $C_{11}H_{11}NO$: C, 76.31; H, 6.36; N, 8.09. Found: C, 76.29; H, 6.21; N, 8.08.

Enzymatic hydrolysis of (±)-*cis*-2-phenylcyclopropanecarbonitrile (4a). *(+)-(1R,2S)-2-Phenylcyclopropanecarboxamide (**5a**).* 41% yield from 0.2 mmol of racemic nitrile **4a** in 7 days; ee > 99% (chiral HPLC); mp 86–88 °C; $[\alpha]_D^{25} = +47.3$ ($c = 0.55$, $CHCl_3$); ^1H NMR δ 7.18–7.31 (m, 5H, Ar–H), 5.58 (br s, 1H, N*H*H), 3.60 (br s, 1H, NH*H*), 2.54 (q, J 8.7, 1H, H_2), 1.92–2.00 (m, 1H, H_1), 1.66–1.73 (m, 1H, H_{3a}), 1.30–1.38 (m, 1H, H_{3b}); IR (KBr) 3398, 3223 (NH_2), 1633, 1604; MS (EI) m/z 161 (M^+, 40%), 144 (29), 118 (23), 117 (100), 116 (25), 115 (49). Anal. calcd for $C_{10}H_{11}NO$: C, 74.51; H, 6.88; N, 8.69. Found: C, 74.76; H, 6.84; N, 8.62.

*(+)-(1S,2R)-2-Phenylcyclopropanecarboxylic acid (**6a**).* 49% yield from 1 mmol of racemic nitrile **4a** in 7 days; ee > 99% (chiral HPLC); mp 72–74 °C [lit.[40] 105.0–105.6 °C (DL)]; $[\alpha]_D^{25} = +26.2$ ($c = 2.06$, $CHCl_3$) [lit.[41] $[\alpha]_D^{25} = +15$ ($c = 2.0$, $CHCl_3$); ^1H NMR δ 7.60 (br s, 1H, COOH), 7.18–7.28 (m, 5H, Ar–H), 2.62 (q, J 8.7, 1H, H_2), 1.99–2.07 (m, 1H, H_1), 1.62–1.68 (m, 1H, H_{3a}), 1.33–1.40 (m, 1H, H_{3b}); IR (KBr) 2568–3463 (COOH), 1690 (C=O), 1437, 1295, 1224; MS (EI) m/z 162 (M^+, 41%), 144 (19), 117 (100), 116 (30),

115 (56). Anal. calcd for $C_{10}H_{10}O_2$: C, 74.06; H 6.21. Found: C, 74.21; H 6.26.

*(−)-(1R,2S)-2-Phenylcyclopropanecarbonitrile (**4a**).* 49% yield recovered from 0.5 mmol of racemic nitrile **4a** in 2 days; ee > 99% (chiral HPLC); mp 44–45 °C [lit. 37 °C (DL)[30b]; 59–60 °C (-) (1*R*, 2*S*) enantiomer[42]]; $[\alpha]_D^{25} = -23.6$ ($c = 1.1$, $CHCl_3$) [lit.[42] $[\alpha]_D^{25} = -21.8$ ($c = 0.3584$, CH_3OH)]; ^1H NMR δ 7.26–7.40 (m, 5H, Ar–H), 2.55 (q, J 8.2, 1H, H_2), 1.80–1.88 (m, 1H, H_1), 1.49–1.59 (m, 2H, CH_2); IR (KBr) 2231 (CN); MS (EI) m/z 143 (M^+, 100%), 142 (23), 116 (87), 115 (93). Anal. calcd for $C_{10}H_9N$: C, 83.88; H, 6.34; N, 9.78. Found: C, 83.83; H, 6.27; N, 9.75.

