JOC*Note*

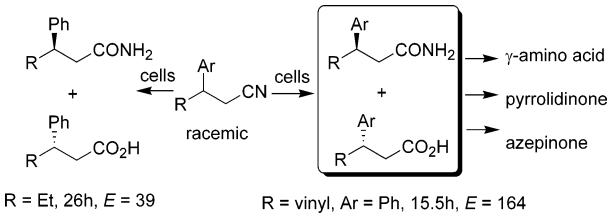
An Unusual β-Vinyl Effect Leading to High Efficiency and Enantioselectivity of the Amidase, Nitrile Biotransformations for the Preparation of Enantiopure 3-Arylpent-4-enoic Acids and Amides and Their Applications in Synthesis

Ming Gao, De-Xian Wang, Qi-Yu Zheng, 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

Received August 10, 2006



Biotransformations of 3-arylpent-4-enenitriles catalyzed by *Rhodococcus erythropolis* AJ270, a nitrile hydratase/amidase-containing microbial whole-cell catalyst were studied, and an unusual β-vinyl effect of the substrate on the biocatalytic efficiency and enantioselectivity of the amidase was observed. While 3-arylpent-4-enenitriles and 3-phenylpentanenitrile were efficiently hydrated by the action of the less *R*-enantioselective nitrile hydratase, the amidase showed greater activity and higher enantioselectivity against 3-arylpent-4-enoic acid amides than 3-arylpentanoic acid amides. Under very mild conditions, nitrile biotransformations provided an efficient synthesis of highly enantiopure (*R*)-3-arylpent-4-enoic acids and (*S*)-3-arylpent-4-enoic acid amides, and their applications were demonstrated by the synthesis of chiral γ-amino acid, 2-pyrrolidinone, and 2-azepinone derivatives.

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 effective and environmentally benign methods for the production of carboxylic acids and their amide derivatives.[1] One of the well-known examples is the industrial production of acrylamide from biocatalytic hydration of acrylonitrile.[2] 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.[3,4] The distinct features of enzymatic transformations of nitriles are the straightforward generation of enantiopure amides, valuable organonitrogen compounds in synthetic chemistry, in addition 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 nitriles including α-aminonitriles,[6] α-alkyl-[7] and α-allyl-substituted arylacetonitriles,[8] cyclopropanecarbonitriles,[9] and oxiranecarbonitriles[10] into the corresponding useful polyfunctionalized chiral carboxylic acids and amides. In contrast to the successful enantioselective nitrile biotransformations for the preparation of chiral carboxylic acids and amide derivatives bearing an α-stereocenter,[3−10] 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[11−15] except for some biocatalytic desymmtrization reactions of 3-substituted glutoranitrile derivatives.[16] For instance, biotransformations of the Baylis−Hillman nitriles[11] and its one-carbon homologated nitriles[12] gave moderate enantioselectivity whereas β-phenylbutyronitrile,[13] β-,[14] γ-, or δ-hydroxylated alkanenitriles[15] gave no or extremely low enantiocontrol. It is generally believed that the movement of a stereocenter from the reactive site (α-position to functional group) to a remote place gives rise to the decrease of enantioselectivity in asymmetric reactions. However, this notion may not be true for

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

(1) (a) Faber, K. *Biotransformations in Organic Chemistry*, 3rd ed.; Spring-Verlag: New York, 1997; pp 136−145. (b) Kobayashi, M.; Shimizu, S. *FEMS Microbiol. Lett.* **1994**, *120*, 217 and references therein. (c) Meth-Cohn, O.; Wang, M.-X. *J. Chem. Soc., Perkin Trans. 1* **1997**, 1099. (d) Meth-Cohn, O.; Wang, M.-X. *J. Chem. Soc., Perkin Trans. 1* **1997**, 3197 and references therein.

(2) Nagasawa, T.; Schimizu, H.; Yamada, H. *Appl. Microbiol. Biotechnol.* **1993**, *40*, 189.

