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Dramatic Enhancement of Enantioselectivity of Biotransformations of β-Hydroxy Nitriles Using a Simple *O*-Benzyl Protection/Docking Group

Da-You Ma, Qi-Yu Zheng, De-Xian Wang, and Mei-Xiang Wang*

Beijing National Laboratory for Molecular Sciences, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100080, China

mxwang@iccas.ac.cn

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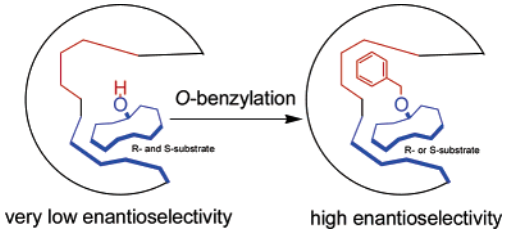
ABSTRACT



Catalyzed by the *Rhodococcus erythropolis* AJ270 whole cell catalyst, the *O*-benzylated β-hydroxy alkanenitriles underwent remarkably high enantioselective biotransformations, whereas the biotransformations of free β-hydroxy alkanenitriles gave very low enantioselectivity. The easy manipulations of *O*-protection and *O*-deprotection, excellent chemical and enantiomeric yields of biotransformations, along with the scalability render this enzymatic transformation attractive and practical for the synthesis of highly enantiopure β-hydroxy alkanoic acids and their amide derivatives.

Molecular recognition of an enzyme toward a substrate is a crucial step in enantiospecific or highly enantioselective biocatalytic transformations.[1] When a non-natural substrate is used, a general practice in synthetic biotransformations, low enantioselectivity, and slow reaction is always encountered because of nonspecific interaction between the enzyme and the substrate. To circumvent these problems, structural modifications of enzymes by means of site-directed mutagenesis[2] and directed evolution,[3] etc. have enjoyed the success in upgrading the performance of enzymes. As an alternative to biotechnological approaches, substrate engineering has been shown to be useful in improving the efficiency and selectivity of biocatalysis. Substrate engineering, in contrast to protein engineering, modifies the structure of small organic molecular substrates to best fit into the active site of the enzyme. The nonbiological approaches are therefore relatively simple, easy to handle, and cost-effective. One excellent example of substrate engineering has been demonstrated by Griengl, who introduced the docking or protecting group concept in biohydroxylation reaction of non-natural substrates.[4−6] Despite the new concept, successful applications are rare in terms of catalytic efficiency and,

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particularly, enantioselectivity. In most cases of biohydroxylation, even with the use of a chiral protecting group, the enantiomeric excess values of products hardly exceed 90%.[4−7]

Biotransformations of nitriles, either through a direct conversion from a nitrile to a carboxylic acid catalyzed by a nitrilase or through the nitrile hydratase-catalyzed hydration of a nitrile followed by the amide hydrolysis catalyzed by the amidase,[8] are an effective and environmentally benign method for the production of carboxylic acids and their amide derivatives.[9] Recent studies have demonstrated that biotransformations of nitriles complement the existing asymmetric chemical and enzymatic methods for the synthesis of chiral carboxylic acids and their derivatives bearing an α-stereocenter.[10−13]

Optically active α-unsubstituted β-hydroxy carboxylic acids and their derivatives are key intermediates in the synthesis of natural products and biologically important compounds.[14] Much effort has been devoted to their synthesis, and synthetic methods involving catalytic asymmetric hydrogenation,[14a,15] reduction,[16] Mukaiyama-aldol reaction,[17] and Reformatsky reaction[18] have been developed. Biocatalytic reduction of β-ketoesters,[19] kinetic resolution of secondary alcohols,[20] and deracemization[21] have proved useful, as well. Considering the easy availability of β-hydroxy alkanenitriles, which can be readily prepared from condensation of acetonitrile and various aldehydes,[22] we envisioned that highly efficient and enantioselective biotransformations of nitriles would provide a convenient and unique approach to optically active β-hydroxy carboxylic acids and their amide derivatives. This has led us to undertake the current study, and herein we report a successful docking/protecting group strategy to dramatically enhance the enantioselectivity of biotransformations of nitriles. It has been reported[23] that enzyme-catalyzed kinetic resolution of β-substituted nitriles gave generally unsatisfactory enantioselectivity. Desymmetrization of prochiral 3-hydroxyglutaronitrile yielded poor enantiocontrol, and the enantioselectivity was improved by using either a 3-benzylated substrate[24] or an engineered nitrilase.[25]

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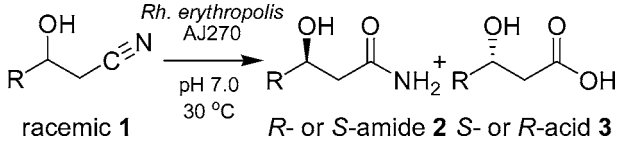
Incubated with *Rhodococcus erythropolis* AJ270[12] microbial whole cells under mild conditions, racemic β-hydroxy alkanenitriles **1a** and **1c** were transformed into the corresponding amides and acids within a very short time. Unfortunately, the enantiocontrol of the reaction was very disappointing (entries 1 and 2, Table 1). This result was not

