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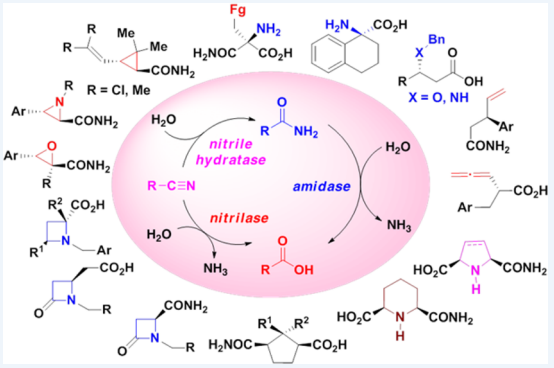
Enantioselective Biotransformations of Nitriles in Organic Synthesis

Mei-Xiang Wang*

MOE Key Laboratory of Bioorganic Phosphorus Chemistry and Chemical Biology, Department of Chemistry, Tsinghua University, Beijing 100084, China

Collaborative Innovation Center of Chemical Science and Engineering, Tianjin 300072, China

CONSPECTUS: The hydration and hydrolysis of nitriles are valuable synthetic methods used to prepare carboxamides and carboxylic acids. However, chemical hydration and hydrolysis of nitriles involve harsh reaction conditions, have low selectivity, and generate large amounts of waste. Therefore, researchers have confined the scope of these reactions to simple nitrile substrates.



However, biological transformations of nitriles are highly efficient, chemoselective, and environmentally benign, which has led synthetic organic chemists and biotechnologists to study these reactions in detail over the last two decades. In nature, biological systems degrade nitriles via two distinct pathways: nitrilases catalyze the direct hydrolysis of nitriles to afford carboxylic acids with release of ammonia, and nitrile hydratases catalyze the conversion of nitriles into carboxamides, which then furnish carboxylic acids via hydrolysis in the presence of amidases. Researchers have subsequently developed biocatalytic methods into useful industrial processes for the manufacture of commodity chemicals, including acrylamide.

Since the late 1990s, research by my group and others has led to enormous progress in the understanding and application of enantioselective biotransformations of nitriles in organic synthesis. In this Account, I summarize the important advances in enantioselective biotransformations of nitriles and amides, with a primary focus on research from my laboratory. I describe microbial whole-cell-catalyzed kinetic resolution of various functionalized nitriles, amino- and hydroxynitriles, and nitriles that contain small rings and the desymmetrization of prochiral and meso dinitriles and diamides. I also demonstrate how we can apply the biocatalytic protocol to synthesize natural products and bioactive compounds.

These nitrile biotransformations offer an attractive and unique protocol for the enantioselective synthesis of polyfunctionalized organic compounds that are not readily obtainable by other methods. Nitrile substrates are readily available, and the mild reaction conditions are specific toward cyano and amido functional groups without interfering with other reactive functional groups. I anticipate that further advances in this field will lead to new and engineered nitrile-hydrolyzing enzymes or catalytic systems with improved activity and altered selectivity. These advances will broaden the scope of these transformations and their applications in organic synthesis.

1. INTRODUCTION

Nitriles are invaluable intermediates in synthesis because they are readily available from many simple, straightforward, and cost-effective synthetic methods and can be converted into a range of diverse organic compounds because of the unique and versatile reactivity of the cyano functional group.[1,2] Among various functional group transformations, hydration and hydrolysis of nitriles represent probably the simplest reactions. However, they are the most frequently used processes both in academia and industry to produce carboxamides and carboxylic acids, respectively. Chemical hydration and hydrolysis generally require rather harsh conditions such as the use of strong acids, bases, heavy-metal catalysts or oxidants, and elevated temperatures.[1,3] The conditions are therefore confined to simple nitriles and are not compatible to reactants that contain labile groups. Because of a lack of selectivity, chemical methods also give mixtures of carboxamide and carboxylic acid products in many cases. They are even more problematic in regioselective hydration and hydrolysis of dinitriles. Moreover, chemical hydration and hydrolysis of nitriles inevitably generate a large amount of waste, causing environmental and safety concerns.

In nature, nitriles are hydrated and hydrolyzed by enzymes in different biological systems (Figure 1).[4] In 1958, Thimann and Mahadevan[5] observed an enzymatic reaction that converted indoleacetonitrile into indoleacetic acid. In 1964 they reported the isolation of a nitrile-hydrolyzing enzyme from barley leaves.[6] Some 16 years later, during the study of microbial cell-catalyzed hydrolysis of nitriles, Asano, Tani, and Yamada[7] discovered that a nitrile hydratase in combination with an amidase involved in *Arthobacter* sp. J-1 was responsible for the biocatalytic degradation of acetonitrile to acetic acid through an acetamide intermediate. Ever since, a large number of biological systems, predominantly microorganisms, have been found to exhibit

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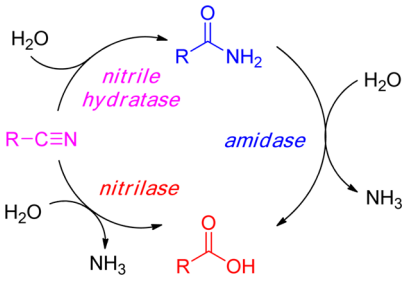


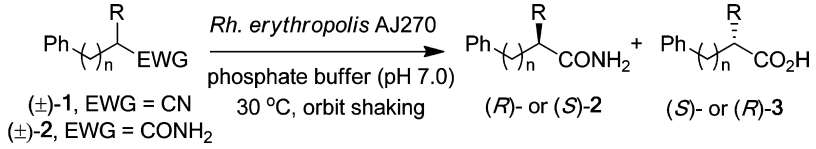
Figure 1. Two distinct pathways for nitrile biodegradation.