(3) For reviews, see: (a) Sugai, T.; Yamazaki, T.; Yokoyama, M.; Ohta, H. *Biosci. Biotech. Biochem.* **1997**, *61*, 1419 and references therein. (b) Martinkova, L.; Kren, V. *Biocatal. Biotrans.* **2002**, *20*, 73. (c) Wang, M.-X. *Top. Catal.* **2005**, *35*, 117.

(4) For recent examples, see: (a) DeSantis, G.; Zhu, Z.; Greenberg, W. A.; Wong, K.; Chaplin, J.; Hanson, S. R.; Farwell, B.; Nicholson, L. W.; Rand, C. L.; Weiner, D. P.; Robertson, D. E.; Burk, M. J. *J. Am. Chem. Soc.* **2002**, *124*, 9024. (b) Effenberger, F.; Oβwald, S. *Tetrahedron: Asymmetry* **2001**, *12*, 279. (c) Hann, E. C.; Sigmund, A. E.; Fager, S. K.; Cooling, F. B.; Gavagan, J. E.; Ben-Bassat, A.; Chauhan, S.; Payne, M. S.; Hennessey, S. M.; DiCosimo, R. *Adv. Synth. Catal.* **2003**, *345*, 775. (d) Preiml, M.; Hillmayer, K.; Klempier, N. *Tetrahedron Lett.* **2003**, *44*, 5057. (e) Yokoyama, M.; Kashiwagi, M.; Iwasaki, M.; Fushuku, K.; Ohta, H.; Sugai, T. *Tetrahedron: Asymmetry* **2004**, *15*, 2817.

(5) (a) Blakey, A. J.; Colby, J.; Williams, E.; O'Reilly, C. *FEMS Microbiol. Lett.* **1995**, *129*, 57. (b) Colby, J.; Snell, D.; Black, G. W. *Monatsh. Chem.* **2000**, *131*, 655. (c) O'Mahony, R.; Doran, J.; Coffey, L.; Cahill, O. J.; Black, G. W.; O'Reilly, C. *Antonie van Leeuwenhoek* **2005**, *87*, 221.

(6) (a) Wang, M.-X.; Lin, S.-J. *J. Org. Chem.* **2002**, *67*, 6542. (b) Wang, M.-X.; Lin, S.-J.; Liu, J.; Zheng, Q.-Y. *Adv. Synth. Catal.* **2004**, *346*, 439.

(7) (a) Wang, M.-X.; Lu, G.; Ji, G.-J.; Huang, Z.-T.; Meth-Cohn, O.; Colby, J. *Tetrahedron: Asymmetry* **2000**, *11*, 1123. (b) Wang, M.-X.; Li, J.-J., Ji, G.-J.; Li, J.-S. *J. Mol. Cat. B: Enzym.* **2001**, *14*, 77.

(8) (a) Wang, M.-X.; Zhao, S.-M. *Tetrahedron Lett.* **2002**, *43*, 6617. (b) Wang, M.-X.; Zhao, S.-M. *Tetrahedron: Asymmetry* **2002**, *13*, 1695.

(9) (a) Wang, M.-X.; Feng, G.-Q. *Tetrahedron Lett.* **2000**, *41*, 6501. (b) Wang, M.-X.; Feng, G.-Q. *New J. Chem.* **2002**, 1575. (c) Wang, M.-X.; Feng, G.-Q. *J. Org. Chem.* **2003**, *68*, 621−624. (d) Wang, M.-X.; Feng, G. Q. *J. Mol. Catal. B: Enzym.* **2002**, *18*, 267. (e) Wang, M.-X.; Feng, G.-Q.; Zheng, Q.-Y. *Adv. Synth. Catal.* **2003**, *345*, 695; (f) Wang, M.-X.; Feng, G.-Q.; Zheng, Q.-Y. *Tetrahedron: Asymmetry* **2004**, *15*, 347.

