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Remarkable Electronic and Steric Effects in the Nitrile Biotransformations for the Preparation of Enantiopure Functionalized Carboxylic Acids and Amides: Implication for an Unsaturated Carbon−Carbon Bond Binding Domain of the Amidase

Ming Gao, De-Xian Wang, Qi-Yu Zheng, Zhi-Tang Huang, and Mei-Xiang Wang*

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

mxwang@iccas.ac.cn

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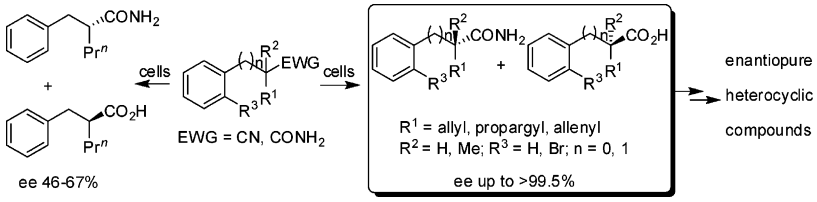

Biotransformations of various functionalized racemic nitriles catalyzed by *Rhodococcus erythropolis* AJ270, a nitrile hydratase/amidase-containing microbial whole-cell catalyst, were studied. While the nitrile hydratase exhibits high catalytic efficiency but very low enantioselectivity against almost all nitrile substrates examined, the amidase is very sensitive toward the structure of the amides. The release of the steric crowdedness around the stereocenter of the substrates and the introduction of an unsaturated carbon−carbon bond into the substrates led to the significant acceleration of the reaction rate and the dramatic enhancement of the enantioselectivity. Nitrile biotransformations provide a unique and high-yielding synthetic route to highly enantiopure carboxylic acids and amides functionalized with an allyl, propargyl, allenyl, or vinyl group. The synthetic applications have been demonstrated by the synthesis of enantiopure heterocyclic compounds including iodoenol γ-lactone, γ-lactam, and 3-allyl-1-phenyl-3,4-dihydro-1*H*-quinolin-2-one derivatives.

Introduction

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, have become the effective and environmentally benign methods for the production of carboxylic acids and their amide derivatives.[1] One well-known example is the industrial production of acrylamide from biocatalytic hydration of acrylonitrile.[2] Owing to the enantioselectivity of the nitrilase, nitrile hydratase, and amidase, biotransformations of nitriles complement the existing asymmetric chemical and enzymatic methods for the synthesis of chiral carboxylic acids and their derivatives.[3,4] One of the distinct features of enzymatic transformations of nitriles is the straightforward generation of enantiopure amides, valuable organo-nitrogen compounds in synthetic chemistry, in addition

* To whom correspondence should be addressed. Tel.: +86-10-62565610. Fax: +86-10-62564723.

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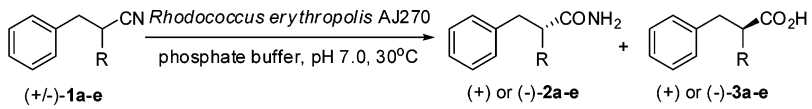
TABLE 1. Enantioselective Biotransformations of Racemic α-Substituted 2-Phenylpropanenitriles 1a−e[a]



entry	1	R	time (h)	2	yield (%)[a]	ee (%)[b]	3	yield (%)[a]	ee (%)[b]	E[c]
1	**1**[d]	$H_2C{=}CHCH_2$	5.5	(*R*)-(−)-**2a**	49	95.4[e]	(*S*)-(+)-**3a**	47	>99.5	>200
2	**1b**	$HC{\equiv}CCH_2$	14.5	(*R*)-(−)-**2b**	48	>99.5	(*S*)-(−)-**3b**	50	94.5	>200
3	**1c**	$H_2C{=}C{=}CH$	2.5	(*S*)-(+)-**2c**	42	98.5	(*R*)-(+)-**3c**	54	82.3	52
4	**1c**	$H_2C{=}C{=}CH$	4[f]	(*S*)-(+)-**2c**	48	>99.5	(*R*)-(+)-**3c**	46	92.5	144
5	**1d**	$H_2C{=}CH$	0.58	(*S*)-(+)-**2d**	48	90.0	(*R*)-(−)-**3d**	51	83.1[e]	32
6	**1d**	$H_2C{=}CH$	1[f]	(*S*)-(+)-**2d**	50	87.0	(*R*)-(−)-**3d**	46	90.9[e]	60
7	**1e**	$CH_3CH_2CH_2$	96	(*R*)-(−)-**2e**	47	66.5[e]	(*S*)-(+)-**3e**	47	52.8[e]	6.3

[a] Isolated yield. [b] Determined by chiral HPLC analysis. [c] Enantiomeric ratio (E) was calculated according to ref 12. [d] Nitrile (2 mmol) was incubated with *R. erythropolis* AJ270 cells (2 g wet weight) in phosphate buffer (50 mL, pH 7.0) at 30 °C. Reaction time was optimized to the completion of nitrile conversion and roughly 50% conversion of the amide. [e] Determined by chiral GC analysis. [f] Biotransformation was carried out at 20 °C.

to the formation of enantiopure carboxylic acids. For example, we[3c] have shown that *Rhodococcus erythropolis* AJ270,[5] a nitrile hydratase/amidase-containing whole-cell catalyst, is able to efficiently and enantioselectively transform different racemic nitriles including ones bearing an α-stereocenter[6] or a β-stereocenter,[7] cyclopropanecarbonitriles,[8] oxiranecarbonitriles,[9] aziridinecarbonitriles,[10] and the prochiral dinitriles[11] into the corresponding carboxylic acids and amides.