Enzymatic hydrolysis of (±)-*cis*-2-(4-chlorophenyl)cyclopropanecarbonitrile (4b). *(+)-(1R,2S)-2-(4-Chlorophenyl)-cyclopropanecarboxamide (**5b**).* 7 days (18%); ee > 99% (chiral HPLC); mp 120–122 °C; $[\alpha]_D^{25} = +67.7$ ($c = 0.65$, $CHCl_3$); ^1H NMR δ 7.27 (d, J 8.4, 2H, Ar–H), 7.22 (d, J 8.4, 2H, Ar–H), 6.26 (br s, 1H, NH*H*), 3.75 (br s, 1H, N*H*H), 2.57 (q, J 8.4, 1H, H_2), 2.98–2.05 (m, 1H, H_1), 1.72–1.79 (m, 1H, H_{3a}), 1.40–1.46 (m, 1H, H_{3b}); IR (KBr) 3410, 3222 (NH_2), 1618; MS (EI) m/z 197 ($M^+ + 2$, 14), 195 (M^+, 36%), 178 (29), 151 (48), 117 (53), 116 (51), 115 (100). Anal. calcd for $C_{10}H_{10}ClNO$: C, 61.39; H, 5.15; N, 7.16. Found: C, 61.49; H, 5.12; N, 7.09.

*(+)-(1R,2S)-2-(4-Chlorophenyl)cyclopropanecarboxylic acid (**6b**).* 7 days (8%); ee > 99% (chiral HPLC); mp 88–90 °C [lit.[40] 128.1–129.1 °C (DL)]; $[\alpha]_D^{25} = +16$ ($c = 0.45$, $CHCl_3$); ^1H NMR δ 7.22 (d, J 8.5, 2H, Ar–H), 7.17 (d, J 8.5, 2H, Ar–H), 3.80 (br s, 1H, COOH), 2.59 (q, J 8.6, 1H, H_2), 2.03–2.10 (m, 1H, H_1), 1.61–1.68 (m, 1H, H_{3a}), 1.36–1.43 (m, 1H, H_{3b}); IR (KBr) 2555–3463 (COOH), 1696 (C=O), 1493, 1438, 1230; MS (EI) m/z 198 ($M^+ + 2$, 12), 196 (M^+, 36%), 178 (15), 153 (22), 151 (65), 116 (55), 115 (100). Anal. calcd for $C_{10}H_9ClO_2$: C, 61.08; H, 4.61. Found: C, 60.96; H, 4.70.

*cis-2-(4-Chlorophenyl)cyclopropanecarbonitrile (**4b**).* 7 days (67%); ee 6% (chiral HPLC); mp 49–50 °C (DL); $[\alpha]_D^{25} \approx 0$; ^1H NMR δ 7.32 (d, J 8.4, 2H, Ar–H), 7.20 (d, J 8.3, 2H, Ar–H), 2.51 (q, J 8.0, 1H, H_2), 1.81–1.89 (m, 1H, H_1), 1.50–1.58 (m, 2H, CH_2); IR (KBr) 2235 (CN); MS (EI) m/z 179 ($M^+ + 2$, 10), 177 (M^+, 31%), 142 (80), 115 (100). Anal. calcd for $C_{10}H_8ClN$: C, 67.64; H, 4.51; N, 7.89. Found: C, 67.43; H, 4.74; N, 7.80.

Enzymatic hydrolysis of (±)-*cis*-2-(2-chlorophenyl)cyclopropanecarbonitrile (4d). *(−)-(1R,2S)-2-(2-Chlorophenyl)-cyclopropanecarboxamide (**5d**).* 7 days (15%); ee > 99% (chiral HPLC); oil; $[\alpha]_D^{25} = -70$ ($c = 1.5$, $CHCl_3$); ^1H NMR δ 7.16–7.34 (m, 4H, Ar–H), 5.60 (br s, 1H, NH*H*), 5.28 (br s, 1H, N*H*H), 2.55 (q, J 8.4, 1H, H_2), 2.02–2.13 (m, 1H, H_1), 1.75–1.81 (m, 1H, H_{3a}), 1.29–1.38 (m, 1H, H_{3b}); IR (KBr) 3470, 3404 (NH_2), 1663, 1612; MS (EI) m/z 197 ($M^+ + 2$, 7), 195 (M^+, 21%), 160 (100), 151 (23), 117 (54), 116 (38), 115 (63). ESI-FTMS calcd for $C_{10}H_{10}ClNO$: 196.0524 [$M^+ + 1$]; found 196.052 [$M^+ + 1$].