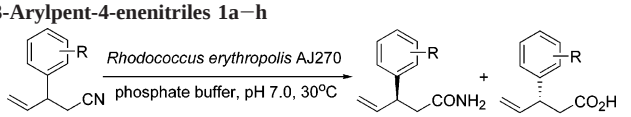
(10) (a) Wang, M.-X.; Lin, S.-J.; Liu, C.-S.; Zheng, Q.-Y.; Li, J.-S. *J. Org. Chem.* **2003**, *68*, 4570. (b) Wang, M.-X.; Deng, G.; Wang, D.-X. Zheng, Q.-Y. *J. Org. Chem.* **2005**, *70*, 2439.

10.1021/jo061664f CCC: $33.50
Published on Web 11/11/2006

enzymatic reactions since the chiral recognition site of an enzyme might be located in some distance to the catalytic center. In other words, the chiral recognition between the enzyme and a substrate might occur at a remote pocket from the reaction site. If this hypothesis works, it could lead to highly enantioselective biocatalytic reactions of the substrates that bear a remotely positioned chiral center. Very recently, we[17] have shown that a simple *O*-benzyl protection group on β-hydroxy nitriles acted probably as a docking group to enhance the chiral recognition by the enzymes and therefore resulted in the dramatic increase of enantioselectivity of biocatalysis. To further test this hypothetic remote chiral recognition mechanism, and also to explore the application of nitrile biotransformations in the synthesis of enantiopure valuable and polyfunctionalized intermediates, we undertook the investigation of biotransformation of 3-arylpent-4-enenitriles. Different from the previously used protection/docking strategy,[17,18] we introduced a vinyl substituent into the β-position of 3-arylpropionitriles, hoping that such an intrinsic β-functional moiety might increase the substrates' affinity and chiral interactions with the enzymes and therefore lead to highly efficient and enantioselective biotransformations. Gratifyingly, the presence of a β-vinyl group within the nitrile substrates plays indeed a significant role in the improvement of biocatalytic efficiency and enantioselectivity. Herein, we report the highly enantioselective biotransformations of 3-arylpent-4-enenitriles and an unusual β-vinyl substituent effect on the activity and selectivity of the amidase involved in the *R. erythropolis* AJ270 strain. The applications of the biotransformation products in the synthesis of chiral γ-amino acid and chiral *N*-heterocyclic compounds will also be discussed.

Initially, we tested the biocatalytic hydrolysis of racemic 3-phenylpent-4-enenitrile **1a** in the presence of *R. erythropolis* AJ270 whole cells. Under the very mild conditions, **1a** was found to undergo efficient reaction to yield optically active carboxylic acid **2a** and amide **3a**. The nitrile hydratase catalyzed hydration was very fast, and the complete conversion of nitrile **1a** (40 mM) into the amide **2a** was effected within several hours, while the hydrolysis of amide **2a** into acid **3a**, with the aid of the amidase, took a slightly longer period (entries 1 and 2, Table 1). Excellent chemical yields and enantiomeric excess values were obtained for the (*S*)-3-phenylpent-4-enoic acid amide **2a** and (*R*)-3-phenylpent-4-enoic acid **3a** after 15.5 h incubation with the cell catalyst (entry 3, Table 1). Under identical conditions, a more efficient and highly enantioselective biotransformation was observed when the concentration of **1a** was halved (entry 4, Table 1).

TABLE 1. Enantioselective Biotransformations of Racemic 3-Arylpent-4-enenitriles 1a−h



1a-h (*S*)-amide **2-h** (*R*)-acid **3-h**

				2		**3**	
entry	**1**	R	reaction conditions[a] (mmol, h)	yield[b] (%)	ee[c] (%)	yield[b] (%)	ee[c] (%)
1	**1a**	H	2, 0.5[d]	74.5	0	3	98.8
2	**1a**	H	2, 9	72	44.1	26	98.4
3	**1a**	H	2, 15.5	48	95.2	47	95.4
4	**1a**	H	1, 6.5	50	90.5	46	94.6
5	**1a**	H	13, 24.3[e]	45	>99.5	49	92.5
6	**1b**	4-OMe	1, 9	49	>99.5	50	90.0
7	**1c**	4-Me	1, 10.5	49	94.9	50	95.0
8	**1d**	4-F	1, 4.75	50.5	94.5	48	90.0
9	**1e**	4-Cl	1, 5.5	49	97.4	50	91.0
10	**1f**	3-Cl	1, 8.5	47	93.2	49	94.2
11	**1g**	2-Cl	1, 10[f]	25	>99.5	55	57.1
12	**1g**	2-Cl	0.5, 3.5	51	78.9	44	92.9
13	**1g**	2-Cl	0.5, 4	42	>99.5	55	77.3
14	**1h**	4-Br	1, 9	49	59.0	48	58.1