catalytic nitrile-hydrolyzing activity.[8,9] Molecular genetics and biochemistry studies have convincingly established two distinct pathways for biocatalytic conversion of a nitrile into its carboxylic acid (Figure 1). While nitrilase (E.C. 3.5.5.1) catalyzes the direct hydrolytic reaction of a nitrile to afford a carboxylic acid with the release of ammonia (pathway A),[10,11] nitrile hydratase (E.C. 4.2.1.84) catalyzes the hydration of a nitrile to form a carboxamide, which undergoes further hydrolysis to give a carboxylic acid product with the help of amidase (E.C. 3.5.1.4).[12] Interestingly, both biocatalytic pathways have been found to operate in some wide-type microbial strains, although most of the microorganisms isolated contain either nitrilase or nitrile hydratase/amidase. In comparison with chemical hydration and hydrolysis, biotransformations of nitriles effected by microbial whole-cell catalysts or isolated enzymes proceed under very mild conditions to furnish the desired carboxamide and carboxylic acid products in a highly selective manner. As typical environmentally benign processes, nitrile biotransformations have been operated successfully in industry to manufacture commodity chemicals.[13] For example, the annual production of acrylamide from microbial hydration of acrylonitrile in China has already reached 400 million kilograms,[14] and it has become probably the largest biocatalytic process in the world.

One of the prominent features of biotransformations is their enantioselectivity. As a result of the intrinsic enantiotopic nature of proteins, nitrilases, nitrile hydratases, and amidases have been shown to be enantioselective biocatalysts, and they are able to catalyze the transformations of nitriles and amides in highly enantioselective fashion. A salient advantage of nitrile biotransformations is the straightforward generation of enantiopure carboxamides, which are valuable nitrogenous compounds in synthesis and applications, in addition to the formation of enantiopure carboxylic acids. Admittedly, although the investigation of enantioselective biotransformations of nitriles may date back to the mid-1980s,[15] early examples were limited to kinetic resolution of structurally simple nitriles. In contrast to the fruitful study of other hydrolytic enzymes such as lipases, esterases, and proteases in organic synthesis, there were few systematic studies of enantioselective biocatalysis of nitrile reactions. To our delight, there has been enormous progress since the beginning of this century due to our[8] and others' endeavors.[9]

Isolated by Colby and his colleagues[16] from a soil sample in northeast England, *Rhodococcus erythropolis* AJ270 is a nitrile hydratase/amidase-containing microorganism. The nitrile hydratase has been purified, and X-ray crystallography has revealed an iron-containing active site of the enzyme structure.[17] The microbial cells are easily cultured on a large scale, and the enzymatic activity remains without noticeable decay after storage of the enzyme at −20 °C for several months. Our initial studies indicated that *Rhodococcus erythropolis* AJ270 is a robust whole-cell catalyst that catalyzes the hydrolysis of a wide variety of aliphatic and aromatic nitriles[18] and dinitriles.[19] Starting in the later 1990s, we became interested in the exploration of enantioselective biotransformations using *Rhodococcus erythropolis* AJ270 whole cells as a catalyst. In this Account, I summarize important advances in enantioselective biotransformations of nitriles and amides with a primary focus on our own research

Table 1. Biotransformations of Nitriles (±)-1 and Amides (±)-2[a]



1	*n*	R	*t* (h)	(*R*) or (*S*)-2 (% yield) (% ee)	(*S*) or (*R*)-3 (% yield) (% ee)	E^b
1a	0	Me	10	(*R*)-**2a** (42) (>99.5)	(*S*)-**3a** (48) (90.0)	99
1b	0	Et	96	(*R*)-**2b** (34) (96.0)	(*S*)-**3b** (40) (>99.5)	>200
1c	0	*i*-Pr	120	(*R*)-**2c** (47) (>99.5)	(*S*)-**3c** (46) (>99.5)	>200
1d	0	*n*-Pr	168	(*R*)-**2d** (trace) (−)	(*S*)-**3d** (2) (>99.5)	–
1e	0	allyl	75	(*R*)-**2e** (48) (>99.5)	(*S*)-**3e** (49) (>99.5)	>200
1f	0	propargyl	144	(*R*)-**2f** (49) (98.6)	(*S*)-**3f** (51) (94.8)	>200
1g	1	allyl	5.5	(*R*)-**2g** (49) (95.4)	(*S*)-**3g** (47) (>99.5)	>200
1h	1	propargyl	14.5	(*R*)-**2h** (48) (95.4)	(*S*)-**3h** (50) (94.5)	>200
1i[c]	1	allenyl	4	(*S*)-**2i** (48) (>99.5)	(*R*)-**3i** (46) (92.5)	144
1j[c]	1	vinyl	1	(*S*)-**2j** (50) (87.0)	(*R*)-**3j** (46) (90.9)	60
1k	1	*n*-Pr	96	(*R*)-**2k** (47) (66.5)	(*S*)-**3k** (47) (52.8)	6.3
2b	0	Et	120	(*R*)-**2b** (47 (>99.5)	(*S*)-**3b** (51) (>99.5)	>200
2g	1	allyl	5.3	(*R*)-**2g** (44) (>99.5)	(*S*)-**3g** (50) (95.7)	>200
2h	1	propargyl	14	(*R*)-**2h** (46) (>99.5)	(*S*)-**3h** (50) (94.9)	>200
2k	1	*n*-Pr	81	(*R*)-**2k** (47) (46.1)	(*S*)-**3k** (49) (46.5)	4.3

[a]Substrate (2 mmol) was incubated with *Rhodococcus erythropolis* AJ270 cells (2 g wet weight) in potassium phosphate buffer (pH 7.0, 0.1 M, 50 mL) at 30 °C. [b]Enantiomeric ratio.[23] [c]The reaction was performed at 20 °C.