*(+)-(1S,2R)-2-(2-Chlorophenyl)cyclopropanecarboxylic acid (**6d**).* 7 days (33%); ee > 99% (chiral HPLC); mp 84–85 °C; $[\alpha]_D^{25} = +96.6$ ($c = 2.75$, $CHCl_3$); ^1H NMR δ 7.19–7.38 (m, 4H, Ar–H), 3.04 (br s, 1H, COOH), 2.63–2.67 (m, 1H, H_2), 2.20–2.27 (m, 1H, H_1), 1.67–1.73 (m, 1H, H_{3a}), 1.46–1.54 (m, 1H, H_{3b}); IR (KBr) 2566–3500 (COOH), 1696 (C=O), 1447, 1325, 1233; MS (EI) m/z 198 ($M^+ + 2$, 21), 196 (M^+, 62%), 153 (18), 151 (55), 117 (24), 116 (52), 115 (100). Anal. calcd for $C_{10}H_9ClO_2$: C, 61.08; H, 4.61. Found: C, 60.87; H, 4.55.

*(−)-(1R,2S)-2-(2-Chlorophenyl)cyclopropanecarbonitrile (**4d**).* 7 days (45%); ee 49% (chiral HPLC); mp 43–45 °C; $[\alpha]_D^{25} = -76$ ($c = 1.5$, $CHCl_3$); ^1H NMR δ 7.21–7.48 (m, 4H, Ar–H), 2.69 (q, J 8.2, 1H, H_2), 1.96–2.04 (m, 1H, H_1), 1.56–1.66 (m, 2H, CH_2); IR (KBr) 2231 (CN); MS (EI) m/z 179 ($M^+ + 2$, 17), 177 (M^+, 49%), 142 (83), 115 (100). Anal. calcd for $C_{10}H_8ClN$: C, 67.64; H, 4.51; N, 7.89. Found: C, 67.28; H, 4.54; N, 7.78.

Enzymatic hydrolysis of (±)-*cis*-2-(4-fluorophenyl)cyclopropanecarbonitrile (4e). *(+)-(1R,2S)-2-(4-Fluorophenyl)cyclopropanecarboxamide (**5e**).* 7 days (38%); ee > 99% (chiral HPLC); mp 64–66 °C; $[\alpha]_D^{25} = +55$ ($c = 0.80$, $CHCl_3$); ^1H NMR δ 6.93–7.24 (m, 4H, Ar–H), 6.15 (br s, 1H, N*H*H); 4.80 (br s, 1H, NH*H*), 2.55 (q, J 8.4, 1H, H_2), 1.96–2.04 (m, 1H, H_1), 1.66–1.72 (m, 1H, H_{3a}), 1.34–1.42 (m, 1H, H_{3b}); IR (KBr) 3415, 3215 (NH_2), 1652, 1617; MS (EI) m/z 179 (M^+, 60%), 162 (34), 135 (100), 133 (52). Anal. calcd for $C_{10}H_{10}FNO$: C, 67.03; H; 5.62; N, 7.82. Found: C, 66.96; H, 5.57; N, 7.85.

*(+)-(1S,2R)-2-(4-Fluorophenyl)cyclopropanecarboxylic acid (**6e**).* 7 days (46%); ee > 99% (chiral HPLC); mp 97–99 °C; $[\alpha]_D^{25} = +26.6$ ($c = 1.5$, $CHCl_3$); ^1H NMR δ 8.30 (br s, 1H, COOH), 6.89–7.19 (m, 4H, Ar–H), 2.57 (q, J 8.6, 1H, H_2), 1.97–2.04 (m, 1H, H_1), 1.57–1.63 (m, 1H, H_{3a}), 1.32–1.39 (m, 1H, H_{3b}); IR (KBr) 2578–3505 (COOH), 1671 (C=O), 1515, 1424, 1372, 1231; MS (EI) m/z 180 (M^+, 36%), 162 (18), 135 (100), 134 (27), 133 (50). Anal. calcd for $C_{10}H_9FO_2$: C, 66.66; H, 5.03. Found: C, 66.62; H, 4.98.