Although a number of enantioselective nitrile biotransformations have been reported, the structures of nitrile substrates are rather simple and limited.[3,4] Biotransformations of nitriles which contain additional functional group(s) have been largely unexplored. Except for the prediction model[8−10] proposed for the biotransformations of nitriles bearing a three-membered ring such as cyclopropane, oxirane, and aziridine, the understanding of the efficiency and enantioselectivity of biocatalytic nitrile transformations still remains challenging. To explore the potential of nitrile biotransformations in the synthesis of enantiopure functionalized carboxylic acids and their amide derivatives, and also to examine the factors governing the efficiency and enantioselectivity of the biocatalysis, we undertook a systematic study of the *R. erythropolis* AJ270 catalyzed hydrolysis of nitrile substrates containing either a saturated or an unsaturated aliphatic group. We have discovered that the release of the steric crowdedness around the stereocenter greatly improved the reaction velocity. More significantly, the introduction of an unsaturated moiety such as an alkene, alkyne, and allene into the substrates led to a dramatic enhancement of the efficiency and enantioselectivity of the amidase-catalyzed reaction. Herein we report the highly efficient nitrile biotransformations for the preparation of enantiopure functionalized carboxylic acids and amides and the implication for a binding domain of the amidase for an unsaturated carbon−carbon bond of the substrate. Applications of the biotransformation products in the synthesis of chiral heterocyclic compounds will also be discussed.

Results and Discussion

Very recently, we[7d] found that the biotransformations of 3-phenylpent-4-enenitrile, the substrate that contains a β-stereocenter, proceeded much more efficiently and enantioselectively than that of 3-phenylpentanenitrile. Intrigued by the vinyl effect on the acceleration of reaction rate and the enhancement of enantioselectivity, we began our investigation of the biotransformations of 2-benzylpent-4-enenitrile **1a**. To understand and to compare the effect of other unsaturated functional groups on the biocatalytic efficiency and enantiocontrol, racemic α-substituted 2-phenylpropanenitriles **1b−e** bearing, respectively, a propargyl, allenyl, vinyl, and propyl group were prepared and subjected to biotransformations. We were surprised to observe a remarkable beneficial effect of an unsaturated group on the biotransformations of nitriles (Table 1). For example, catalyzed by the *R. erythropolis* AJ270 whole cells under very mild conditions, 2-benzylpent-4-enenitrile **1a** underwent very rapid and highly enantioselective hydrolysis. After 5 h of incubation, almost quantitative yields of *R*-(−)-2-benzylpent-4-enamide **2a** and (*S*)-(+)-2-benzylpent-4-enoic acid **3a** were obtained with enantiomeric excess values of 95.4% and >99.5%, respectively (entry 1, Table 1). When the allyl group of **1a** was replaced by a propargyl group, biotransformations of **1b** took slightly longer

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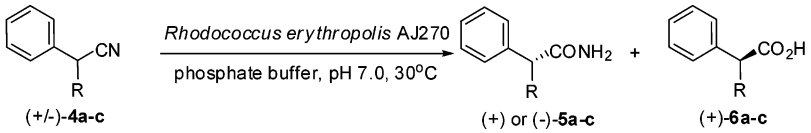
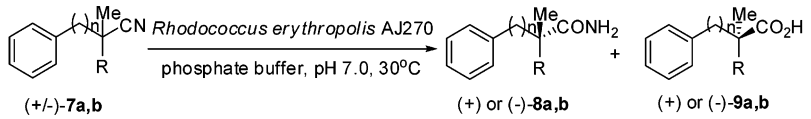
TABLE 2. Enantioselective Biotransformations of Racemic α-Substituted Phenylacetonitriles 4a−c[a]



entry	4	R	time	5	yield (%)[b]	ee (%)[c]	6	yield (%)[b]	ee (%)[c]	E[d]
1	**4a**	$H_2C{=}CHCH_2$	75 h	(*R*)-(−)-**5a**	48	>99.5	(*S*)-(+)-**6a**	49	95.5	>200
2	**4b**	$HC{\equiv}CCH_2$	6 days	(*R*)-(−)-**5b**	49	98.6	(*S*)-(+)-**6b**	51	94.8	>200
3	**4c**	$CH_3CH_2CH_2$	7 days[e]	**5c**	trace	−	(*S*)-(+)-**6c**	2	>99.5	−

[a] Nitrile (2 mmol) was incubated with *R. erythropolis* AJ270 cells (2 g wet weight) in phosphate buffer (50 mL, pH 7.0) at 30 °C. Reaction time was optimized to the completion of nitrile conversion and roughly 50% conversion of the amide. [b] Isolated yield. [c] Determined by chiral HPLC analysis. [d] Enantiomeric ratio (E) was calculated according to ref 12. [e] Optically inactive nitrile **4c** (83%, ee 0%) was recovered.

TABLE 3. Enantioselective Biotransformations of Racemic Nitriles Bearing a Quaternary Stereocenter 7a,b[a]



entry	7	n	R	time (h)	8	yield (%)[b]	ee (%)[c]	9	yield (%)[b]	ee (%)[c]	E[d]
1	**7a**	1	$H_2C{=}CHCH_2$	168[e]	(−)-**8a**[f]	36	26.1	(+)-**9a**[f]	33	>99.5	−
2	**7a**	1	$H_2C{=}CHCH_2$	54[g]	(−)-**8a**	48	94.6	(+)-**9a**	49	93.2	98
3	**7b**	0	$H_2C{=}CH$	168	(*R*)-(+)-**8b**	81	15.7	(*S*)-(−)-**9b**	15	>99.5	−
4	**7b**	0	$H_2C{=}CH$	168[h]	(*R*)-(+)-**8b**	69	34.8	(*S*)-(−)-**9b**	30	>99.5	−
5	**7b**	0	$H_2C{=}CH$	148[g]	(*R*)-(+)-**8b**	51	94.0	(*S*)-(−)-**9b**	48	>99.5	>200

