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Progress of Enantioselective Nitrile Biotransformations in Organic Synthesis

Mei-Xiang Wang*

Abstract: Recent progress of enantioselective biotransformations of nitriles including various functionalized nitriles, β-hydroxy and β-amino nitriles, oxirane- and aziridine-containing carbonitriles is summarized in this short review article.

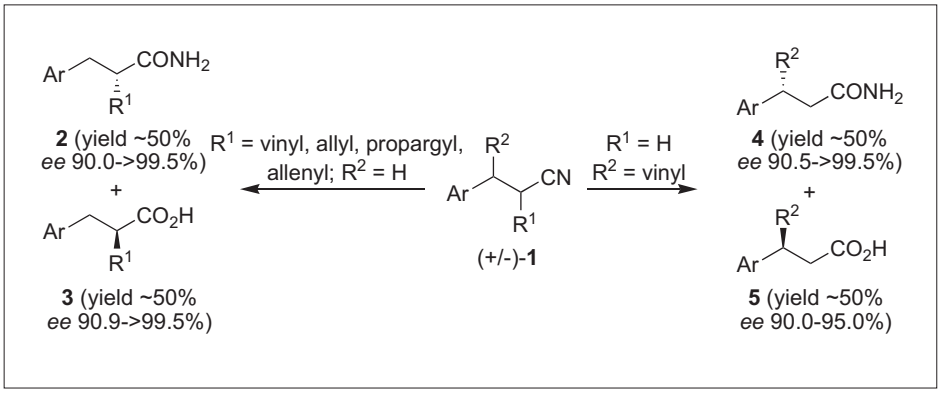
Keywords: Amidase · Amide · Carboxylic acid · Nitrile biotransformations · Nitrile hydratase

Nitrile biotransformations have been revealed to proceed through two distinct pathways.[1] Catalyzed by nitrilase, nitriles are converted directly into the corresponding carboxylic acids, while in the presence of nitrile hydratase, nitriles are hydrated to give amides which undergo further hydrolysis with the aid of amidase to afford carboxylic acids. Until now a large number of nitrile-hydrolyzing microorganisms have been reported[1,2] and microbial hydrolysis of nitriles has been utilized in industry[3] and in academic research[4] to produce amide and carboxylic acid products. In comparison to conventional chemical hydration and hydrolysis of nitriles, the salient advantages of nitrile biotransformations include high catalytic efficiency, excellent chemo-, regio- and enantioselectivities, and very mild reaction conditions. Most noticeably, nitrile biotransformations have been shown to display intriguing and high enantioselectivity that enables the synthesis of enantiopure carboxylic acids and amides, synthetically valuable organonitrogen compounds, which are not readily available from chemical synthesis. For years, we[4b] have been studying the enantioselective biotransformations of nitriles using *Rhodococcus erythropolis* AJ270,[5] a nitrile-hydratase/amidase-containing microbial whole cell catalyst. In this short review article, I will summarize recent progress of enantioselective nitrile biotransformations with a primary focus on our own work.[6]

1. Biotransformations of Functionalized Nitriles

Although many enantioselective nitrile biotransformations are documented in literature, the structures of the nitrile substrates investigated are rather simple and limited.[2–4] To explore synthetic potentials of enantioselective biotransformations of nitriles, we[7–10] have in recent years studied the functionalized nitrile substrates in microbial cell catalyzed reactions. The introduction of additional functional groups into nitrile and amide substrates also allows us to examine the factors that govern the efficiency and enantioselectivity, and therefore helps us to have a deep insight into the mechanism of biocatalysis.