*(−)-(1R,2S)-2-(4-Fluorophenyl)cyclopropanecarbonitrile (**4e**).* 7 days (11%); ee > 99% (chiral HPLC); oil; $[\alpha]_D^{25} = -29.6$ ($c = 0.475$, $CHCl_3$); ^1H NMR δ 7.03–7.28 (m, 4H, Ar–H), 2.53 (q, J 8.0, 1H, H_2), 1.80–1.86 (m, 1H, H_1), 1.47–1.57 (m, 2H, CH_2); IR (KBr) 2238 (CN); MS (EI) m/z 161 (M^+, 100%), 134 (67), 133 (83). Anal. calcd for $C_{10}H_8FN$: C, 74.52; H, 5.00; N, 8.69. Found: C, 74.55; H, 5.38; N, 8.75.

Enzymatic hydrolysis of (±)-*cis*-2-(4-methylphenyl)cyclopropanecarbonitrile (4f). *(+)-(1R,2S)-2-(4-Methylphenyl)cyclopropanecarboxamide (**5f**).* 11% yield from 0.25 mmol of racemic nitrile **4f** in 7 days; ee > 99% (chiral HPLC); mp 122–124 °C; $[\alpha]_D^{25} = +36$ ($c = 0.5$, $CHCl_3$); ^1H NMR δ 7.30 (br s, 1H, N*H*H), 7.12 (d, J 8.0, 2H, Ar–H), 7.07 (d, J 8.0, 2H, Ar–H), 6.20 (br s, 1H, NH*H*), 2.60 (q, J 8.5, 1H, H_2), 2.29 (s, 3H, CH_3), 2.04–2.12 (m, 1H, H_1), 1.68–1.74(m, 1H, H_{3a}), 1.34–1.41 (m, 1H, H_{3b}); IR (KBr) 3416, 3221 (NH_2), 1634; MS (EI) m/z 175 (M^+, 48%), 158 (36), 131 (100), 117 (30), 116 (30), 115 (45). Anal. calcd for $C_{11}H_{13}NO$: C, 75.40; H, 7.48; N, 7.99. Found: C, 75.68; H, 7.51; N, 8.09.

*cis-2-(4-Methylphenyl)cyclopropanecarbonitrile (**4f**).* 80% yield recovered from 0.25 mmol of racemic nitrile **4f** in 7 days; ee 8% (chiral HPLC); mp 43–44 °C (DL); $[\alpha]_D^{25} \approx 0$; ^1H NMR δ 7.155 (s, 5H, Ar–H), 2.49 (q, J 8.2, 1H, H_2), 2.33 (s, 3H, CH_3), 1.75–1.82 (m, 1H, H_1), 1.44–1.54 (m, 2H, CH_2); IR (KBr) 2230 (CN); MS (EI) m/z 157 (M^+, 100%), 156 (90), 142 (73), 129 (46), 115 (89). Anal. calcd for $C_{11}H_{11}N$: C, 84.08; H, 7.01; N, 8.91. Found: C, 84.14; H, 7.06; N, 8.81.

Enzymatic hydrolysis of (±)-*cis*-2-(4-methoylphenyl)cyclopropanecarbonitrile (4g). *(+)-(1R,2S)-2-(4-Methoylphenyl)cyclopropanecarboxamide (**5g**).* 18% yield from 0.25 mmol of racemic nitrile **4g** in 7 days; ee > 99% (chiral HPLC); mp 125–127 °C; $[\alpha]_D^{25} = +66$ ($c = 4.5$, $CHCl_3$); ^1H NMR δ 7.18 (d, J 8.6, 2H, Ar–H), 6.86 (d, J 8.6, 2H, Ar–H), 6.30 (br s, 2H, NH_2), 3.80 (s, 3H, CH_3O), 2.63 (q, J 8.5, 1H, H_2), 2.04–2.12 (m, 1H, H_1), 1.66–1.73 (m, 1H, H_{3a}), 1.39–1.46 m, 1H, H_{3b}); IR (KBr) 3463, 3357 (NH_2), 1652; MS (EI) m/z 191 (M^+, 60%), 174 (52), 147 (100). Anal. calcd for $C_{11}H_{13}NO_2$: C, 69.09; H, 6.85; N, 7.32. Found: C, 69.09; H, 6.69; N, 7.17.