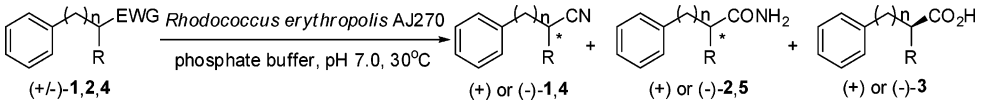
[a] Nitrile (2 mmol) was incubated with *R. erythropolis* AJ270 cells (2 g wet weight) in phosphate buffer (50 mL, pH 7.0) at 30 °C. Reaction time was optimized to the completion of nitrile conversion and roughly 50% conversion of the amide. [b] Isolated yield. [c] Determined by chiral HPLC analysis. [d] Enantiomeric ratio (E) was calculated according to ref 12. [e] Optically active nitrile (*S*)-(+)-**7a** (27%, ee 84.1%) was recovered. [f] Absolute configuration was not determined. [g] Nitrile (0.5 mmol) was used. [h] Nitrile (1 mmol) was used.

time (14.5 h) to afford highly enantiopure amide (*R*)-(−)-**2b** and acid (*S*)-(−)-**3b** in excellent yields (entry 2, Table 1). More efficient biotransformations were observed for the allenyl and vinyl substituted substrates. The conversion of nitrile **1c** into almost quantitative yields of highly enantiopure (*S*)-(+)-**2c** and (*R*)-(+)-**3c** finished in 4 h even at lower temperature (20 °C) (entries 3−4, Table 1), whereas (*S*)-(+)-**2d** and (*R*)-(−)-**3d** were obtained in excellent chemical yields with high enantiomeric purity from the interaction of racemic nitrile **1d** with microbial cells within 1 h (entry 5, Table 1). In sharp contrast to the functionalized nitrile substrates **1a**−**d**, however, biotransformations of 2-benzylpentanenitrile **1e** took 4 days to yield the corresponding amide (*R*)-(−)-**2e** and acid (*S*)-(+)-**3e** with only moderate enantioselectivity (entry 7, Table 1).

To further examine the generality of the interesting unsaturated group effect, we studied the biotransformations of α-substituted phenylacetonitriles **4**, the analogues of **1**. As tabulated in Table 2, biotransformations of α-allylated phenylacetonitrile **4a** proceeded faster than that of α-propargylated analogue **4b**, and both reactions produced highly enantiopure (*R*)-amide and (*S*)-acid products in almost quantitative yields (entries 1 and 2, Table 2). The microbial hydrolysis of **4c**, however, appeared extremely sluggish, giving, after 7 days, a trace amount of amide and acid (*S*)-**6c** (2%) (entry 3, Table 2) along with the recovery of starting nitrile **4c** in 83% yield (entry 3, Table 2). The results demonstrated again the favorable effect of an unsaturated moiety in the substrate on reaction efficiency. It should be noted that biotransformations of all α-substituted phenylacetonitriles **4** (Table 2) proceeded much slower than that of α-substituted 2-phenylpropanenitrile homologues **1** (Table 1). This indicated convincingly that the insertion of a methylene between phenyl and the α-stereocenter led to a tremendous increase of conversion rate. In other words, the release of steric crowdedness around the α-stereocenter resulted in the enhancement of the efficiency of biocatalytic hydrolysis. The steric effect on the biotransformations of nitriles was then further demonstrated by the reaction of nitriles bearing a fully substituted α-stereocenter. As exemplified by the reaction of racemic 2-benzyl-2-methylpent-4-enenitrile **7a**, the analogue of **1a**, only by using a dilute concentration of the substrate did biotransformations go to completion in a lengthy period of time (54 h) to afford products (−)-**8a** and (+)-**9a** in high yields with excellent enantiocontrol (entries 1 and 2, Table 3). Further increase of the crowdedness of the fully substituted α-stereocenter of the substrate of **7a** by knocking off two methylenes—one is in between phenyl and the chiral center and the other is in between vinyl and the chiral center—only under conditions of dilute substrate concentration (0.5 mmol) and of a very long interaction time (148 h) effected the biotransformations of **7b** with the formation of highly enantiopure products (*R*)-(+)-**8b** and (*S*)-(−)-**9b** in very good yields (entries 3−5, Table 3).

Since *R. erythropolis* AJ270 is a nitrile hydratase/amidase-containing microbial strain,[5] the kinetic resolutions of nitrile and amide catalyzed, respectively, by the nitrile hydratase and amidase were then conducted in order to clarify the roles that the nitrile hydratase and the amidase played in the nitrile biotransformations aforementioned. As indicated by the results in Table 4, the nitrile hydratase-catalyzed hydration reaction

TABLE 4. Enantioselective Biotransformations of Racemic Nitriles 1 and 4 and Amide 2[a]



entry	subs	*n*	R	EWG	time	nitrile (yield %)[b] (ee %)[c]	amide (yield %)[b] (ee %)[c]	acid (yield %)[b] (ee %)[c]	*E*[d]
1	**1a**	1	$H_2C{=}CHCH_2$	CN	1 min	(*R*)-**1a** (42) (18.3)	(*S*)-**2a** (54) (6.3)[f]	n.o.[e]	1.3
2	**1b**	1	$HC{\equiv}CCH_2$	CN	1 min	(*R*)-**1b** (41) (24.4)	(*S*)-**2b** (55) (7.5)	n.o.	1.4
3	**1c**	1	$H_2C{=}C{=}CH$	CN	1 min[g]	(*S*)-**1c** (50) (13.0)	(*R*)-**2c** (44) (37.4)	n.o.	2.5
4	**1e**	1	$CH_3CH_2CH_2$	CN	10 min	(*R*)-**1e** (46) (18.9)	(*S*)-**2e** (45) (14.7)[f]	n.o.	1.6
5	**4a**	0	$H_2C{=}CHCH_2$	CN	5 min	(*R*)-**4a** (51) (4.5)	(*S*)-**5a** (44) (6.2)	n.o.	1.2
6	**4b**	0	$HC{\equiv}CCH_2$	CN	5 min	**4b** (42) (0)	(*S*)-**5b** (56) (6.4)	n.o.	–
7	**2a**	1	$H_2C{=}CHCH_2$	$CONH_2$	5.3 h	–	(*R*)-**2a** (44) (>99.5)[f]	(*S*)-**3a** (50) (95.7)	>200
8	**2b**	1	$HC{\equiv}CCH_2$	$CONH_2$	14 h	–	(*R*)-**2b** (46) (> 99.5)	(*S*)-**3b** (50) (94.9)	>200
9	**2e**	1	$CH_3CH_2CH_2$	$CONH_2$	81 h	–	(*R*)-**2e** (47) (46.1)[f]	(*S*)-**3e** (49) (46.5)[f]	4.3