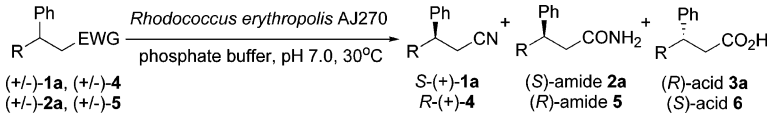
[a] Biotransformation was carried out in a suspension of *Rhodococcus erythropolis* AJ270 cells (2 g wet weight) in phosphate buffer (50 mL, pH 7.0) at 30 °C. [b] Isolated yield. [c] Determined by HPLC analysis using a chiral column (see the Supporting Information). [d] Nitrile **1a** (yield 18%, ee 15.8%) was recovered. [e] In this multigram-scale biotransformations, 14 g of wet weight cells was used. [f] Nitrile **1g** (yield 11%, ee 39.4%) was recovered.

To examine the scope of the reaction and the influence of the substituent attached on the benzene ring on the efficiency and enantioselectivity of biotransformations, a number of racemic 3-arylpent-4-enenitriles **1b**−**h** were subjected to incubation with *R. erythropolis* AJ270 (Table 1). As illustrated by the results compiled in Table 1, all substrates, irrespective of the nature of the substituent on the benzene ring, underwent efficient biotransformations within about 10 h to afford almost quantitative yields of the corresponding amide and acid products, except 3-(2-chlorophenyl)pent-4-enenitrile **1g**. which required a lower concentration to fulfill effective hydrolysis (entries 11−13). It indicates that only when the bulkiness of the benzene ring at the ortho position increased did hydrolysis proceed slowly. The steric effect, on the other hand, did not influence the enantioselectivity of the biotransformations, as almost all substrates gave excellent chemical yields of the products with high enantiomeric purity. The only exception is the hydrolysis of 3-(4-bromophenyl)pent-4-enenitrile **1h** which showed moderate enantiocontrol (entry 14, Table 1).

Encouraged by the highly efficient and enantioselective biotransformations of 3-arylpent-4-enenitriles, we then extended study to racemic 3-phenylpentanenitrile **4**, a substrate devoid only of a double bond compared to 3-phenyl-pent-4-enenitrile **1a**. To shed light on the stereochemical outcomes, and to examine the effect of the β-vinyl group on the biocatalytic