*(+)-(1S,2R)-2-(4-Methoylphenyl)cyclopropanecarbonitrile (**4g**).* 77% yield recovered from 0.25 mmol of racemic nitrile **4g** in 7 days; ee 17% (chiral HPLC); oil; $[\alpha]_D^{25} = +4.8$ ($c = 1.45$, $CHCl_3$); ^1H NMR δ 7.21 (d, J 8.6, 2H, Ar–H), 6.90 (d, J 8.6, 2H, Ar–H), 3.81 (s, 3H, CH_3O), 2.52 (q, J 8.0, 1H, H_2), 1.77–1.84 (m, 1H, H_1), 1.48–1.53 (m, 2H, CH_2); IR (KBr) 2237

(CN); MS (EI) m/z 173 (M^+, 100%), 172 (29), 158 (26), 130 (39), 121 (25). Anal. calcd for $C_{11}H_{11}NO$: C, 76.31; H, 6.36; N, 8.09. Found: C, 76.40; H, 6.32; N, 8.17.

Preparation of (+)-(1*S*,2*R*)-*trans*-2-phenylcyclopropylamine (7)

To a mixture of (+)-(1*S*,2*S*)-2-phenylcyclopropanecarboxylic acid (**3a**, 70 mg, 1.43 mmol, ee 82%) and triethylamine (61 mg, 0.604 mmol) in dry acetone (3 ml) at −10 °C was added ethyl chloroformate (71 mg, 0.656 mmol). After 2 h stirring, a solution of NaN_3(49 mg) in water (400 µl) was added and the mixture was stirred for another 1 h. The resulting suspension was poured into cold water (10 ml) and was extracted with ether (4 × 10 ml). The organic layers were combined and dried with $MgSO_4$. After filtration and removal of the solvent in vacuum, the residue was then dissolved in anhydrous toluene (10 ml) and heated on an oil bath (90 °C) until no more N_2 was evolved (about 3 h). The toluene was then removed in vacuum and the resulting residue was dissolved again in dry ^{t}BuOH (10 ml) and refluxed for 23 h. Concentration of the mixture followed by column chromatography using silica gave pure *tert*-butyl carbamate as a white solid. The solid was mixed with THF (1 ml) and aqueous hydrochloric acid (33%, 1 ml) and was warmed to 40 °C for 30 min. After cooling to room temperature, the mixture was basified to pH 13–14 by slowly adding aqueous NaOH (20%), extracted with ether (4 × 10 ml) and dried with anhydrous $MgSO_4$. Evaporation of the solvent, after filtration through a short pad of basic aluminum oxide, afforded **7** as a colorless oil (25 mg, 68% yield); $[\alpha]_D^{25} = +93.8$ ($c = 1.3$, $CHCl_3$) [lit.[28] $[\alpha]_D^{20} + 134.9$ ($c = 0.85$, $CHCl_3$), ee > 99%]. Treatment of **7** with hydrogen chloride in dry ether gave (+)-(1*S*, 2*R*)-*trans*-2-phenylcyclopropylamine hydrochloride as a white solid (27 mg): ee 91% (chiral HPLC); mp 141–143 °C; $[\alpha]_D^{25} + 47$ ($c = 1.1$, CH_3OH); ^{1}H NMR (**7**·HCl, 300 MHz, D_2O): δ 7.00–7.21 (m, 5H, Ar–H), 4.67 (s, 3H, NH_3^+), 2.66–2.72 (m, 1H, PhCH), 2.21–2.27 (m, 1H, CHN), 1.19–1.28 (m, 1H, CH*H*), 1.12–1.19 (m, 1H, C*H*H); IR (KBr, **7**·HCl) 2095 (br, s), 1604, 1498; MS (EI) m/z 133 (78%), 132 (100), 115(52), 103(15), 91(19), 77(23).