[a] Substrate (2 mmol) was incubated with a suspension of *R. 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] Enantiomeric ratio (*E*) was calculated according to ref 12. [e] Product was not observed. [f] Determined by chiral GC analysis. [g] The reaction was carried out at 20 °C.

proceeded extremely fast, and ca. 50% conversion of nitrile was completed during 1−10 min irrespective of the structures (entries 1−6, Table 4). No appreciable effect of either the unsaturated functionality or the steric crowdedness was observed on the activity and enantioselectivity of the nitrile hydratase. In contrast, the efficiency of the kinetic resolution of amide was drastically influenced by the structure of the substrate. Racemic amides having an allyl (**2a**) or a propargyl (**2b**) substituent, for example, led to the rapid hydrolysis (entries 7 and 8). For propyl-substitued 2-phenylpropanamide **2e**, on the contrary, more than 3 days was required to yield effective kinetic resolution (entry 9, Table 4). Most noticeably, all enzymatic nitrile hydration reactions gave products with very low enantiomeric excess values regardless of whether the substrates **1** and **4** contained an unsaturated or a saturated substituent (entries 1−6, Table 4). However, a huge difference in enantiocontrol was evidenced in the amidase-catalyzed hydrolysis between amides substituted by an unsaturated and a saturated group (entries 7−9). This has been clearly exemplified by the kinetic resolution of allyl- (**2a**) and propargyl- (**2b**) substituted amides which gave excellent enantioselectivity with an enantiomeric ratio (*E*)[12] of >200 (entries 7 and 8, Table 4), whereas the reaction of propyl-substitued 2-phenylpropanamide **2e** led to very low enantioselectivity ($E = 4.3$) (entry 9, Table 4).

The outcomes of highly efficient nitrile hydration reactions with very low enantioselectivity from current study are in agreement with almost all previous observations that nitrile hydratases are a type of highly active and less selective enzyme. These properties of the nitrile hydratase, such as having a broad substrate spectrum and possessing low or none enantioselectivity, are determined intrinsically by its enzyme structure in which there is a spacious pocket near the active site.[13] Except for nitrile **7a** (entry 1, Table 3), electronic and steric differences among all nitriles **1a**−**e**, **4a**−**c**, and **7b** were not recognized or differentiated by the nitrile hydratase, and almost identical biocatalytic hydration reactions therefore resulted (entries 1−6, Table 4).

The high sensitivity of the amidase toward the structure of amides is intriguing. To interpret the biocatalysis of various racemic amides, especially the amides bearing a three-membered ring, amidase of a deep buried and highly steric demanding active site has been proposed.[8−10] This hypothetic amidase model can best explain its different enzymatic efficiencies toward different amides **2**, **5**, and **8**. The larger the steric hindrance, the slower the amide hydrolysis. With the increase of the steric crowdedness around the α-stereocenter in the order of **2**, **5**, and **8**, the amidase-catalyzed hydrolysis proceeded in a decreasing order (Tables 2 and 3). However, the rationalization for the remarkably different catalytic efficiencies and enanti-

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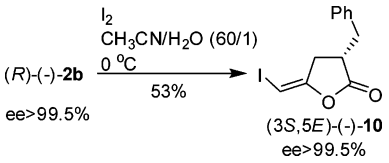
oselectivities of the amidase displayed for unsaturated and saturated nitrile substrates remains challenging at this stage because of the lack of a molecular structure of the amidase. Since the allyl, propargyl, and allenyl are not considerably different from propyl in terms of the van der Waals volume,[14] the great difference in reaction velocity and enantioselectivity between unsaturated three-carbon substituents and propyl is most likely due to the electronic effect. The more efficient reaction of the allyl (49 Å^3) substituted substrate than the propargyl (47.8 Å^3) substituted one (entries 1 and 2 in Table 2, and entries 7 and 8 in Table 4) also suggested the electronic effect. If the steric effect played an important role, the propargyl substituted substrates would exceed allyl substituted analogues because the propargyl group (47.8 Å^3) is slightly smaller than the allyl group (49 Å^3).[14] The enhancement of enantioselectivity was also observed previously in the biotransformations of 3-arylpent-4-enecarboxamides[7d] and α-allyl-α-methylmalononitrile,[4e] the substrates that contain a vinyl substituent. The preferential and highly enantioselective hydrolysis of unsaturated amide substrates over the saturated one strongly implies that the amidase involved in *R. erythropolis* AJ270 may have another domain for hydrophobic interaction. This binding domain, which is slightly away from the site where the amide is susceptible for hydrolysis, seems capable of discriminating the substituents of electronic difference, such as between saturated and unsaturated three-carbon segments. As a consequence, in addition to the interaction between the active site of the amidase and the amido and/or aryl moieties of the substrate, the selective binding of an unsaturated carbon−carbon bond with the specific binding domain of the enzyme may contribute greatly to the acceleration of the reaction and to the drastic improvement of the enantioselectivity.

