JOC Article

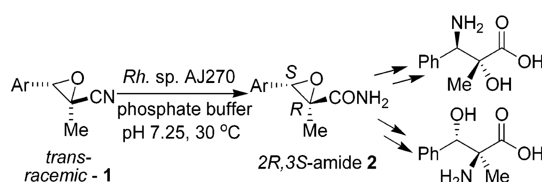
Nitrile Biotransformations for Highly Enantioselective Synthesis of Oxiranecarboxamides with Tertiary and Quaternary Stereocenters; Efficient Chemoenzymatic Approaches to Enantiopure α-Methylated Serine and Isoserine Derivatives

Mei-Xiang Wang,* Gang Deng, De-Xian Wang, and Qi-Yu Zheng

Laboratory for Chemical Biology, Center for Molecular Science, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100080, People's Republic of China

mxwang@iccas.ac.cn

Received October 4, 2004



Biotransformations of a number of differently substituted and configured oxiranecarbonitriles using *Rhodococcus* sp. AJ270, a microbial whole-cell catalyst that contains nitrile hydratase/amidase, were studied. While almost all trans-configured 3-aryl-2-methyloxiranecarbonitriles and 2,3-dimethyl-3-phenyloxiranecarbonitrile were efficiently hydrated by the action of the less enantioselective nitrile hydratase, the amidase exhibited excellent 2*S*,3*R*-enantioselectivity against 2-methyl-3-(para-substituted-phenyl)oxiranecarboxamides. Under very mild conditions, biotransformations of nitriles provided an efficient and practical synthesis of 2*R*,3*S*-(−)-3-aryl-2-methyloxiranecarboxamides, electrophilic epoxides with tertiary and quaternary stereocenters, in excellent yield with enantiomeric excess greater than 99.5%. The synthetic applications of the resulting enantiomerically pure epoxides were demonstrated by convenient and straightforward syntheses of polyfunctionalized chiral molecules possessing a quaternary stereocenter such as *R*-(+)-2-hydroxy-2-methyl-3-phenylpropionic acid, 2*R*,3*R*-(−)-3-amino-2-hydroxy-2-methyl-3-phenylpropionic acid, and 2*S*,3*S*-(+)-2-amino-3-hydroxy-2-methyl-3-phenylpropionic acid, employing the regio- and stereospecific epoxide ring opening reactions of 2*R*,3*S*-(−)-2-methyl-3-phenyloxiranecarboxamide as the key steps.

Introduction

Enantiomerically pure electrophilic epoxides with tertiary and quaternary stereocenters are versatile and powerful intermediates in the synthesis of a wide range of chiral molecules bearing both tertiary and quaternary stereocenters in vicinal positions upon the regio- and stereoselective ring opening reactions of epoxides by various nucleophiles. The resulting highly functionalized organic compounds, which are hard to obtain by other synthetic methods, are not only useful in synthetic chemistry but are also valuable entities in medicinal chemistry.[1−3] Although the preparation of enantiopure epoxide compounds has been well developed by Sharpless and others, no general and single approach stands out for the synthesis of optically active electrophilic epoxides.[4] It is even more challenging to synthesize chiral electro-

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

(1) For a useful review, see: Jung, M. In *Chemistry and Biochemistry of Amino Acids*; Barret, G. C., Ed.; Chapman and Hall: New York, 1985; pp 22−296.

(2) Paul, P. K. C.; Sukumar, M.; Bardi, R.; Piazzesi, A. M.; Valle, G.; Toniolo, C.; Balaram, P. *J. Am. Chem. Soc.* **1986**, *108*, 6363 and references therein.

(3) (a) Ojima, I.; Wang, T.; Delaloge, F. *Tetrahedron Lett.* **1998**, *39*, 3663. (b) Denis, J.-N.; Fkyerat, A.; Gimbert, Y.; Coutterez, C.; Mantellier, P.; Jost, S.; Greene, A. E. *J. Chem. Soc., Perkin Trans. 1* **1995**, 1811.

(4) For a recent review, see: (a) Porter, M. J.; Skidmore, J. *Chem. Commun.* **2000**, 1215. For examples of asymmetric epoxidation of α,β-unsaturated ketones, see: (b) Enders, D.; Zhu, J.; Raabe, G. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1725. (c) Yu, H.-B.; Zheng, X.-F.; Lin, Z.-M.; Hu, Q.-S.; Huang, W.-S.; Pu, L. *J. Org. Chem.* **1999**, *64*, 8149. (d) Watanabe, S.; Kobayashi, Y.; Arai, T.; Sasai, H.; Bougauchi, M.; Shibasaki, M. *Tetrahedron Lett.* **1998**, *39*, 7353. (e) Watanabe, S.; Arai, T.; Sasai, H.; Bougauchi, M.; Shibasaki, M. *J. Org. Chem.* **1998**, *63*, 8090. (f) Elston, C. L.; Jackson, R. F. W.; MacDonald, S. J. F.; Murray, P. J. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 410. (g) Wang, Z.-X.; Miller, S. M.; Anderson, O. P.; Shi, Y. *J. Org. Chem.* **1999**, *64*, 6443 and references therein.