(11) Wang, M.-X.; Wu, Y. *Org. Biomol. Chem.* **2003**, *1*, 535.
(12) Zhao, S.-M.; Wang, M.-X. *Chin. J. Chem.* **2002**, *20*, 1291.
(13) Gradley, M. L.; Knowles, C. J. *Biotechnol. Lett.* **1994**, *16*, 41.
(14) (a) Klempier, N.; de Raat, A.; Faber, K.; Griengl, H. *Tetrahedron Lett.* **1991**, *32*, 341. (b) In the case of biotransformation of β-benzoyloxypentanenitrile, good enantioselectivity was obtained. Yokoyama, M.; Imai, N.; Sugai, T. Ohta, H. *J. Mol. Catal. B: Enzym.* **1996**, *1*, 135.
(15) Taylor, S. K.; Chmiel, N. H.; Simons, L. J.; Vyvyan, J. R. *J. Org. Chem.* **1996**, *61*, 9084.
(16) (a) Kakeya, H.; Sakai, N.; Sano, A.; Yokoyama, M.; Sugai, T.; Ohta, H. *Chem. Lett.* **1991**, 1823. (b) Crosby, J. A.; Parratt, J. S.; Turner, N. J. *Tetrahedron: Asymmetry* **1992**, *3*, 1547. (c) Maddrell, S. J.; Turner, N. J.; Kerridge, A.; Willetts, A. J.; Crosby, J. *Tetrahedron Lett.* **1996**, *37*, 6001. (d) Vink, M. K.; Schortinghuis, C. A.; Luten, J.; van Maarseveen, J. H.; Schoemaker, H. E.; Hiemstra, H.; Rutjes, F. P. J. T. *J. Org. Chem.* **2002**, *67*, 7869. (e) DeSantis, G.; Wong, K.; Farwell, B.; Chatman, K.; Zhu, Z.; Tomlinson, G.; Huang, H.; Tan, X.; Bibbs, L.; Chen, P.; Kretz, K.; Burk, M. J. *J. Am. Chem. Soc.* **2003**, *125*, 11476. (f) Robertson, D. E.; Chaplin, J. A.; DeSantis, G.; Podar, M.; Madden, M.; Chi, E.; Richardson, T.; Milan, A.; Miller, M.; Weiner, D. P.; Wong, K.; McQuaid, J.; Farwell, B.; Preston, L. A.; Tan, X.; Snead, M. A.; Keller, M.; Mathur, E.; Kretz, P. L.; Burk, M. J.; Short, J. M. *Appl. Environ. Microbiol.* **2004**, *70*, 2429. (g) Wang, M.-X.; Liu, C.-S.; Li, J.-S. *Tetrahedron: Asymmetry* **2001**, *12*, 3367. (h) Wang, M.-X.; Liu, C.-S.; Li, J.-S.; Meth-Cohn, O. *Tetrahedron Lett.* **2000**, *41*, 8549.
(17) Ma, D.-Y.; Zheng, Q.-Y.; Wang, D.-X.; Wang, M.-X. *Org. Lett.* **2006**, *8*, 3231.
(18) (a) Braunegg, G.; De Raadt, A.; Feichtenhofer, S.; Griengl, H.; Kopper, I.; Lehmann, A.; Weber, H.-J. *Angew. Chem., Int. Ed.* **1999**, *38*, 2763. (b) De Raadt, A.; Griengl, H.; Weber, H. *Chem. Eur. J.* **2001**, *7*, 27.

TABLE 2. Enantioselective Biotransformations of Racemic Nitriles 1a and 4 and Amides 2a and 5



					1 or **4**		**2** or **5**		**3** or **6**	
ent	subs	R	EWG	time[a]	yield, %[b]	ee, %[c]	yield, %[b]	ee, %[c]	yield, %[b]	ee, %[c]
1	**1a**	vinyl	CN	30 min	18	15.8	74.5	<1	3	98.8
2	**1a**	vinyl	CN	15.5 h			47.5	95.2	47	95.4
3	**2a**	vinyl	$CONH_2$	15.5 h			48	97.1	49	95.1
4	**4**	Et	CN	20 min	18	13.4	79	<1	trace	N.D.
5	**4**	Et	CN	26 h			48	85.8	47	82.2
6	**5**	Et	$CONH_2$	23 h			48	81.6	48	87.6

[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 HPLC or GC analysis using the chiral columns (see the Supporting Information).