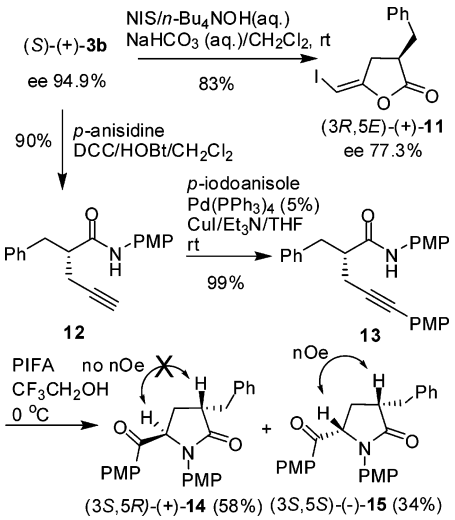
The last but not least issue that needs addressing is the enantioselection of both the nitrile hydratase and amidase against their substrates. As summarized in Table 4, regardless of the *R*- and *S*-designations, the amide products preferentially formed from the nitrile hydratase-catalyzed kinetic resolution of nitriles (entries 1−6, Table 4) had the same stereochemistry as the acid products derived from the kinetic resolution of amides catalyzed by the amidase (entries 7−9, Table 4). This shows that it is the sequential actions of the less enantioselective nitrile hydratase and the highly enantioselective amidase involved in *R. erythropolis* AJ270 that account for the excellent enantioselectivity of the overall nitrile biotransformations (Tables 1−3).

As multifunctional molecules, highly enantiopure carboxylic acids and amides obtained from the current nitrile biotransformation study are useful chiral intermediates. On the basis of chemical manipulations, the synthesis of various chemical entities is feasible. Our interest in optically active heterocyclic compounds of synthetic and biological significance has led us to explore their potential in the preparation of enantiopure lactones and lactams. 3-Alkyl-5-halomethylene-γ-lactones and 3-alkyl-5-alkynylmethylene-γ-lactones are known as haloenol lactones and ynenol lactones, respectively. The haloenol lactones are the precursors to ynenol lactones through the coupling reaction with alkynes. The biochemical studies have shown that haloenol lactones[15] and ynenol lactones[16] are potent inactivators

SCHEME 1. Synthesis of Enantiopure γ-Lactone from Iodolactonization of Amide (*R*)-(−)-2b



SCHEME 2. Synthesis of Enantiopure γ-Lactams



of glutathione *S*-transferase and serine proteases, respectively. Interestingly, no effect of chirality of the compounds on the biochemical property was investigated. Treatment of enantiopure 2-benzylpent-4-ynamide (*R*)-(−)-**2b** with iodine in a mixture of acetonitrile and water (60:1) at 0 °C afforded (3*S*,5*E*)-3-benzyl-5-(iodomethylene)dihydrofuran-2(3*H*)-one **10** as the sole product in 53% (Scheme 1). It should be pointed out that no iodolactonization of 2-benzylpent-4-ynoic acid (*S*)-(−)-**3b** took place under the identical conditions. Following an extensively used method[14] by employing *N*-iodosuccinimide (NIS), iodolactonization of acid (*S*)-(−)-**3b** (ee 94.9%) did give the desired product **11**. However, partial racemization of the product **11** (ee 77.3%) occurred inevitably because of the basic reaction condition (Scheme 2). For the preparation of optically active γ-lactams, the fundamental skeleton for pyrrolidine alkaloids, acid (*S*)-(−)-**3b** was first converted into the amide **13** via intermediate **12** followed by the Sonogashira cross-coupling reaction.[17] Using the method developed recently by Telitu and Domonguez and their co-workers,[18] amide compound **13** was transformed into (3*S*,5*R*)-3-benzyl-5-(4-methoxybenzoyl)-1-(4-methoxyphenyl)pyrrolidin-2-one **14** (58%) and (3*S*,5*S*)-3-benzyl-5-(4-methoxybenzoyl)-1-(4-methoxyphenyl)pyrrolidin-2-one **15** (34%) when treated with a hypervalent iodine reagent phenyliodine(III)bistrifluoroacetate (PIFA) in trifluoroethanol. The relative stereochemistry of the products **14** and **15** was assigned by means of nuclear Overhauser effect (Scheme 2).

Racemic 3-alkyl-1-aryl-3,4-dihydro-1*H*-quinolin-2-one derivatives have been discovered very recently as potent and

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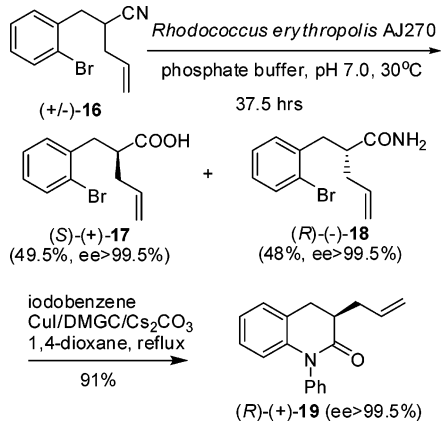
SCHEME 3. Synthesis of Enantiopure Dihydroquinolin-2-one



selective norepinephrine reuptake inhibitors by Lilly.[19] We envisioned that chemoenzymatic synthesis involving nitrile biotransformations would provide a unique approach to enantiomerically pure 3-funcationalized 1-aryl-3,4-dihydro-1*H*-quinolin-2-ones, and therefore it would allow the development of chiral drugs for depression and attention-deficit/hyperactivity disorder. Starting with the biotransformations of racemic 2-(2-bromobenzyl)pent-4-enenitrile (±)-**16** using the *R. erythropolis* AJ270 whole cell catalyst, (*R*)-2-(2-bromobenzyl)pent-4-enamide **18** was obtained in an almost quantitative yield with ee of >99.5%. Catalyzed by the CuI/*N*,*N*-dimethylglycine catalyst,[20] intra- and intermolecular *N*-arylation reactions of (*R*)-(−)-**18** in the presence of iodobenzene were conducted in a one-pot operation to yield, without racemization, (*R*)-3-allyl-1-phenyl-3,4-dihydro-1*H*-quinolin-2-one **19** in 91% yield (Scheme 3). It is noteworthy that the enantiomer of (*R*)-(+)-**19** might be easily available when the other nitrile biotransformation product, namely, (*S*)-2-(2-bromobenzyl)pent-4-enoic acid (*S*)-(+)-**17** is used. The allyl substituent in the product would serve as a key functional group for further chemical elaborations.