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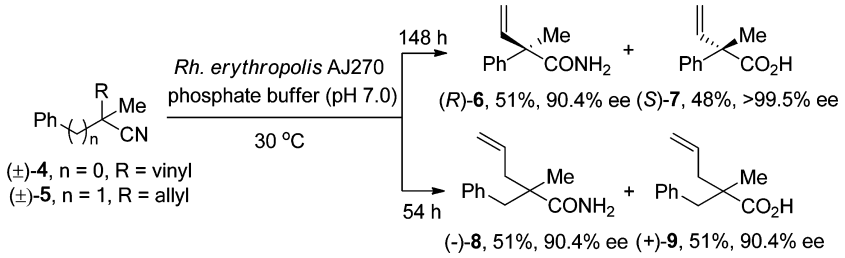

Figure 2. Biotransformations of quaternary-carbon-bearing nitriles (±)-**4** and (±)-**5**.

work. Applications of the biocatalytic protocol in the synthesis of natural products and bioactive compounds are also highlighted.

2. BIOTRANSFORMATIONS OF RACEMIC NITRILES

2.1. Functionalized Nitriles

The *Rhodococcus erythropolis* AJ270 biocatalyst is able to catalyze biotransformations of various α-alkylated benzyl cyanides[20,21] and β-phenylpropionitriles[22] under mild conditions (Table 1). Both the reaction rate and enantioselectivity of the overall nitrile biotransformation are strongly dependent upon the structure of the substrate. The results summarized in Table 1 show explicitly that increasing the steric hindrance of the substrate leads to a decrease in the reaction velocity. Replacement of a simple alkyl group such as an *n*-Pr by a functional one such as allyl, propargyl, or allenyl, however, facilitates the transformation. Except for 2-benzylpentanenitrile, biotransformations of all of the racemic nitriles, especially ones containing an unsaturated carbon−carbon bond, afford high enantioselectivities with enantiomeric ratios (*E*)[23] of up to >200. The outcomes of controlled kinetic resolutions of racemic nitriles and amides reveal convincingly that the nitrile hydratase, irrespective of the structure of the substrate, exhibits high enzymatic activity but virtually no enantioselectivity. The amidase, on the contrary, is enantioselective in general, displaying substrate-dependent activity and enantioselectivity. The formation of highly enantiopure carboxamides and carboxylic acids from nitriles therefore originates from tandem catalysis by the nitrile hydratase and amidase, with the latter playing a dominant role. The intriguing and beneficial effect of an unsaturated carbon−carbon bond on the biotransformation probably implies a binding domain of the amidase toward alkene, alkyne, and allene moieties, which reinforces the chiral recognition between the enzyme and the substrate.

Figure 2 illustrates further examples of steric and electronic effects on biocatalysis. In comparison with 2-benzylpent-4-enenitrile (**1g**), the introduction of a methyl moiety at the α-position diminishes the reaction rate of (±)-**5**. Further increasing the steric hindrance by removal of methylenes in (±)-**5** leads to a slower transformation of (±)-**4**. Nevertheless, these biotransformations furnish the formation of the quaternary-carbon-bearing functionalized carboxylic acids and amide derivatives with ee up to >99.5%.

It is generally believed that asymmetric catalysis results in a decrease of enantiocontrol if a stereogenic center moves from near the reactive site (e.g., from the α-position relative to the functional group) to a remote place.[24] However, this notion may be invalid in biocatalysis, as the enantiotopic recognition site or binding domain may be located some distance away from the catalytic center. Inspired by the beneficial effect of a carbon−carbon double bond on the biocatalysis, biotransformations of 3-arylpent-4-enenitriles (±)-**10** were investigated.[25] Gratifyingly, almost all of the nitriles tested underwent efficient and enantioselective reactions to give (*S*)-3-arylpent-4-enamides (*S*)-**11** and (*R*)-3-arylpent-4-enoic acids (*R*)-**12** (Figure 3). It

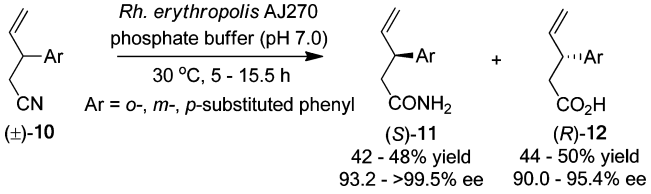

Figure 3. Biotransformations of 3-arylpent-4-enenitriles (±)-**10**.

should be mentioned that the β-vinyl moiety plays a paramount role in achieving high efficiency and enantioselectivity of the amidase, as the biotransformations of racemic 3-phenylpentanenitrile and 3-phenylpentanamide gave only moderate enantioselectivity.[25]

2.2. Amino and Hydroxy Nitriles

Biotransformations provide a powerful route to various α-amino acids, including nonproteinogenic and functionalized ones.[26−28] Incubation of α-arylglycine- and α-cyclohexylglycinenitriles with *Rhodococcus erythropolis* AJ270 whole cells very rapidly produces (*R*)- or D-amino amides and (*S*)- or L-amino acids in nearly quantitative yields with ee values of up to >99.5%[26] (Figure 4).

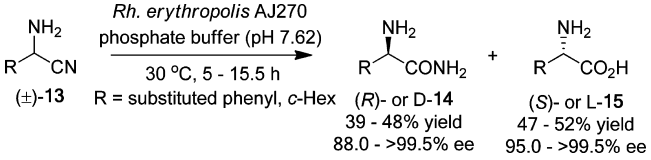

Figure 4. Biotransformations of α-amino nitriles (±)-**13**.