Table 1. Biotransformations of β-Hydroxy Alkanenitriles **1**[a]



entry	1	R	concn (mmol)	time (min)	**2** (yield %)[b] (ee %)[c]	**3** (yield %)[b] (ee %)
1	**a**	Me	2	20	*R*-**2a** (36) (12.0)	*S*-**3a** (12) (17.8)[d]
2	**c**	^{i}Pr	2	20	*S*-**2c** (41) (2.0)	*R*-**3c** (31) (3.0)[e]
3[f]	**g**	*c*-Pen	2	50	*S*-**2g** (13) (11.5)	*R*-**3g** (32) (6.4)[d]

[a] Biotransformation was carried out in a suspension of *Rh. erythropolis* AJ270 cells (2 g wet weight) in phosphate buffer (50 mL, pH 7.0) at 30 °C. [b] Isolated yield. [c] Determined by chiral HPLC analysis of the corresponding β-benzyloxy alkaneamides. [d] Determined by chiral HPLC analysis of the corresponding methyl ester of β-benzyloxy alkanoic acid. [e] Determined by chiral HPLC analysis of the benzyl ester. [f] Nitrile (49% yield, 2.8% ee) was recovered.

unexpected, however, as the movement of the stereocenter from the α to β position to the functional group of the substrate generally has a detrimental effect on enantioselectivity of biocatalytic resolution of nitriles.[23] Further metabolization and hydrophilic nature of the polar products posed further problems in the isolation of β-hydroxy carboxylic acids and amides from aqueous reaction media. Increasing the bulkiness of the substrate led to a sluggish conversion of nitrile **1g** (entry 3, Table 1). As indicated by the enantiomeric excess values of amide and acid products and of the nitrile recovered (Table 1), neither the nitrile hydratase nor the amidase exhibited even moderate enantioselectivity against β-hydroxy alkanenitriles and alkaneamides, respectively.

To increase the enantioselectivity of the biotransformations, and also to circumvent the problems, such as further metabolization and hydrophilicity of β-hydroxy alkanoic acids, we decided to protect the β-hydroxy group of the substrates. The following criteria should be considered while choosing a protecting group. First, both the introduction and the removal of a protection group should be readily carried out under mild conditions and cause no contamination to the products. Second, under the deprotection conditions, no racemization is allowed. In addition, because a whole cell biocatalyst is used, the protection group should be stable and resistant to other enzymes, such as esterases. Furthermore, large protection groups are not favorable because they result in the deficiency of biocatalysis. Last but not least, an intrinsically UV-active protection group is beneficial because it will facilitate the detection and visualization of both substrates and products and, therefore, simplify the monitoring of the biotransformation. Taking the aforementioned factors into consideration, the benzyl group was finally selected as the protection group. Gratifyingly, the introduction of a benzyl protection group led to a dramatic enhancement of enantioselectivity of the biotransformations, although the protection group was remote from the functional group.

As illustrated in Table 2, all of the β-benzyloxy alkaneni-

Table 2. Biotransformations of β-Benzyloxy Alkanenitriles **4**[a]

entry	4	R	concn (mmol)	time (h)	**5** (yield %)[b] (ee %)[c]	**6** (yield %)[b] (ee %)[c]	E[d]
1	**a**	Me	2	1.75	*R*-**5a** (47) (92.0)	*S*-**6a** (49) (86.2)	43
2	**b**	Et	2	5.5	*R*-**5b** (46) (95.4)	*S*-**6b** (47) (83.4)	39
3	**b**[e]	Et	12	10	*R*-**5b** (41) (94.4)	*S*-**6b** (49) (92.4)	85
5	**c**	^{i}Pr	2	25.5	*S*-**5c** (47) (95.0)	*R*-**6c** (47) (91.0)	78
4	**d**	allyl	2	28	*R*-**5d** (47) (91.0)	*S*-**6d** (48) (86.4)	41
6	**e**	*c*-Pr	2	21	*S*-**5e** (47) (92.4)	*R*-**6e** (48) (93.8)	106
7	**f**	*c*-Bu	2	24	*S*-**5f** (49) (95.6)	*R*-**6f** (47) (94.4)	127
8	**g**	*c*-Pen	1	70	*S*-**5g** (48) (95.4)	*R*-**6g** (51) (85.4)	45

[a] Biotransformation was carried out in a suspension of *Rh. erythropolis* AJ270 cells (2 g wet weight) in phosphate buffer (50 mL, pH 7.0) at 30 °C. [b] Isolated yield. [c] Determined by chiral HPLC analysis. [d] E value was calculated according to ref 27. [e] A suspension of 5 g wet weight cells in 125 mL of phosphate buffer was used, and nitrile (10% yield, 93.0% ee) was recovered.