We have found that *Rhodococcus erythropolis* AJ270 is an efficient whole cell biocatalyst able to catalyze the hydrolysis of functionalized nitrile substrates under very mild conditions.[7] When incubated with microbial cells in phosphate buffer (pH 7.0) at 30 °C, for example, racemic nitriles **1** (R^2 = H) that bear an α-positioned vinyl, allyl, propargyl or allenyl substituent undergo highly efficient and enantioselective biotransformations.[7] Within 1 to 14.5 h, the reaction gives excellent yield of amides **2** and acids **3** with enantiomeric excess values (*ee*) ranging from 90% to >99.5% (Scheme 1). As indicated by the results of biocatalytic resolution of racemic nitriles into amides and of racemic amides into acids, the overall enantioselectivity of nitrile biotransformations originates from a consecutive action of *a less enantioselective nitrile hydratase* and *a highly enantioselective amidase* in *Rhodococcus erythropolis* AJ270. It is very important to address that when the vinyl, allyl, propargyl or allenyl functional group is replaced by an alkyl group such as *n*-propyl, the same biocatalytic hydrolysis of nitrile proceeds at a very slow rate with only modest enantioselectivity. Four days' incubation of racemic nitrile (R^1 = *n*-Pr, R^2 = H, Ar = Ph) with cells leads to the formation of *R*-(–)-2-benzylpentamide (47% yield, 66.5% *ee*) and *S*-(+)-2-benzylpentanoic acid (47% yield, 52.8% *ee*). The unusual beneficial effect of an unsaturated carbon–carbon bond on the reaction efficiency and enantioselectivity has also been observed in the biotransformations of racemic 3-arylpent-4-enenitriles **1** (R^1 = H, R^2 = vinyl).[8] For example, almost all substrates tested are transformed within several hours into amides **4** and acids **5** in almost quantitative yields with high enantiopurity (Scheme 1).



Scheme 1. Enantioselective nitrile biotransformations of functionalized nitriles.

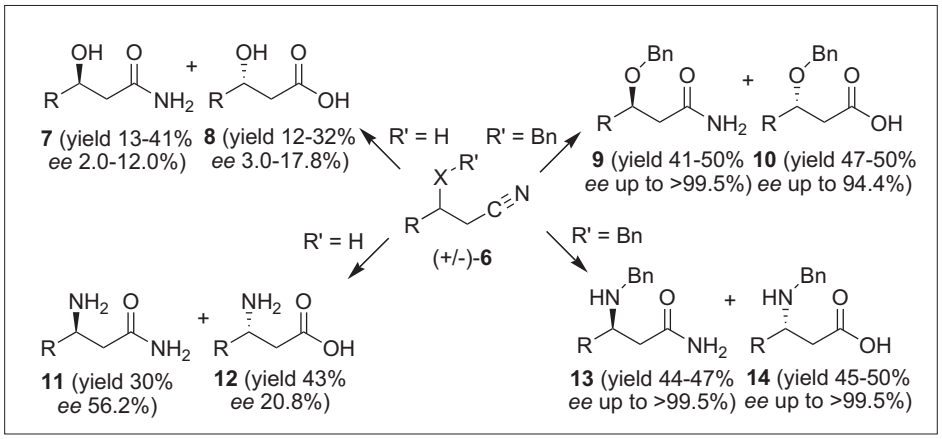
*Correspondence: Prof. Dr. M.-X. Wang
Beijing National Laboratory for Molecular Sciences
CAS Key Laboratory of Molecular Recognition and Function
Institute of Chemistry
Chinese Academy of Sciences
Beijing 100190
Tel.: +8610 6256 5610
Fax: +8610 6256 4723
E-mail: mxwang@iccas.ac.cn

On the contrary, it takes more than one day to convert racemic 3-phenylpentanenitrile **1** (R^1 = H, R^2 = Et) into the corresponding *R*-3-phenylpentamide and *S*-3-phenylpentanoic acid products with *ee* of 85.8% and 82.2%, respectively.