efficiency and enantioselectivity of both the nitrile hydratase and the amidase within *R. erythoropolis* AJ270, cell-catalyzed kinetic resolution of racemic amide **2a** and **5** was also investigated (Table 2). Surprisingly, under conditions identical to those for the reaction of **1a**, biotransformation of **4** proceeded slowly with only modest enantioselectivity. For example, only after 26 h incubation were equal amounts of optically active (*R*)-amide **5** and (*S*)-acid **6** obtained with ee values of 85.8% and 82.2%, respectively (entry 5, Table 2). Careful examination of the nitrile hydration reaction of racemic **1a** and **4** revealed that the nitrile hydratase in the microbial cells exhibited high enzymatic activity but low enantioselectivity toward both 3-phenylpent-4-enenitrile **1a** and 3-phenylpentanenitrile **4**, as the hydration of **1a** and **4** took place very rapidly and the ee values for both recovered nitriles **1a** and **4** were around 15% (entries 1 and 4, Table 2). In other words, the nitrile hydratase showed loose discrimination power between β-vinyl and β-ethyl substituents within the substrates **1a** and **4**, respectively. On the other hand, as indicated in Table 2, the kinetic resolution of racemic amide **2a** finished in 15.5 h, furnishing excellent enantioselectivity with an enantiomeric ratio (*E*)[19] up to 164 (entry 3, Table 2), whereas the kinetic resolution of racemic amide **5** took almost 1 day to afford modest enantioselectivity with *E* around 39 (entry 6, Table 2). It is obvious that the overall efficiency and enantioselectivity of the nitrile biotransformations (entries 2 and 5) were determined by a combination of the effects of the nitrile hydratase and the amidase, with the latter playing a dominant role. The different efficiencies and enantioselectivities shown by the amidase against between 3-phenylpent-4-enoic acid amide **2** and 3-phenylpentanoic acid amide **5** strongly suggested that the amidase involved in *R. erythropolis* AJ270 is highly sensitive toward the structures of the substrates. A slight structure variation of the substrate, such as the change from a β-ethyl in amide **5** to a β-vinyl in amide **2**, led to a significant enhancement of the biocatalytic efficiency and enantioselectivity. It is worth noting that, to the best of our knowledge, it is rare that a carbon−carbon double bond at the β-position to the amido functional group can facilitate the amidase-catalyzed hydrolysis and can drastically increase the enantioselectivity.

The outcomes of the current study are in agreement with almost all previous observations that nitrile hydratases are a type of highly active and less selective enzyme against a wide variety of nitrile substrates containing either an α-[3] or a β-chiral center.[11−15] These properties of the nitrile hydratase, such as having a broad substrate spectrum and possessing low or none enantioselectivity, are determined by its enzyme structure in which there is a spacious pocket near the active site.[20] In other words, marginal difference in structures between nitriles **1a** and **4** was not recognized by the nitrile hydratase, and almost identical biocatalytic hydration reactions were therefore effected.[21]

The high sensitivity of the amidase toward the structure of the amide substrates is intriguing. To interpret the biocatalysis of various racemic amides bearing an α-stereocenter, amidase of a deeply buried and highly steric demanding active site has been proposed.[9] Taking the similarity between 3-phenylpent-4-enoic acid amide **2a** and 3-phenylpentanoic acid amide **5** into consideration, the rationalization for the different catalytic efficiencies and enantioselectivities of the amidase displayed for two substrates remains a challenge at this stage because of the lack of molecular structure of the amidase.[22] However, it is most probably that the amidase may have another binding domain slightly away from the amido reactive site. This binding domain seems able to discriminate the substituents of slight electronic and/or steric difference, such as between vinyl and ethyl. 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 the β-vinyl with the specific vinyl binding domain of the enzyme may contribute greatly to the acceleration of the reaction and to the drastic improvement of the enantioselectivity. Much more effort is certainly needed to elucidate the amidase's action on the molecular level. Nevertheless, the outcomes of our study have shown that, in contrast to the general notion, the biotransformations of nitriles and amides are in fact useful in the preparation of highly enantiopure carboxylic acids and amides with a β-chiral center, provided the substrates are carefully designed.

As multifunctionalized molecules, chiral 3-aryl-pent-4-enoic acids and their amide derivatives are useful in organic synthesis.