Preparation of (−)-(1*R*,2*R*)-*trans*-1-aminomethyl-2-phenylcyclopropane (8)

(−)-(1*R*,2*R*)-2-phenylcyclopropanecarboxamide (**2a**, 84 mg, 0.52 mmol, ee > 99%) and $LiAlH_4$(85 mg) were added to 20 ml of freshly distilled anhydrous THF under N_2 protection. The mixture was heated to reflux for 24 h and then allowed to cool down to room temperature. After the reaction was carefully quenched with a small amount of ice water, the mixture was filtered through a short pad of basic aluminum oxide and the filter cake was thoroughly washed with THF. The filtrate was concentrated in vacuum to give **8** as a colorless oil (66 mg, 85%); ee > 99% (chiral HPLC analysis of the corresponding hydrochloride salt); $[\alpha]_D^{25}$ −43.75 ($c = 1.6$, CH_3OH); ^{1}H NMR δ 7.02–7.27 (m, 5H, Ar–H), 2.66–2.78 (m, 2H, CH_2NH_2), 2.19 (br s, 2H, NH_2), 1.72–1.78 (m, 1H, PhCH), 1.26–1.32 (m, 1H, $CHCH_2NH_2$), 0.83–0.95 (m, 2H, CH_2); MS (EI) m/z 147 (M^+, 4%), 130 (12), 129 (18), 115 (25), 106 (92). FAB-MS calcd. for $C_{10}H_{13}N$ ($M^+ + 1$): 148.11121; found ($M^+ + 1$) 148.1121.