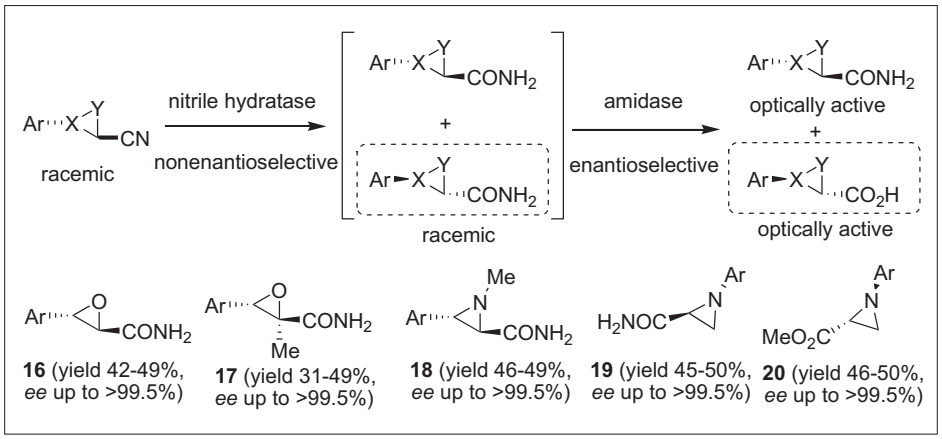
It has been proposed that the amidase in *Rhodococcus erythropolis* AJ270 may contain a binding domain specific to the unsaturated carbon–carbon bond of the amide substrate. It is most likely that the binding to the unsaturated carbon–carbon bond functionality further strengthens the interaction or the recognition of the amidase with the amide substrate, leading to the significant acceleration of the reaction rate and the remarkable enhancement of the enantioselectivity.[7] The resulting enantiopure functionalized carboxylic acids and amide derivatives have been employed as useful intermediates in the synthesis of various chiral heterocycles.[7,8]

2. Biotransformations of β-Hydroxy and β-Amino Nitrile Derivatives

In contrast to the successful enantioselective nitrile biotransformations for the preparation of chiral carboxylic acids and amide derivatives that bear an α-stereocenter, biotransformations of substrates having a chiral center remote from the cyano or the amido functional group have been reported to proceed with, in most cases, disappointingly low enantioselectivity and chemical yield.[11,12] Biotransformations of the Baylis-Hillman nitriles and the one carbon homologated nitriles, for example, give only moderate enantioselectivity[12] whereas β-phenylbutyronitrile,[11a] β-, γ- or δ-hydroxylated nitriles yield no or extremely low enantiocontrol.[11d] Recent studies[13] of *Rhodococcus erythropolis* AJ270 catalyzed reactions of racemic β-hydroxy nitriles also show poor enantioselectivity. Because of the high polarity and further degradation of β-hydroxy acids, the chemical yields of the products are also low (Scheme 2). It is generally believed indeed that the movement of a stereocenter from the reactive site (α-position to functional group) to a remote place results in the decrease of enantioselectivity in catalytic asymmetric reactions. To circumvent this problem, we[13,14] have developed a protection/docking strategy[15] based on the hypothesis that the chiral recognition site of an enzyme might locate in some distance to the catalytic center. It has been found[13,14] that a very simple and convenient benzyl protection on hydroxyl group increases dramatically the enantioselectivity of biotransformation. The *ee* values of the resulting β-benzyloxy amides **9** and acids **10** are up to >99.5% and 99.4%, respectively, compared to the biotransformations of β-hydroxy nitriles that give products **7** and **8** with *ee* values below 20% (Scheme 2). The excellent enantioselectivity is attributable most probably to the enhanced chiral recognition between the amidase and the O-protected β-hydroxy amides. In addition, introduction of an O-benzyl group also facilitates the detection and isolation of the products because of the UV activity and the increased molecular hydrophobicity, respectively. The protection/docking protocol using benzyl group is also found very effective in the biotransformation of β-amino nitriles.[14] For example, the *Rhodococcus erythropolis* AJ270-catalyzed hydrolysis of racemic nitrile **6** (R = Me, R' = H) gives low enantioselectivity. The same reaction of N-benzylated analogs **6** affords high yields of amides **13** and acids **14** with *ee* up to >99.5%. As useful chiral building blocks, the benzyl protected β-hydroxy and β-amino acids and their amide derivatives can be applied directed in organic synthesis or they can undergo convenient catalytic hydrogenolysis to yield debenzylated acids and amide products.[13,14]



Scheme 2. Enantioselective biotransformations of β-hydroxy and β-amino nitrile derivatives.