Conclusion

In summary, we have shown the remarkable steric and electronic effects in nitrile biotransformations catalyzed by the nitrile hydratase/amidase containing *R. erythropolis* AJ270 microbial cell catalyst. The nitrile hydratase involved in *R. erythropolis* AJ270 exhibits high catalytic efficiency but very low enantioselectivity against almost all nitrile substrates studied. The amidase, on the contrary, is very sensitive toward the structure of the amides. Increase of the steric crowdedness around the stereocenter of the substrates gives rise to the noticeable decrease of the reaction rate. In addition, hydrolysis of amides bearing an unsaturated carbon−carbon bond proceeds much faster than that of analogous amide substituted by a saturated alkyl moiety. Moreover, the replacement of an unsaturated carbon−carbon bond substituent such as an allyl, propargyl, or allenyl by a saturated propyl group in substrate results in a considerably diminished enantioselectivity of the amide hydrolysis. The outcomes imply a possible binding domain of the amidase for 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 dramatic enhancement of the enantioselectivity. Nitrile biotransformations provide a unique and high-yielding synthetic route to highly enantiopure carboxylic acids and amides functionalized with an allyl, propargyl, allenyl, or vinyl group. The synthetic applications of the nitrile biotransformations have been demonstrated by the conversion of the functionalized acid and amide products into the enantiopure heterocyclic compounds including iodoenol γ-lactone, γ-lactam, and 3-allyl-1-phenyl-3,4-dihydro-1*H*-quinolin-2-one derivatives using convenient chemical transformations.

Experimental Section

General Procedure for the Biotransformations of Nitriles or Amides. To an Erlenmeyer flask (150 mL) with a screw cap were added *Rhodococcus* sp. AJ270 cells[5] (2 g wet weight), which were prepared in bulk from a 5 L fermentor using acetamide as both the carbon and nitrogen source and stored at −20 °C in a freezer,[1c] 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. Racemic nitriles or amide (2 mmol) 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 and HPLC, was quenched after a specified period of time (see Tables 1−4) by removing the biomass through a Celite pad filtration. The resulting aqueous solution was adjusted to pH 12 with aqueous NaOH solution (2 N) and extracted with ethyl ether (3 × 50 mL). After drying ($MgSO_4$) and after the solvent was removed under vacuum, the residue was subjected to a silica gel column using a mixture of petroleum ether and ethyl acetate as the mobile phase to give pure amide product and, in some case, the recovered nitrile. The aqueous phase was then adjusted to pH 2 with hydrochloric acid (2 N) and extracted with ethyl ether (3 × 50 mL). The organic phase was combined. After drying ($MgSO_4$) and after the solvent was removed under vacuum, the residue was chromatographed on a silica gel column using a mixture of petroleum ether and ethyl acetate as an eluant to give pure acid product. The structures of all products were fully characterized by spectroscopic data and microanalyses. The absolute configurations of amide and acid products were determined by reducing the unsaturated carbon−carbon bond into the saturated one and then by comparing the directions of their optical rotations with that of the authentic samples (see Supporting Information). The ee values were determined using HPLC and GC analyses using chiral stationary phase (see Supporting Information).

Biotransformations of racemic 2-benzylpent-4-ynenitrile **1b** gave (*R*)-(−)-2-benzylpent-4-ynamide (*R*)-(−)-**2b** and (*S*)-(−)-2-benzylpent-4-ynoic acid (*S*)-(−)-**3b**. (*R*)-(−)-**2b:** mp 76−77 °C; $[\alpha_D^{25}] = -28.8$ (*c* 2.5, CH_2Cl_2); ee >99.5% (HPLC analysis); ^{1}H NMR ($CDCl_3$/300 MHz) δ 7.33−7.20 (m, 5H), 5.45 (br s, 2H, $CONH_2$), 3.01−2.87 (m, 2H), 2.67−2.58 (m, 1H), 2.55−2.37 (m, 2H), 2.09 (t, 1H, *J* = 2.6 Hz); ^{13}C NMR (75 MHz/$CDCl_3$) δ 175.5, 138.8, 129.0, 128.6, 126.7, 81.8, 70.6, 47.9, 37.8, 21.2; IR (KBr) 3446, 3223 ($CONH_2$), 3309 (≡C−H), 1656 cm^{-1}; MS (EI) *m*/*z* 187 (M^+, 5%), 148 (100), 131 (47), 91 (40), 28 (69). Anal. Calcd. for $C_{12}H_{13}NO$: C, 76.98; H, 7.00; N, 7.48. Found: C, 76.92; H, 6.99; N, 7.48. (*S*)-(−)-**3b**: oil; $[\alpha_D^{25}] = -12.0$ (*c* 2.0, $CHCl_3$); ee 94.5% (HPLC analysis) [lit.[21] $[\alpha_D^{26}] = -10.9$ (*c* 1.24, $CHCl_3$); ee 96%]; ^{1}H NMR ($CDCl_3$/300 MHz) δ 10.66 (br s, 1H, COOH), 7.26−7.13 (m, 5H), 3.07−3.00 (m, 1H), 2.93−2.89 (m, 1H), 2.86−2.77 (m, 1H), 2.39−2.33 (m, 2H), 2.00 (t, 1H, *J* = 2.6 Hz); ^{13}C NMR (75 MHz/$CDCl_3$) δ 179.5, 138.0, 129.1, 128.6, 126.8, 80.8, 70.7, 45.8, 36.3, 20.0; IR (KBr) 3411−2882 (br, COOH), 1713 cm^{-1};

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MS (EI) *m*/*z* 188 (M^+, 1%), 143 (47), 91 (100). Anal. Calcd. for $C_{12}H_{12}O_2$: C, 76.57; H, 6.43. Found: C, 76.56; H, 6.46.