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References

1 (*a*) C.-H. Wong and G. M. Whitesides, *Enzymes in Synthetic Organic Chemistry*, Pergamon Press, Oxford, 1994; (*b*) K. Drauz and H. Waldmann, *Enzyme Catalysis in Organic Chemistry*, VCH, Weinheim, 1995; (*c*) K. Faber, *Pure Appl. Chem.*, 1997, **69**, 1613; (*d*) H.-J. Rehm and G. Reed, *Biotechnology*, Wiley-VCH, Weinheim, 1998, vol. 8a; (*e*) W.-D. Fessner, *Biocatalysis: From Discovery to Application*, Springer Verlag, Berlin and Heidelberg, 1999.
2 (*a*) J. B. Jones, *Aldrichim. Acta*, 1993, **26**, 105; (*b*) E. Schoffers, A. Golebiowski and C. R. Johnson, *Tetrahedron*, 1996, **52**, 3769.
3 (*a*) T. Sugai, T. Yamazaki, M. Yokoyama and H. Ohta, *Biosci., Biotechnol., Biochem.*, 1997, **61**, 1419; (*b*) J. Crosby, J. Moiliet, J. S. Parratt and N. J. Turner, *J. Chem. Soc., Perkin Trans. 1*, 1994, 1679.
4 Recent examples of biotransformation of nitriles (*a*) J. E. Gavagan, S. K. Fager, R. D. Fallon, P. W. Folsom, F. E. Herkes, A. Eisenberg, E. C. Hann and R. DiCosmo, *J. Org. Chem.*, 1998, **63**, 4792; (*b*) K. Matoishi, A. Sano, N. Imai, T. Yamazaki, M. Yokoyama, T. Sugai and H. Ohta, *Tetrahedron: Asymmetry*, 1998, **9**, 1097; (*c*) F. Effenberger and B. W. Graef, *J. Biotechnol.*, 1998, **60**, 165; (*d*) M. A. Wegman, U. Heinemann, A. Stolz, F. van Rantijk and R. A. Sheldon, *Org. Process Res. Dev.*, 2000, **4**, 318; (*e*) F. B. Cooling, S. K. Fager, R. D. Fallon, P. W. Folsom, F. G. Gallagher, J. E. Gavagan, E. C. Hann, F. E. Herkers, R. L. Phillips, A. Sigmund, I. N. Wanger, W. Wu and R. DiCosmo, *J. Mol. Catal. B: Enzym.*, 2001, **11**, 295.
5 Z. Rappoport, *The Chemistry of the Cyano Group*, Wiley Interscience, New York, 1970.
6 (*a*) D. Evgred and S. Harnett, *Cyanide Compounds in Biology* (Ciba Foundation Symposium 140), Wiley, Chichester, 1988; (*b*) J.-C. Jallegeas, A. Arnaud and P. Galzy, *Adv. Biochem. Eng.*, 1980, **12**, 1; (*c*) J. L. Legras, G. Chuzel, A. Arnaud and P. Galzy, *World J. Microbiol. Biotechnol.*, 1990, **6**, 83.
7 (*a*) D. B. Harper, *Biochem. Soc. Trans.*, 1976, **4**, 502; (*b*) D. B. Harper, *Biochem. J.*, 1977, **165**, 309; (*c*) M. Kobayashi and S. Shimizu, *FEMS Microbiol. Lett.*, 1994, **120**, 217.
8 (*a*) Y. Asano, Y. Tani and H. Yamada, *Agric. Biol. Chem.*, 1980, **44**, 2251; (*b*) Y. Asano, K. Fujishiro, Y. Tani and H. Yamada, *Agric. Biol. Chem.*, 1982, **46**, 1165; (*c*) Y. Asano, M. Tachibana, Y. Tani and H. Yamada, *Agric. Biol. Chem.*, 1982, **46**, 1175.
9 For a useful overview, see: O. Meth-Cohn and M.-X. Wang, *J. Chem. Soc., Perkin Trans. 1*, 1997, 1099 and references cited therein.
10 For a useful overview, see: O. Meth-Cohn and M.-X. Wang, *J. Chem. Soc., Perkin Trans. 1*, 1997, 3197 and references cited therein.
11 M. Kobayashi and S. Shimizu, *Curr. Opin. Chem. Biol.*, 2000, **4**, 95.
12 (*a*) T. Nagasawa, S. Schimizu and H. Yamada, *Appl. Microbiol. Biotechnol.*, 1993, **40**, 189; (*b*) M. Kobayashi, T. Nagasawa and H. Yamada, *Trends Biotechnol.*, 1992, **40**, 189.
13 A. J. Blakey, J. Colby, E. Williams and C. O'Reilly, *FEMS Microbiol. Lett.*, 1995, **129**, 57.
14 M.-X. Wang, G. Lu, G.-J. Ji, Z.-T. Huang, O. Meth-Cohn and J. Colby, *Tetrahedron: Asymmetry*, 2000, **11**, 1123.
15 M.-X. Wang, J.-J. Li, G.-J. Ji and J. S. Li, *J. Mol. Catal. B: Enzym.*, 2001, **14**, 77.
16 M.-X. Wang and S.-J. Lin, *Tetrahedron Lett.*, 2001, **42**, 6925.
17 (*a*) M.-X. Wang, C.-S. Liu, J.-S. Li and O. Meth-Cohn, *Tetrahedron Lett.*, 2000, **41**, 8549; (*b*) M.-X. Wang, C.-S. Liu and J.-S. Li, *Tetrahedron: Asymmetry*, 2001, **12**, 3367.
18 Part of this work has appeared as a preliminary communication: M.-X. Wang and G.-Q. Feng, *Tetrahedron Lett.*, 2000, **41**, 6501.
19 (*a*) M. P. Doyle and D. C. Forbes, *Chem. Rev.*, 1998, **98**, 911; (*b*) M. P. Doyle and M. N. Protopopova, *Tetrahedron*, 1998, **54**, 7919; (*c*) V. K. Singh, A. D. Gupta and G. Sekar, *Synthesis*, 1997, 137; (*d*) M. Lautens, W. Klute and W. Tam, *Chem. Rev.*, 1996, **96**, 49.
20 (*a*) C. Kaiser, B. M. Lester, C. L. Zirkle, A. Burger, C. S. Davis, T. J. Della and L. Zirngibl, *J. Med. Pharm. Chem.*, 1962, **5**, 1243; (*b*) C. L. Zirkle, C. Kaiser, D. H. Tedeschi, R. E. Tedeschi and A. Burger, *J. Med. Pharm. Chem.*, 1962, **5**, 1265.
21 L.-E. Arvidsson, J. L. G. Johansson, K. Svensson, S. Hjorth, T. Magnusson, A. Carlsson, P. Lindberg, B. Andersson, D. Sanchez, H. Wikstrom and S. Sundell, *J. Med. Chem.*, 1988, **31**, 92.
22 H. Nozaki, S. Moriuti, H. Takaya and R. Noyori, *Tetrahedron Lett.*, 1966, 5239.
23 T. Aratani, *Pure Appl. Chem.*, 1985, **57**, 1839.