Scheme 3. Enantioselective biotransformations of oxirane- and aziridine-2-carbonitriles.

3. Biotransformations of Nitriles Bearing a Three-membered (Hetero)cyclic Ring

Chiral cyclopropane structures are prevalent in natural products and synthetic pharmaceuticals. Biotransformations of nitriles can provide a valuable approach to enantioenriched cyclopropane carboxylic acids and their amide derivatives.[16,17] Furthermore, diverse cyclopropane carbonitriles and carboxamides can serve as substrates to probe the catalytic features of the nitrile hydratase and the amidase. On the basis of the reaction efficiency and enantioselectivity of *Rhodococcus erythropolis* AJ270 catalyzed biotransformations of a large number of differently substituted and configured racemic 2-arylcyclopropanecarbonitriles and carboxamides,[16] it is concluded[16c] that a readily accessible reactive site is embedded within the spacious pocket of the nitrile hydratase while the amidase probably comprises a relatively deep-buried and size-limited enantioselective active site. Correlation of the reaction profile with the nature of the substituents and their substitution pattern

lead to the proposal of a predictive mode of the reaction efficiency and enantioselectivity.[16c,17a] To our delight, biocatalytic reactions of the substrates including chrysanthemic nitriles[16c] and geminally dihalogenated cyclopropanecarbonitriles and amides[17a,b] have been found to follow the prediction mode. Very recently, we have further demonstrated that the prediction mode works well with other three-membered heterocyclic nitrile substrates.[18–21] Illustrated in Scheme 3 are enantioselective biotransformations of oxirane- and aziridine-containing nitriles and amides. Racemic 3-aryloxirane-2-carbonitriles,[18] 3-aryl-2-methyloxirane-2-carbonitriles,[19] 1-arylaziridine-2-carbonitrile,[20] 3-aryl-1-methylaziridine-2-carbonitriles[21] and their amides undergo efficient biotransformations to afford highly enantiopure products in excellent yields. It should be pointed out that, with the exception of biotransformations of racemic 1-arylaziridine-2-carbonitriles which give both amides **19** and acids (isolated as methyl ester **20**),[20] reaction of all substrates do not allow the isolation of acid products because they undergo spontaneous decomposition under reaction conditions.[18,19,21]

It is interesting to note that the amidase involved in *Rhodococcus erythropolis* AJ270 is able to recognize all carboxamides with a *trans*-arylated three-membered ring in the same steric sense. In other words, irrespective of the nature of the three-membered ring, all racemic amides are kinetically resolved into the optically active amides and acids by the amidase following the same chiral selection mode (Scheme 3). This fits well with the hypothesis that the amidase might comprise a deeply buried and highly steric-demanding active site. The highly enantiopure oxirane- and aziridine-containing carboxamides and esters listed in Scheme 3 are not readily available from other conventional chemical synthesis. They are unique and valuable intermediates in the synthesis of unusual hydroxyl acids,[18,19] amino acids and diamino acids.[20,21] Using enantiopure oxirane-carboxamide **16** as the key starting material, we[22,23] have very recently accomplished the synthesis of all naturally occurring clausena alkaloids isolated from *Clausena lansium* (lour.) Skeels, a fruit tree widely distributed in southern China, and of a related balasubramide isolated from *Clausena indica* in Sri Lanka.

4. Outlook

Owning to the easy preparation of virtually all kinds of nitrile substrates, availability of nitrile-hydrolyzing biocatalysts and very mild reaction conditions, efficient and enantioselective nitrile biotransformations will find wide applications in the synthesis of highly enantiopure carboxylic acids and amide derivatives that are not readily accessible by conventional synthetic methods.

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