Because α-amino nitriles derived from ketones and from methylamine undergo decomposition to give cyanide, which inhibits the activity of the nitrile hydratase, an iron-containing metalloenzyme, biotransformations of α-alkyl-α-amino nitriles and α-methylamino nitriles were not successful. Taking the advantage of the activity and enantioselectivity of the amidase enabled the development of the *Rhodococcus erythropolis* AJ270-catalyzed kinetic resolution of amides.[27,28] Figure 5 shows examples of the synthesis of enantiopure tetrasubstituted α-amino amides (*R*)-**16** and (*R*)-**17** and α-amino acids (*S*)-**18** and (*S*)-**19**.

Extending the biotransformations of α-amino nitriles to β-hydroxy nitriles (±)-**20** (R^1 = alkyl, R^2 = H)[29] and β-amino nitriles (±)-**21** (R^1 = alkyl, R^2 = H)[30] leads to poor enantioselectivity. Because of the high polarity and further degradation of acids, the chemical yields of the products **22** to **25** are also very low. To circumvent these problems, a very simple

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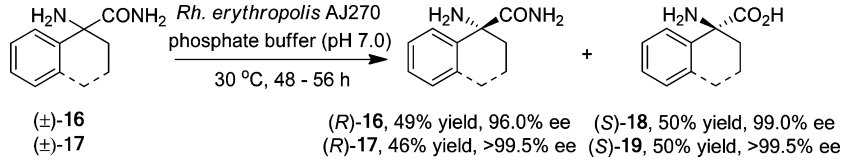

Figure 5. Biotransformations of α-substituted α-amino amides (±)-**16** and (±)-**17**.

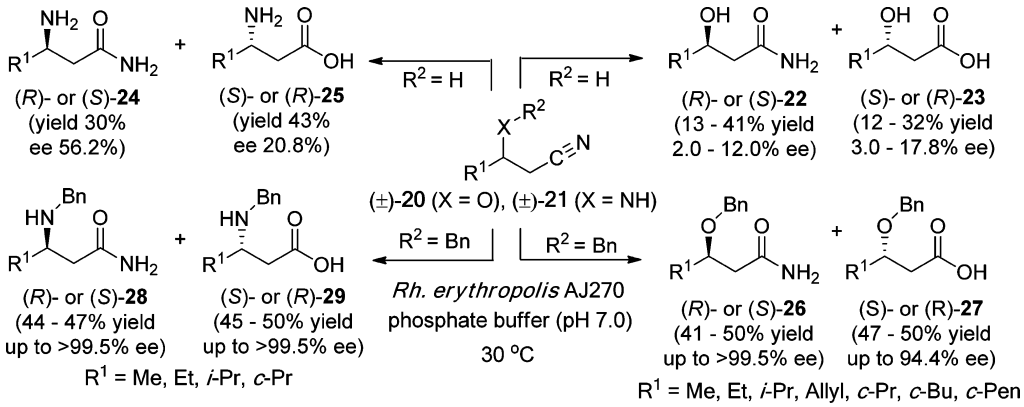

Figure 6. Biotransformations of β-amino and β-hydroxy nitriles (±)-**20** and (±)-**21**.

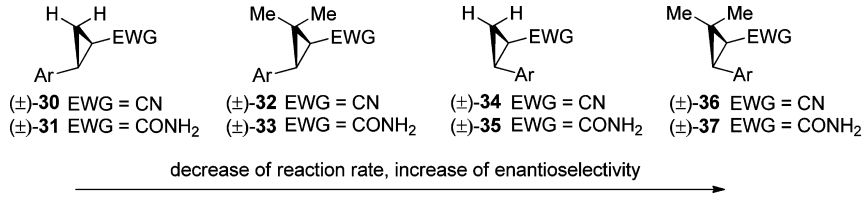

Figure 7. Effect of the substrate on the catalytic efficiency and enantioselectivity of biotransformations of nitriles and amides.

and effective protection/docking strategy was established on the basis of the hypothesis that the chiral recognition site of the enzyme might be situated some distance from the catalytic center. Protecting the hydroxyl group with a benzyl group increases the enantioselectivity dramatically, with the ee values of up to >99.5% and 99.4% for the resulting β-benzyloxy amides **26** and β-benzyloxy acids **27**, respectively. The same protection/docking protocol also works effectively in the biotransformation of N-benzylated β-amino nitriles (±)-**21** (R^1 = alkyl, R^2 = Bn) which affords high yields of β-amino amides **28** and β-amino acids **29** with ee values of up to >99.5% (Figure 6).

The use of benzyl as a docking group not only enhances the enantioselectivity but also results in the improvement of the chemical yield because of the augmented molecular weight and hydrophobicity of the products, which alleviate their further degradation and facilitate their recovery from aqueous media, respectively. Furthermore, the benzyl protecting group benefits the monitoring of the reaction because of its UV activity. The resulting β-benzyloxy and β-benzylamino acids and amides can be applied directly in synthesis or can be converted to β-amino and β-hydroxy acids and amides by convenient catalytic hydrogenolysis.

Rhodococcus erythropolis AJ270 is tolerant to organoazides. Biotransformations of α-branched β-azidopropionitriles therefore provides an alternative chemoenzymatic method for the synthesis of both antipodes of highly enantiopure β-amino acids via β-azido acids and amides.[31]

2.3. Nitriles Bearing Small Rings

Using an array of trans- and cis-configured arylcyclopropanecarbonitriles and amides as substrates, we probed the actions of the nitrile hydratase and amidase of *Rhodococcus erythropolis* AJ270.[32,33] As summarized in Figure 7, the biotransformations of trans-configured nitriles and carboxamides proceed much more effectively than those of the corresponding cis-configured analogues. Increasing the steric bulkiness of the substrate by installing a pair of geminal methyls on the cyclopropane ring led to a decrease of hydrolysis. As an extreme example, the presence of a substituent larger than fluorine on the phenyl ring in cis-configured 2-arylcyclopropanecarbonitriles (±)-**36** and amides (±)-**37** inhibits the biotransformations.