24 (*a*) D. Müller, G. Umbricht, B. Weber and A. Pfaltz, *Helv. Chim. Acta*, 1991, **74**, 232; (*b*) U. Leutenegger, G. Umbricht, C. Fahrni, P. von Matt and A. Pfaltz, *Tetrahedron*, 1992, **48**, 2143; (*c*) A. Pfaltz, *Acc. Chem. Res.*, 1993, **26**, 339.

25 (*a*) R. E. Lowenthal, A. Abiko and S. Masamune, *Tetrahedron Lett.*, 1990, **31**, 6005; (*b*) R. E. Lowenthal and S. Masamune, *Tetrahedron Lett.*, 1991, **32**, 7373.

26 (*a*) D. A. Evans, K. A. Woerpel, M. M. Hinman and M. M. Faul, *J. Am. Chem. Soc.*, 1991, **113**, 726; (*b*) D. A. Evans, K. A. Woerpel and M. J. Scott, *Angew. Chem., Int. Ed. Engl.*, 1992, **31**, 430.

27 (*a*) T. Uchida, R. Irie and T. Katsuki, *Tetrahedron*, 2000, **56**, 3501; (*b*) T. Niimi, T. Uchida, R. Irie and T. Katsuki, *Tetrahedron Lett.*, 2000, **41**, 3647.

28 R. Csuk, M. J. Schabel and Y. von Scholz, *Tetrahedron: Asymmetry*, 1996, **7**, 3505.

29 S. C. Stinton, *Chem. Eng. News*, 1994, **72**, 38.

30 (*a*) C. Kaiser, B. M. Trost, J. Beeson and J. Weinstock, *J. Org. Chem.*, 1965, **30**, 3972; (*b*) C. R. Johnson and P. Rogers, *J. Org. Chem.*, 1973, **38**, 1793.

31 J. H. Hall and M. Gisler, *J. Org. Chem.*, 1976, **41**, 3769.

32 (*a*) H. Kakeya, N. Sakai, T. Sugai and H. Ohta, *Tetrahedron Lett.*, 1991, **32**, 1343; (*b*) F. Effenberger and J. Böhme, *Bioorg. Med. Chem.*, 1994, **2**, 715; (*c*) M. A. Cohen, J. S. Parratt and N. J. Turner, *Tetrahedron: Asymmetry*, 1992, **3**, 1543; (*d*) T. Beard, M. A. Cohen, J. S. Parratt, N. J. Turner, J. Crosby and J. Moilliet, *Tetrahedron: Asymmetry*, 1993, **4**, 1085.

33 (*a*) U. M. Teotino, D. D. Bella, A. Gandini and G. Benelli, *J. Med. Chem.*, 1967, **10**, 1091; (*b*) J. Vallgarda, U. Appelberg, L.-E. Arvidsson, S. Hjorth, B. E. Svensson and U. Hacksell, *J. Med. Chem.*, 1996, **39**, 1485.

34 R. F. Borne, M. L. Forrester and I. W. Waters, *J. Med. Chem.*, 1977, **20**, 771.

35 A. Solladie-Cavallo and T. Isarno, *Tetrahedron Lett.*, 1999, **40**, 1579.

36 G. A. Pinna, M. Loruga, G. Paglietti and G. Cignarella, *Farmaco*, 1980, **35**, 684.

37 S. C. Yasui and T. A. Keiderling, *J. Am. Chem. Soc.*, 1987, **109**, 2311.

38 T. A. Wittstruck and E. N. Trachtenberg, *J. Am. Chem. Soc.*, 1967, **89**, 3810.

39 T. J. W. Jorden and G. W. Perold, *Chem. Ber.*, 1953, **86**, 991.

40 R. Fuchs, C. A. Kaplan, J. J. Bloomfield and L. F. Hatch, *J. Org. Chem.*, 1962, **27**, 733.

41 T. Aratani, Y. Nakanisi and H. Nozaki, *Tetrahedron*, 1970, **26**, 1675.

42 W. E. Doering and E. A. Barsa, *Tetrahedron Lett.*, 1978, 2495.