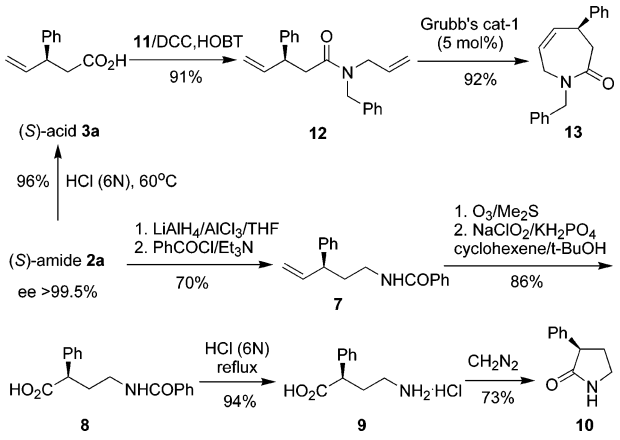
(19) (a) Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294. (b) Program "Selectivity" by Faber, K.; Hoenig, H. http://www.cis.TUGraz.at/orgc/.

(20) (a) Huang, W. J.; Jia, J.; Cummings, J.; Nelson, M.; Schneider, G.; Lindqvist, Y. *Structure* **1997**, *5*, 691. (b) Nagashima, S.; Nakasako, M.; Dohmae, N.; Tsujimura, M.; Takio, K.; Odaka, M.; Yohda, M.; Kamiya, N.; Endo, I. *Nat. Struct. Biol.* **1998**, *5*, 347.

(21) Only in the case of biotransformations of sterically well-defined cyclopropanecarbonitrile substrates was good enantioselectivity obtained. See ref 10b,c.

(22) No molecular structure of amidase involved in nitrile-hydrolyzing microorganisms has been reported. Our study on the isolation and crystallization of the amidase in *R. erythropolis* AJ270 is making progress.

SCHEME 1. Synthetic Applications of Nitrile Biotransformation Products



It was noteworthy that optically active acid **3a**, the only known compound in the literature in the series of **2** and **3**, was obtained from lengthy asymmetric syntheses using chiral auxiliaries.[23] Our nitrile biotransformation approach, however, provided a straightforward and scale-able route to highly enantiopure (*S*)-3-phenylpent-4-enoic acid amide (*S*)-**2a** and (*R*)-3-phenylpent-4-enoic acid (*R*)-**3a** in the laboratory (entry 5, Table 1). Chemical hydrolysis of (*S*)-3-phenylpent-4-enoic acid amide in refluxing hydrochloride (6 N) resulted in a quantitative yield of (*S*)-phenylpent-4-enoic acid (*S*)-**3a**, the antipode of the biotransformation product (*R*)-**3a** (Scheme 1). A large number of structurally diversified chiral organic compounds are feasible on the basis of the functional group transformations of both enantiopure intermediates **2** and **3**.[23,24] To demonstrate their synthetic values, we synthesized chiral γ-amino-α-phenylbutyric acid **9**, a γ^2-amino acid potentially useful for peptide mimetics,[25] simply by reducing the carboxamido group of (*S*)-**2a** followed by oxidation of the vinyl group. Upon treatment of diazomethane, γ-amino acid **9** was easily converted into a five-membered chiral lactam **10**.[26] A seven-membered enantiopure ϵ-lactam, (4*S*)-1-benzyl-4-phenyl-1,3,4,7-tetrahydro-azepin-2(2*H*)-one **13**,[27] was conveniently prepared in an excellent overall yield from the coupling reaction of (*S*)-**3a** with *N*-allylbenzylamine **11** followed by ring-closure metathesis using the first-generation Grubbs' catalyst.[28] Its 4*R*-enantiomer, **15** was also obtained similarly when (*R*)-3-phenylpent-4-enoic acid (*R*)-**3a** was employed as the starting material (see the Supporting Information). It is worth noting that in all chemical transformations depicted in Scheme 1, no racemization was observed.