While being nonenantioselective toward *trans*-2-arylcyclopropanecarbonitriles (±)-**30**, the nitrile hydratase displays moderate to good enantioselectivity toward *trans*-2,2-dimethyl-3-arylcyclopropanecarbonitriles (±)-**32** and excellent enantioselectivity toward *cis*-2-arylcyclopropanecarbonitriles (±)-**34**. The amidase exhibits low 1*S*,2*S* enantioselectivity toward *trans*-2-arylcyclopropanecarboxamides (±)-**31** and high levels of enantioselection for both *trans*-2,2-dimethyl-3-arylcyclopropanecarboxamides (±)-**33** and *cis*-2-arylcyclopropanecarboxamides (±)-**35**. The combined action of the enantioselective nitrile hydratase and amidase is responsible for the highly enantioselective biotransformations of nitriles (±)-**32** and (±)-**34**. In all cases, the influence of the electronic effect of the aryl substituent on the enantioselectivity is negligible.

On the basis of these results, we have proposed a prediction model in terms of reaction efficiency and enantioselectivity based

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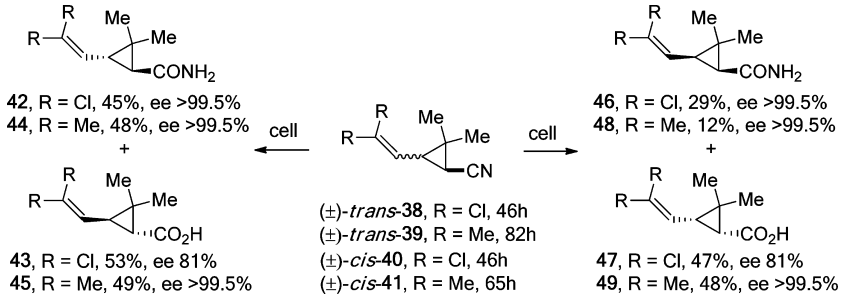

Figure 8. Biotransformations of nitriles (±)-**38**−(±)-**41**.

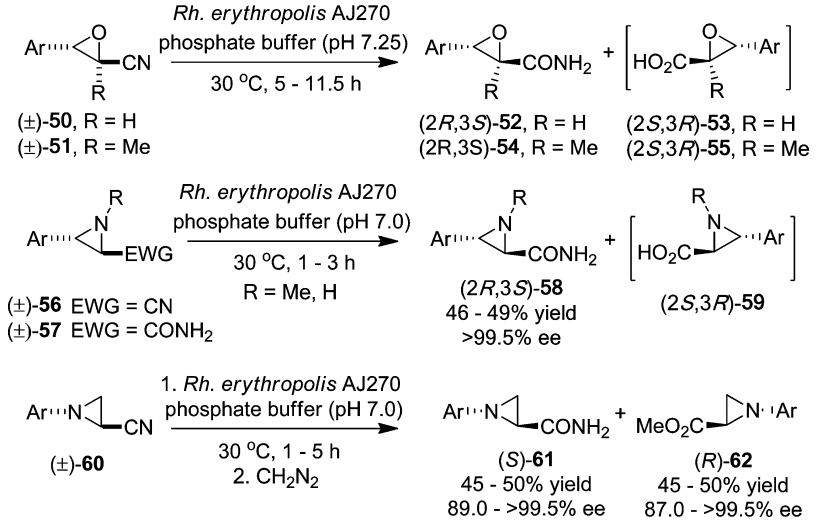

Figure 9. Biotransformations of oxiranecarbonitriles and aziridinecarbonitriles.

on the steric effect of the substituents on the cyclopropane ring.[33,34] The prediction is followed very well by the biocatalytic reactions of other cyclopropanecarbonitriles and amides (Figure 8). Being comparable to (±)-**32** in size, nitrile (±)-**38** undergoes efficient biotransformations to produce amide (1*S*,3*R*)-**42** and acid (1*R*,3*S*)-**43** in good yields with high ee values. Increasing the steric bulkiness of the substituent from chloro to methyl results in a slower enantioselective reaction of (±)-**39**. For the cis-configured substrates (±)-**40** and (±)-**41**, the anticipated slow conversions give low yields of highly enantiopure products.[33b] In the case of 2,2-dimethylcyclopropanecarbonitrile and amide, rapid biotransformations proceed as expected to afford products with moderate ee values.[33a] Another example is the biotransformations of 2,2-dihalo-3-phenylcyclopropanecarbonitriles and amides. Variation of the two germinal hydrogen atoms in (±)-**31** (Ar = Ph) to fluorine and chlorine atoms leads to a gradual decrease in the bioconversion rate. Meanwhile, the selectivity is greatly improved, with the enantiomeric ratio *E* increasing from 8.9 to 74 and 125.[34]

Remarkably, the prediction model works equally well when the biotransformations are extended to three-membered heterocyclic nitriles (Figure 9).[35−37] Both *trans*-3-(*p*-substituted-phenyl)oxirane-2-carbonitriles (±)-**50** and their tetrasubstituted analogues (±)-**51** undergo very efficient biotransformations to afford the corresponding carboxamides (2*R*,3*S*)-**52** and (2*R*,3*S*)-**54**, respectively, in almost quantitative yields with ee values of >99.5%, whereas the cis isomers remain almost intact under identical conditions.[35] The kinetic resolution of 3-arylaziridine-2-carboxamides (±)-**57** occurs as expected, although 3-arylaziridine-2-carbonitriles (±)-**56** are not stable under the biocatalytic conditions[36] (Figure 9). In all cases, acid products are not isolated because they decompose spontaneously in aqueous media. Following the prediction, most 1-arylaziridine-2-carbonitriles (±)-**60** undergo efficient and enantioselective biotransformations to yield amides (*S*)-**61** and acid products that are converted into methyl esters (*R*)-**62** after treatment with CH_2N_2[37] (Figure 9). At the same time, Gotor[38] independently investigated the same reaction using *Rhodococcus rhodochrous* IFO 15564. Notably, the formation of acids was not observed because of further metabolic degradations by cells as proposed by the authors.