Synthesis of (3*S*,5*E*)-3-Benzyl-5-(iodomethylene)dihydrofuran-2(3*H*)-one 10 from Iodolactonization of (*R*)-(−)-2-Benzylpent-4-ynamide (*R*)-(−)-2b. To a mixture of (*R*)-(−)-**2b** (120 mg, 0.642 mmol) in acetonitrile (6.48 mL) and water (108 μL), which was cooled in an ice-bath, was added iodine (326.1 mg, 2 equiv). After the mixture was stirred for 2 h, water (9 mL) was added, and the mixture was kept stirring for another 10 min. The saturated aqueous Na_2SO_3 solution was added until the purple color of the mixture vanished. Extraction with ethyl acetate (3 × 50 mL), drying over with anhydrous $MgSO_4$, and concentration under vacuum gave a residue. Chromatography on a silica gel column using a mixture of petroleum ether and ethyl acetate (35:1) as an eluant afforded pure **10** (107 mg, 53%): oil; $[\alpha_D^{25}] = -32.7$ (*c* 3.0, $CHCl_3$); ee >99.5% (HPLC analysis); ^{1}H NMR ($CDCl_3$/300 MHz) δ 7.29−7.11 (m, 5H), 5.67 (t, 1H, $J = 2.3$ Hz), 3.19−3.07 (m, 2H), 2.89−2.74 (m, 2H), 2.52−2.49 (m, 1H); ^{13}C NMR (75 MHz/$CDCl_3$) δ 176.1, 153.2, 137.1, 128.92, 128.89, 127.2, 53.5, 41.7, 36.5, 32.9; IR (KBr) 1802, 1656 cm^{-1}; MS (EI) *m*/*z* 315 ($M^+ + 1$, 8), 314 (M^+, 100%), 187 (94), 145 (66), 117 (88), 91 (64). Anal. Calcd. for $C_{12}H_{11}IO_2$: C, 45.88; H, 3.53. Found: C, 45.52; H, 3.73.

Synthesis of (3*S*,5*R*)-3-Benzyl-5-(4-methoxybenzoyl)-1-(4-methoxyphenyl)pyrrolidin-2-one 14 and (3*S*,5*S*)-3-Benzyl-5-(4-methoxybenzoyl)-1-(4-methoxyphenyl)pyrrolidin-2-one 15. A solution of DCC (0.525 mmol) and HOBT (0.525 mmol) in CH_2Cl_2 (1 mL) was added to the solution of 2-benzylpent-4-ynoic acid (*S*)-(−)-**3b** (94 mg, 0.5 mmol) in CH_2Cl_2 (1 mL) while cooling in an ice bath. *p*-Anisidine (92.3 mg, 0.75 mmol) solution in CH_2Cl_2 (1 mL) was then added, and the resulting mixture was stirred at room temperature for 24 h. A saturated ammonium chloride solution (10 mL) was then added, and the organic phase was separated. The aqueous phase was extracted with CH_2Cl_2 (3 × 20 mL), and the organic phase was combined and dried over with anhydrous $MgSO_4$. After removal of the solvent, the residue was subjected to silica gel column chromatography with a mixture of petroleum ether and ethyl acetate (4:1) as the eluant to give **12** (131.9 mg, 90%): mp 123−124 °C; $[\alpha_D^{25}] = +54.7$ (*c* 1.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$/300 MHz) δ 7.32−7.20 (m, 7H), 6.97 (br s, 1H, CONH), 6.80 (d, 2H, $J = 8.9$ Hz), 3.76 (s, 3H), 3.06−2.96 (m, 2H), 2.70−2.46 (m, 3H), 2.10−2.09 (m, 1H); ^{13}C NMR (75 MHz/$CDCl_3$) δ 171.3, 156.6, 139.0, 130.4, 129.0, 128.7, 126.7, 122.2, 114.1, 81.9, 70.7, 55.5, 49.5, 38.2, 21.5; IR (KBr) 3430, 3297, 1652 cm^{-1}; MS (EI) *m*/*z* 295 ($M^+ + 2$, 2), 294 ($M^+ + 1$, 13), 293 (M^+, 76%), 254 (63), 123 (100). Anal. Calcd. for $C_{19}H_{19}NO_2$: C, 77.79; H, 6.53; N, 4.77. Found: C, 77.43; H, 6.65; N, 4.82.

Under argon protection, a mixture of **12** (59 mg, 0.2 mmol), *p*-iodoanisole (71 mg, 0.3 mmol), $Pd(PPh_3)_4$ (11.6 mg, 5 mol %), CuI (15.4 mg, 0.08 mmol), and Et_3N (67.1 μL, 0.5 mmol) in dry THF (1 mL) was stirred at room temperature for 1 h. After filtration through a short silica gel (100−200 mesh) pad, the filtrate was concentrated under vacuum and subjected to a silica gel column chromatography using a mixture of petroleum ether and ethyl acetate (3:1) as the mobile phase. Compound **13** (79.3 mg, 99%) was obtained as brown solids: mp 135−136 °C; $[\alpha_D^{25}] = -29.0$ (*c* 2.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$/300 MHz) δ 7.32−7.20 (m, 9H), 7.09 (br s, 1H, CONH), 6.80 (dd, 4H, $J = 8.8$, 2.0 Hz), 3.80 (s, 3H), 3.77 (s, 3H), 3.13−3.00 (m, 2H), 2.81−2.67 (m, 3H); ^{13}C NMR (75 MHz/$CDCl_3$) δ 171.6, 159.4, 156.6, 139.2, 133.0, 130.6, 129.0, 128.7, 126.6, 122.1, 115.4, 114.09, 113.95, 85.7, 82.9, 55.5, 55.3, 49.9, 38.2, 22.5; IR (KBr) 3318, 1653 cm^{-1}; MS (EI) *m*/*z* 401 ($M^+ + 2$, 1), 400 ($M^+ + 1$, 6), 399 (M^+, 20%), 308 (100). HRMS for $C_{26}H_{25}NO_3$: 399.1834. Found: 399.1830.