triles **4**,[26] prepared readily from the benzylation of β-hydroxy alkanenitriles **1**, underwent effective biotransformations to afford optically active β-benzyloxy alkanoic acids **6** and amides **5** with enantiomeric excess values up to 95%. Comparison of the biotransformations of **4a**, **4c**, and **4g** with those of **1a**, **1c**, and **1g** and protection of the hydroxyl by a benzyl group led to a dramatic increase of enantioselectivity with enantiomeric ratio E[27] ranging from 43 to 78. For the substrates, such as **4e** and **4f**, that contains a cyclopropyl and cyclobutyl moiety, respectively, biotransformations gave excellent enantioselectivity with an E over 100 (entries 6 and 7, Table 2). In addition to high reaction efficiency and enantioselectivity, an extra advantage of utilizing the benzyl protection group is the prohibition of product metabolization and the increase of molecular hydrophobicity, both leading to the isolation of products in high yields. Furthermore, biotransformations of *O*-benzylated β-hydroxy alkanenitriles

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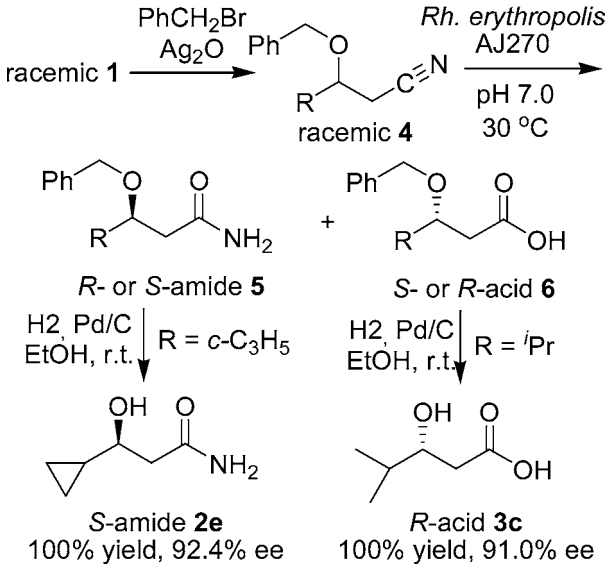
allowed practical synthesis of highly enantiopure β-hydroxy alkanoic acids and their amide derivatives, which was exemplified by the multigram biotransformation of **4b** (entry 3, Table 2). Finally, the protection group was conveniently removed through catalytic hydrogenolysis giving rise to the highly enantiopure β-hydroxy carboxylic acid and amide in quantitative yield (Scheme 1). To demonstrate the further

Scheme 1. Enhancement of Enantioselectivity of Biotransformations of **1** Using a Benzyl Protection Group



advantage of protection strategy, the biotransformation product **5d** was directly employed as the reactant to prepare, via iodolactonization,[13e,24c] lactone **8** which is the essential intermediate for the construction of effective cholesterol-lowering agents Lipitor (atorvastatin) and Crestor (rosuvastatin) (see Supporting Information).[28]

The dramatic enhancement of enantioselectivity of the biotransformation of β-benzyloxy alkanenitriles **4** is extraordinarily intriguing, especially when we consider the introduction of a protecting benzyl group at the hydroxyl oxygen, a position being remote from the reaction site, such as a cyano or an amido group. It is difficult at this current stage to rationalize the effect of a β-positioned benzyl protection group on the enantioselectivity because of the lack of structural information of the enzymes. Nevertheless, much more efficient chiral recognition of the enzymes toward protected β-hydroxy alkanenitriles or amides than free β-hydroxy alkanenitriles or amides might account for the increase of enantioselectivity. In other words, it is most likely that the β-benzyl protection group acts as an excellent docking group, leading to the enhancement of enantioselective interaction of the substrate with the chiral pocket of the enzyme (Figure 1).

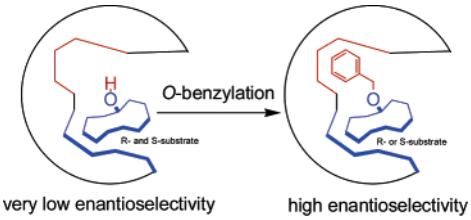


Figure 1. Schematic illustration of enhancement of enantioselectivity through a benzyl protection/docking group.

In summary, we have shown that the introduction of a simple and remote protection/docking benzyl group dramatically increases the enantioselectivity of the biotransformations of β-hydroxy alkanenitriles. The easy protection and deprotection manipulations, high chemical yields, and excellent enantioselectivity along with the scalability render this microbial transformations attractive and practical for the synthesis of highly enantiopure β-hydroxy alkanoic acids and their amide derivatives. The protection/docking strategy may, in general, provide a simple and cost-effective method to improve the enantioselectivity and efficiency of biocatalysis, even if such a docking/protection group is far away from the reactive functional group. Studies toward the understandings of the remarkable protection/docking effect and toward the further improvement of enantioselectivity using a modified benzyl group in the biotransformations of nitriles are actively being investigated in this laboratory.

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Supporting Information Available: Experimental details of biotransformation of nitriles, copies of ^{1}H and ^{13}C NMR spectra of compounds **2**, **3**, and **5**−**8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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