It is worth mentioning that as a dominant enzyme that controls the enantioselectivity, the amidase involved in *Rhodococcus erythropolis* AJ270 tends to recognize all three-membered-ring-containing carboxamides with a *trans*-aryl substituent in the same steric sense. Irrespective of the nature of the three-membered ring, all of the racemic amides are kinetically resolved into the chiral amides and acids by the amidase following the chiral selection mode as depicted in Figure 10. The observations are useful in predicting absolute configurations of the products.

Biotransformations of four-membered N-heterocyclic nitriles and amides provide a unique approach to enantiopure azitidine and β-lactam compounds (Figure 11).[39−41] Under the catalysis of *Rhodococcus erythropolis* AJ270, a variety of 1-arylmethylazetidine-2-carbonitriles along with disubstituted and quaternary-substituted substrates are efficiently transformed into azetidine-2-carboxylic acids (2*R*)-**64** and amide derivatives (2*S*)-**65**.[39] It is interesting to note that in the biotransformations of β-lactam nitriles and amides, the amidase shows high activity to kinetically resolve all of the amides (±)-**67** and (±)-**70**. However, the nitrile

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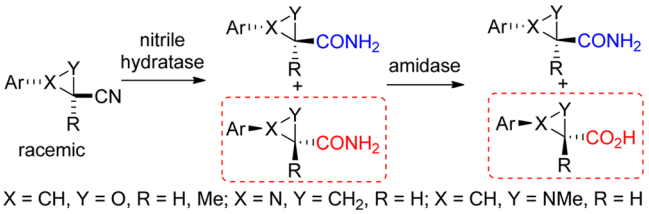

Figure 10. Stereochemical outcomes of biotransformations of trans-configured three-membered-ring-containing nitriles and amides.

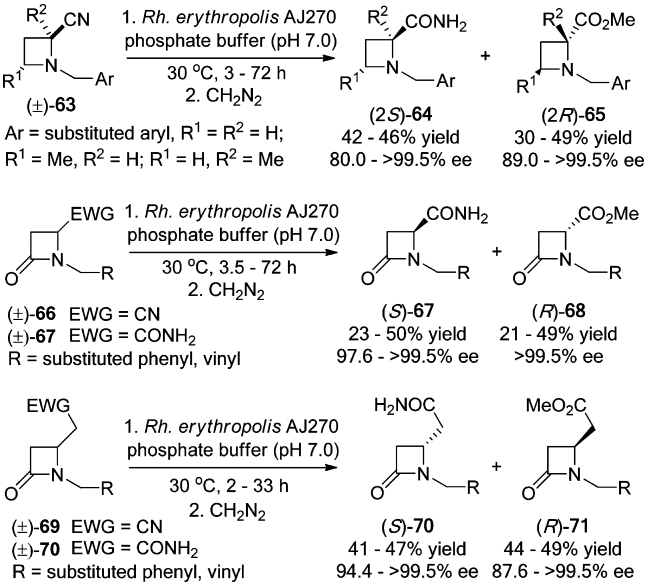

Figure 11. Biotransformations of azetidine-2-carbonitriles and β-lactam carbonitriles and amides.

hydratase, which is generally more tolerant than the amidase toward steric bulkiness of the substrate, exhibits limited activity toward β-lactam nitriles (±)-**66** and (±)-**69**. It is also noteworthy that the amidase is specific for primary amides, as no hydrolysis of the β-lactam bond is observed.[40,41] Furthermore, the amidase displays opposite enantioselections for 4-oxoazetidine-2-carboxamides (±)-**67** and 4-oxoazetidin-2-yl)acetamides (±)-**70** (Figure 11), reflecting its high sensitivity to subtle structural variations of the substrate.

3. BIOCATALYTIC DESYMMETRIZATION OF DINITRILES AND DIAMIDES

3.1. Prochiral Dinitriles and Diamides

Prochiral 3-aryl- and 3-alkyl-substituted glutaronitriles undergo *Rhodococcus erythropolis* AJ270-catalyzed hydrolysis effectively to afford monocyanocarboxylic acids in moderate to good yields. Unfortunately, the enantioselectivity of desymmetrization appears to be low. Intriguingly, the use of an organic additive such as acetone or β-cyclodextrin greatly improves the enantioselectivity. For example, the ee of (*S*)-4-cyano-3-(*p*-tolyl)butanoic acid (*S*)-**73** from the reaction of 3-(*p*-tolyl)-glutaronitrile **72** (R = *p*-tolyl; Figure 12) increases from 64% to 95% in the presence of a very small amount of acetone.[42]

Biocatalytic hydrolysis of prochiral 2,2-disubstituted malononitriles gives a mixture of cyanoacetamide, cyanoacetic acid, malonamic acid, malonamide, and malonic acid products after a lengthy incubation time. However, the amidase-catalyzed desymmetrization of prochiral 2,2-disubstituted malonamides

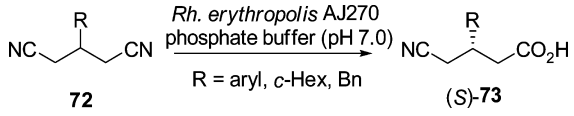

Figure 12. Biotransformations of prochiral glutaronitriles **72**.

is highly efficient and enantioselective.[43,44] As demonstrated in Figure 13, almost all of the 2-aminomalonamide substrates **74** are