In summary, we have shown that biotransformations of racemic 3-arylpent-4-enenitriles, catalyzed by the *R. erythropolis* AJ270 whole cell catalyst under mild conditions, provide an efficient preparation of highly enantiopure (*R*)-3-arylpent-4-enoic acids (*R*)-**3** and (*S*)-3-arylpent-4-enoic acid amides (*S*)-**2**. While the nitrile hydratase exhibits low *R*-enantioselectivity against 3-arylpent-4-enenitriles, the amidase is highly *R*-enantioselective toward 3-arylpent-4-enoic acid amide substrates. Moreover, when the vinyl group in 3-phenylpent-4-enenitrile was replaced by an ethyl group, biotransformations of racemic 3-phenylpentanenitrile **4** proceeded slowly and gave a diminished enantioselectivity, owing to the greater enzyme activity and higher enantioselectivity of the amidase toward 3-phenylpent-4-enoic acid amide **2a** than toward 3-phenylpentanoic acid amide **5**. The results have expanded the application of nitrile and amide biotransformations in the synthesis of carboxylic acids and amides that have a β or even more remote chiral center, provided the substrates are carefully engineered. The resulting enantiopure 3-arylpent-4-enoic acids (*R*)-**3** and amide derivatives (*S*)-**2**, which are not easily available from asymmetric chemical reactions, can serve as the versatile chiral intermediates in organic synthesis. Their synthetic applications have been demonstrated by the convenient preparations of chiral γ-amino acid, five-membered 2-pyrrolidinone and seven-membered 2-azepinone derivatives.

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) 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 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 and 2) 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 (50 mL × 3). After drying ($MgSO_4$) and removing solvent under vacuum, the residue was subjected to a silica gel column using a mixture of petroleum ether and ethyl acetate (from 2:1 to 1:2) as the mobile phase to give pure amide product and, in some cases, the recovered nitrile. The aqueous phase was then adjusted to pH 2 with hydrochloric acid (2 N) and extracted with ethyl ether (50 mL × 3). The organic phase was combined. After drying ($MgSO_4$) and removing solvent under vacuum, the residue was chromatographied on a silica gel column using a mixture of petroleum ether and ethyl acetate (from 20:1 to 4:1) as an eluent to give pure acid product. The structures of all products were fully characterized by spectroscopic data and microanalyses. The absolute configurations of acids (*R*)-**3a**, (*S*)-**3a**, and (*R*)-**3e** were determined by comparing the directions of their optical rotations with that of the authentic samples,[23] while the absolute configurations of other acids (*R*)-**3** were assigned assuming that the introduction of a substituent on the benzene ring did not change the direction of optical rotation. The absolute configuration of amide products was obtained from the optical rotation of their corresponding acids.

Acknowledgment. We thank the MOST (2003CB716005), the NNSC, and the CAS for financial support.

Supporting Information Available: Spectroscopic data of **1**−**3** and **7**−**13**. ^{1}H and ^{13}C NMR spectra of **2a**, **3a**, **10**, and **13**. HPLC analysis of all chiral products. This material is available free of charge via the Internet at http://pubs.acs.org.

JO061664F

(23) (a) Brown, E.; Deroye, C.; Touet, J. *Tetrahedron: Asymmetry* **1998**, *9*, 1605. (b) Mitsui, K.; Sato, T.; Urabe, H.; Sato, F. *Angew. Chem., Int. Ed.* **2004**, *43*, 490.

(24) (a) Karla, R.; Ebert, B.; Thorkildsen, C.; Herdeis, C.; Johansen, T. N.; Nielsen, B.; Krogsgaard-Larsen, P. *J. Med. Chem.* **1999**, *42*, 2053. (b) Belda, O.; Lundgren, S.; Moberg, C. *Org. Lett.* **2003**, *5*, 2275.

(25) For a comprehensive review of peptide mimetics, see: Seebach, D.; Beck, A. K.; Bierbaum, D. J. *Chem. Biodiv.* **2004**, *1*, 1111.

(26) Kim, B. J.; Park, Y. S.; Beak, P. *J. Org. Chem.* **1999**, *64*, 1705.

(27) For a review of medium ring *N*-heterocycles, see: Evans, P. A.; Holmes, A. B. *Tetrahedron* **1991**, *47*, 9131.

(28) (a) Grubbs, R. H.; Miller, S. J.; Fu, G. C. *Acc. Chem. Res.* **1995**, *28*, 446. (b) Fu, G. C.; Nguyen, S. T.; Grubbs, R. H. *J. Am. Chem. Soc.* **1993**, *115*, 9856.