To a solution of **13** (60 mg, 0.15 mmol) in CF_3CH_2OH (1.4 mL), which was cooled in an ice-bath, was added dropwise a solution of PIFA (95 mg, 0.22 mmol) in CF_3CH_2OH (1.6 mL). After being stirred at 0 °C for another 80 min, an aqueous solution of Na_2CO_3 (10%) was added, and the resulting mixture was extracted with dichloromethane (3 × 10 mL). The combined organic phase was washed with brine (20 mL) and dried over with anhydrous $MgSO_4$. The solvent was removed, and the residue was subjected to silica gel column chromatography using a mixture of petroleum ether and ethyl acetate (11:4 − 2:1) to give pure (3*S*,5*R*)-3-benzyl-5-(4-methoxybenzoyl)-1-(4-methoxyphenyl)pyrrolidin-2-one **14** (36.1 mg, 58%) and (3*S*,5*S*)-3-benzyl-5-(4-methoxybenzoyl)-1-(4-methoxyphenyl)pyrrolidin-2-one **15** (21.3 mg, 34%). **14**: mp 148−150 °C; $[\alpha_D^{25}] = +15.0$ (*c* 2.0, C_6H_6); ^{1}H NMR ($CDCl_3$/300 MHz) δ 7.87 (d, 2H, $J = 8.8$ Hz), 7.35−7.20 (m, 7H), 6.93 (d, 2H, $J = 8.8$ Hz), 6.83 (d, 2H, $J = 8.9$ Hz), 5.34 (dd, 1H, $J = 9.6$, 1.8 Hz), 3.86(s, 3H), 3.75 (s, 3H), 3.34 (dd, 1H, $J = 13.9$, 4.2 Hz), 3.07−2.96 (m, 1H), 2.80 (dd, 1H, $J = 13.7$, 9.2 Hz), 2.34−2.23 (m, 1H), 2.17−2.07 (m, 1H); ^{13}C NMR (75 MHz/$CDCl_3$) δ 194.6, 175.4, 164.2, 157.2, 139.0, 131.6, 130.7, 129.1, 128.5, 126.9, 126.5, 124.1, 114.2, 62.1, 55.6, 55.4, 42.6, 36.9, 29.9; IR (KBr) 1688 cm^{-1}; MS (EI) *m*/*z* 416 ($M^+ + 1$, 2), 415 (M^+, 6%), 280 (100). Anal. Calcd. for $C_{26}H_{25}NO_4$: C, 75.16; H, 6.06; N, 3.37. Found: C, 74.96; H, 6.13; N, 3.68. **15**: oil; $[\alpha_D^{25}] = -30.4$ (*c* 2.0, C_6H_6); ^{1}H NMR ($CDCl_3$/300 MHz) δ 7.88 (d, 2H, $J = 8.9$ Hz), 7.35 (d, 2H, $J = 9.0$ Hz), 7.23−7.14 (m, 5H), 6.92 (d, 2H, $J = 8.9$ Hz), 6.84 (d, 2H, $J = 9.0$ Hz), 5.58 (dd, 1H, $J = 8.7$, 6.7 Hz), 3.87(s, 3H), 3.75 (s, 3H), 3.44 (dd, 1H, $J = 14.0$, 3.9 Hz), 3.07−2.96 (m, 1H), 2.72 (dd, 1H, $J = 13.8$, 11.3 Hz), 2.62−2.52 (m, 1H), 1.87−1.78 (m, 1H); ^{13}C NMR (75 MHz/$CDCl_3$) δ 195.3, 175.3, 164.2, 157.3, 139.3, 131.4, 130.7, 128.9, 128.6, 127.3, 126.4, 124.3, 114.2, 62.3, 55.6, 55.4, 44.3, 37.6, 29.4; IR (KBr) 1690 cm^{-1}; MS (EI) *m*/*z* 416 ($M^+ + 1$, 2), 415 (M^+, 7%), 280 (100). HRMS for $C_{26}H_{25}NO_4$: 415.1784. Found: 415.1786.

Synthesis of (*R*)-3-Allyl-1-phenyl-3,4-dihydro-1*H*-quinolin-2-one 19. Under argon protection, a mixture of (*R*)-2-(2-bromobenzyl)pent-4-enamide **18** (ee > 99.5%) (80 mg, 0.3 mmol), CuI (22.9 mg, 0.12 mmol), DMGC (33.5 mg, 0.24 mmol), Cs_2CO_3 (97.7 mg, 0.3 mmol), and iodobenzene (50.4 μL, 0.45 mmol) in dry 1,4-dioxane (12 mL) was gently refluxed for 19 h. After filtration through a short silica gel (100−200 mesh) pad, the filtrate was concentrated under vacuum. The residue was chromatographed on a silica gel column eluted with a mixture of petroleum ether and ethyl acetate (12:1) to give pure **19** (72 mg, 91%) as white solids: mp 77−78 °C; $[\alpha_D^{25}] = +20.0$ (*c* 1.5, $CHCl_3$); ee >99.5% (HPLC analysis); ^{1}H NMR ($CDCl_3$/300 MHz) δ 7.53−7.41 (m, 3H), 7.23−7.19 (m, 3H), 7.05−6.98 (m, 2H), 6.34 (d, 1H, $J = 8.0$ Hz), 5.95−5.81 (m, 1H), 5.14−5.08 (m, 2H), 3.13−3.07 (m, 1H), 2.93−2.70 (m, 3H), 2.31−2.26 (m, 1H); ^{13}C NMR (75 MHz/$CDCl_3$) δ 171.8, 141.3, 138.7, 135.5, 129.8, 129.0, 128.10, 128.06, 127.1, 124.8, 122.9, 117.4, 116.8, 40.6, 34.0, 30.4; IR (KBr) 1676 cm^{-1}; MS (EI) *m*/*z* 265 (M^++2, 2), 264 (M^++1, 16), 263 (M^+, 100%), 180 (50). Anal. Calcd. for $C_{18}H_{17}NO$: C, 82.10; H, 6.51; N, 5.32. Found: C, 82.45; H, 6.57; N, 5.52.

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Supporting Information Available: Preparation of all starting nitriles, spectroscopic data of all products, ^{1}H and ^{13}C NMR spectra of **2**, **3**, **5**, **6**, **8**, **9**, **10**−**15**, and **17**−**19**, HPLC analysis of all chiral products. This material is available free of charge via the Internet at http://pubs.acs.org.

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