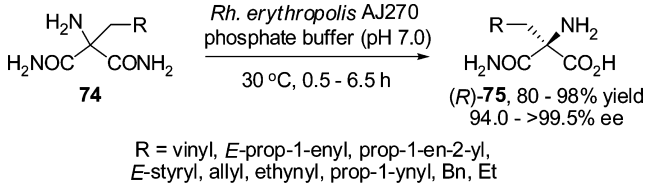

Figure 13. Desymmetrization of 2-amino-substituted malonamides **74**.

converted rapidly into functionalized (*R*)-2-amino-2-carbamoyl-carboxylic acids (*R*)-**75** in high yields with excellent enantioselectivity. The only exception is in the case of 2-amino-2-methylmalonamide, where a fast reaction gives (*R*)-2-amino-2-methylmalonamic acid in 80% yield with 24.8% ee, probably as a result of diminished ability of the amidase to enantiodifferentiate between the sterically similar methyl and amino groups.

3.2. Meso Diamides

We recently found that *Rhodococcus erythropolis* AJ270 is capable of catalyzing the desymmetrization of meso pyrrolidine-2,5-dicarboxamides **76** to yield (2*R*,5*S*)-5-carbamoylpyrrolidine-2-carboxylic acids **77** or esters after esterification with high enantiopurity[45] (Figure 14). Variation of the N-substituent from hydrogen to methyl, allyl, or benzyl results in a decrease in the conversion. The high biocatalytic efficiency and enantioselectivity remain when a double bond (**76e**) or an extra methylene (**76f**) is introduced into the five-membered ring. Further expanding the ring size to azepane (**76g**), however, results in slower transformation and diminished enantioselectivity.

In comparison with N-heterocyclic diamides **76**, the biotransformations of meso cyclopentane-1,3-dicarboxamides **78** proceed more sluggishly because of the steric hindrance of the two substituents at the 2-position.[46] However, the reaction is accelerated effectively when the biocatalyst loading is doubled. Diamides **78a**−**e**, in which the larger group is oriented trans to the amido groups, are biotransformed into, afetr esterification, enantiopure (1*S*,2*S*,3*R*)-3-carbamoylcyclopentanecarboxylic acid benzyl esters **79a**−**e** in excellent yields (Figure 15). The presence of a cis-configured larger group in substrates **78f** and **78g** leads to slower transformations that give moderate yields of the corresponding products in 1 week. Polyfunctionalized chiral cyclopentanes **79** containing three continuous stereogenic centers, including a quaternary carbon, are hardly available by other methods.

4. SYNTHETIC APPLICATIONS

Biotransformations are readily scalable, enabling gram- and multigram-scale syntheses of highly enantiopure compounds. A typical and fascinating example is the production of ca. 20 g of (2*R*,5*S*)-5-carbamoylpyrrolidine-2-carboxylic acid (**77h**) by desymmetrization of meso pyrrolidine-2,5-dicarboxamide.[45b] Many products resulting from biotransformations are unique chiral building blocks, and their applications in the synthesis of

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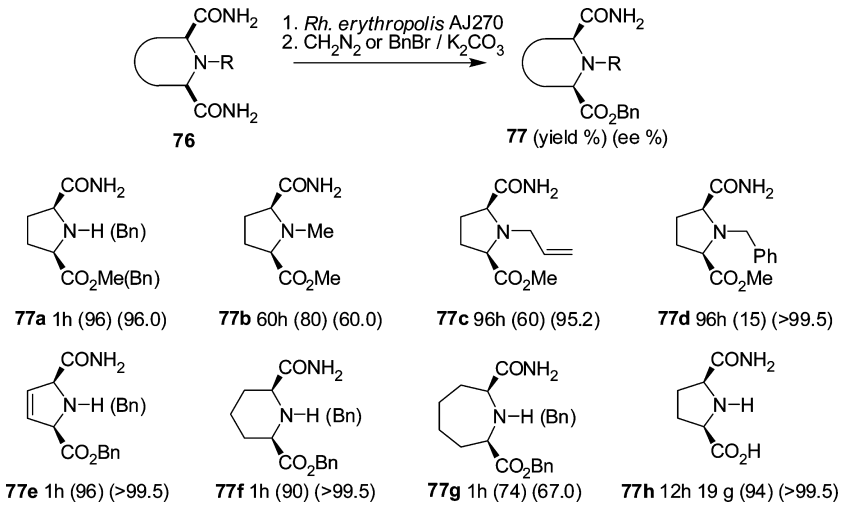

Figure 14. Biotransformations of meso N-heterocyclic diamides **76**.

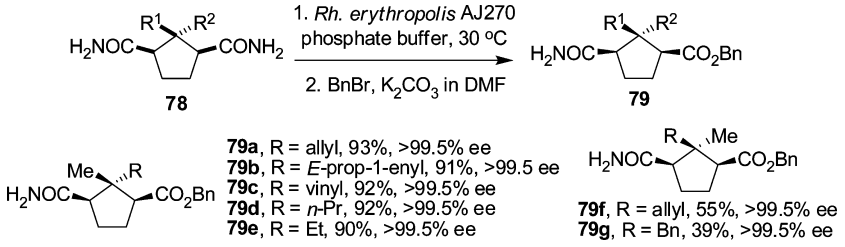

Figure 15. Biotransformations of meso cyclopentane-1,3-dicarboxamides **78**.

natural products and bioactive compounds have been explored. Figure 16 shows the facile synthesis of the *Clausena* alkaloid

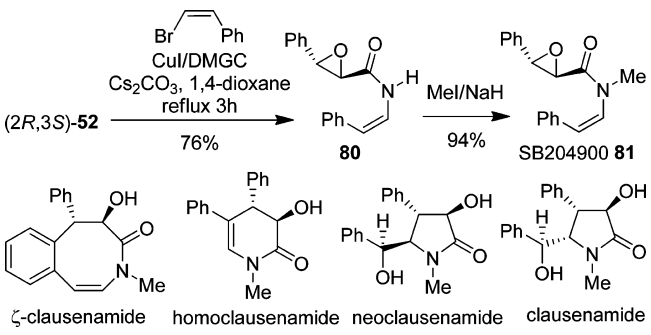

Figure 16. Synthesis of *Clausena* alkaloids.

(2*R*,3*S*)-(−)-SB204900 (**81**) from oxiranecarboxamide (2*R*,3*S*)-**52**. Following our proposed biosynthetic pathways, **81** can be converted selectively into ζ-clausenamide, homoclausenamide, neoclausenamide, or clausenamide. The availability of various oxiranecarboxamides from biotransformations enables the preparation of a focused library of *Clausena* alkaloids and their analogues.[47]

Taking advantage of their polyfunctionality, β-lactam products derived from biotransformations have been utilized in syntheses of novel bicyclic β-lactam compounds of pharmacological relevance. Some typical examples are included in Figure 17. (2*R*,5*S*)-5-Carbamoylpyrrolidine-2-carboxylic acid **77h** from biocatalytic desymmetrization of pyrrolidine-2,5-dicarboxamide has been used as a versatile platform in the synthesis of both antipodes of azanucleoside analogues.[45] Figure 18 depicts the

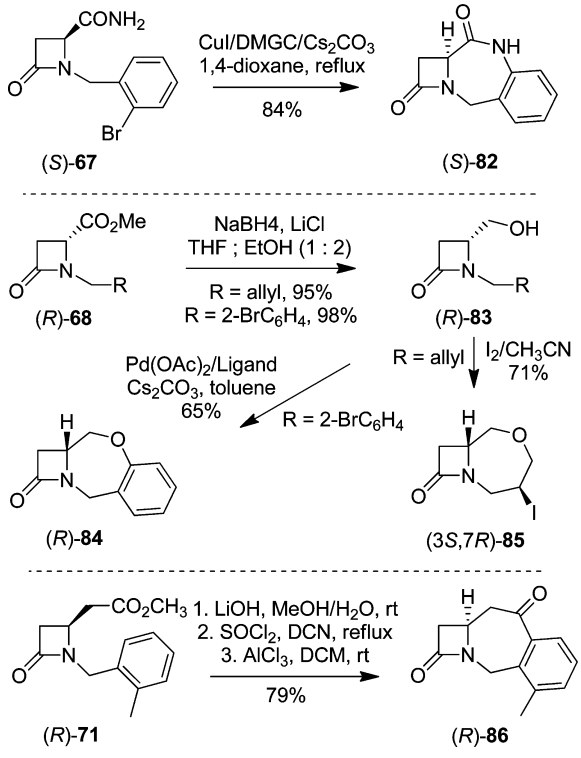

Figure 17. Synthesis of bicyclic β-lactam derivatives.

four-step synthesis of tetrazole-containing nucleoside-like compound **90** from **77h**.

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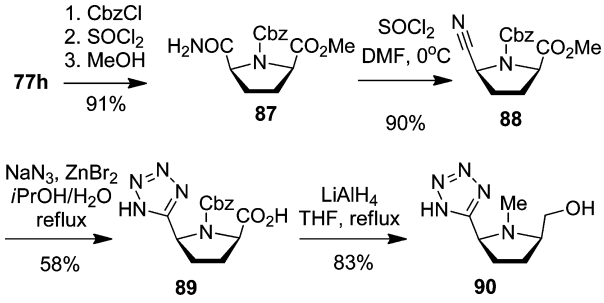

Figure 18. Synthesis of an azanucleoside analogue.

5. CONCLUDING REMARKS

After two decades of development, enantioselective biotransformations of nitriles using nitrile-hydrolyzing microbial whole-cell catalysts or hydratases/amidases and nitrilases have evolved into a powerful method for the preparation of carboxylic acids and amide derivatives with high enantiomeric purity. The easy availability of nitrile substrates by means of various documented syntheses, the mild biocatalytic reaction conditions that are compatible with labile groups, and the specificity of enzymes toward cyano and amido functional groups render nitrile biotransformations an attractive and unique protocol for enantioselective syntheses of polyfunctionalized organic compounds that are not readily obtainable by other chemical and biological catalyzes. It can be anticipated that advances in biotechnology will lead to new and engineered nitrile-hydrolyzing enzymes or catalytic systems of improved activity and altered selectivity that will flourish the synthetic application of enantioselective biotransformations of nitriles in organic synthesis, with the substrate scope being further broadened and the enantioselectivity being still enhanced. The joint efforts of organic and biological chemists will ultimately uncover the mechanism of the fascinating enantioselectivity of enzymatic reactions of nitriles and amides at the molecular level. It is also foreseeable that more and more applications of chiral compounds resulting from enantioselective biotransformations of nitriles in the synthesis of natural products and bioactive compounds will appear in the years to come.

AUTHOR INFORMATION

Corresponding Author

*E-mail: wangmx@mail.tsinghua.edu.cn.

Notes

The authors declare no competing financial interest.

Biography

Mei-Xiang Wang obtained his B.Sc. and Ph.D. from Fudan University in Shanghai and Institute of Chemistry, The Chinese Academy of Sciences (ICCAS) in Beijing, respectively. He used to work at ICCAS and now is a professor in the Department of Chemistry at Tsinghua University, Beijing. His research interests include biocatalysis and biotransformations, macrocyclic and supramolecular chemistry, organic reactions and asymmetric synthesis, anion-π non-covalent bond interactions, and high-valent organocopper chemistry.

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