10.1021/jo0482615 CCC: $30.25 © 2005 American Chemical Society
Published on Web 03/02/2005

philic epoxides with tertiary and quaternary stereocenters.[5−9] Most of the syntheses reported to date are multistep ones either using a chiral auxiliary[5] or starting from Sharpless asymmetric epoxidation of allylic alcohols followed by oxidation of the hydroxy group to the carbonyl.[6] Highly enantioselective Darzens reaction of a camphor-derived sulfonium amide[7] and catalytic asymmetric epoxidation of α,β-unsaturated amides[8] have been reported very recently to provide highly enantiopure oxiranecarboxamides, but neither method gave quaternary carbon-centered epoxide analogues. In the presence of benzylquininium chloride, epoxidation of 2-substituted 1,4-naphthoquinones yielded optically active 2,3-epoxides with enantiomeric excess values less than 45%.[9]

Biotransformations of nitriles, either through a direct conversion from a nitrile to a carboxylic acid catalyzed by a nitrilase[10] or through the nitrile hydratase catalyzed hydration of a nitrile followed by amide hydrolysis catalyzed by amidase,[11] are an effective and environmentally benign method for the production of carboxylic acids and their amide derivatives.[12] 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.[13,14] The distinct features of enzymatic transformations of nitriles are the formation of enantiopure carboxylic acids and the straightforward generation of enantiopure amides, which are valuable organonitrogen compounds in synthetic chemistry. Very recently, we have shown that *Rhodococcus* sp. AJ270,[15] a whole-cell catalyst that contains nitrile hydratase/amidase, is able to efficiently and enantioselectively transform cyclopropanecarbonitriles[16] and oxiranecarbonitriles[17] into the corresponding carboxylic acids and amides. A prediction model for reaction efficiency and enantioselectivity has also been proposed.[16f] To further explore the synthetic potential of the nitrile biotransformations catalyzed by *Rhodococcus* sp. AJ270 and to validate the prediction model for the three-membered substrates, we undertook the current study. In this paper we report an efficient and convenient synthesis of enantiopure oxiranecarboxamides with tertiary and quaternary stereocenters and their applications in the synthesis of α-methylated α-hydroxycarboxylic acid and α-methylated serine and isoserine derivatives.

Results and Discussion

We first examined the reaction of racemic *trans*-2-methyl-3-phenyloxiranecarbonitrile **1a**. Catalyzed by the *Rhodococcus* sp. AJ270 microbial whole-cell catalyst under very mild conditions, nitrile **1a** was very rapidly and effectively hydrolyzed. For example, more than 50% of the nitrile **1a** was hydrated within 5 min and a complete hydration was effected in about 30 min (entries 1 and 2 in Table 1). The enantiomeric excess (ee) values obtained for both the amide **2a** and the recovered nitrile **1a** were extremely low (5%) after 50% hydration (entry 1 in Table 1), indicating that the nitrile hydratase involved in this microbial cell catalyst shows very low enantioselectivity against *trans*-2-methyl-3-phenyloxiranecarbonitrile. Although the subsequent amide hydrolysis was slower than the nitrile hydration, the amidase involved in *Rhodococcus* sp. AJ270 cells catalyzed the biohydrolysis of the resulting amide in a few hours to produce the corresponding enantiomerically pure 2*R*,3*S*-2-methyl-3-phenyloxiranecarboxamide (−)-**2a** in excellent yield (entry 3 in Table 1) and 2*S*,3*R*-2-methyl-3-phenyloxiranecarboxylic acid **3a**, with the latter being not isolable because it underwent a spontaneous decomposition similar to that of its 2*S*,3*R*-2-phenylglycidic acid analogue[17] to form benzyl methyl ketone under the reaction conditions (Scheme 1). To shed further light on the stereochemistry of the reaction, we then investigated the biotransformation of racemic *trans*-2-methyl-3-phenyloxiranecarboxamide **2a** under the identical conditions. It was found that (±)-**2a** was resolved after 7.5 h into optically active 2*R*,3*S*-2-methyl-3-phenyloxiranecarboxamide (−)-**2a** in 44% yield with 81% ee. Again, no

(5) (a) Adam, W.; Pastor, A.; Peters, K.; Peters, E.-M. *Org. Lett.* **2000**, *8*, 1019. (b) Ruano, J. L. G.; Castro, A. M. M.; Ramos, J. H. R.; Flamarique, A. C. R. *Tetrahedron: Asymmetry* **1997**, *8*, 3503. (c) Torres-Valencia, J. M.; Cerda-Garcia-Rojas, C. M.; Joseph-Nathan, P. *Tetrahedron: Asymmetry* **1998**, *9*, 757. (d) Hayashi, M.; Terashima, S.; Koga, K. *Tetrahedron* **1981**, *37*, 2797.

(6) For representative examples, see: (a) Jung, M. E.; Anderson, K. L. *Tetrahedron Lett.* **1997**, *38*, 2605. (b) Jung, M. E.; D'Amico, D. C. *J. Am. Chem. Soc.* **1995**, *117*, 7379. (c) Marshall, J. A.; Trometer, J. D.; Blough, B. E.; Crute, T. D. *J. Org. Chem.* **1988**, *53*, 4274. (d) Molander, G. A.; Shubert, D. C. *J. Am. Chem. Soc.* **1987**, *109*, 576 and references therein.

(7) Aggarwal, V. K.; Hynd, G.; Picoul, W.; Vasse, J.-L. *J. Am. Chem. Soc.* **2002**, *124*, 9964.

(8) (a) Nemoto, T.; Kakei, H.; Gnanadesikan, V.; Tosaki, S.-y.; Ohshima, T.; Shibasaki, M. *J. Am. Chem. Soc.* **2002**, *124*, 14544. (b) Kakei, H.; Nemoto, T.; Ohshima, T.; Shibasaki, M. *Angew. Chem., Int. Ed.* **2004**, *43*, 317.

(9) Pluim, H.; Wynberg, H. *J. Org. Chem.* **1980**, *45*, 2498.

(10) Kobayashi, M.; Shimizu, S. *FEMS Microbiol. Lett.* **1994**, *120*, 217 and references therein.

(11) (a) Meth-Cohn, O.; Wang, M.-X. *J. Chem. Soc., Perkin Trans. 1* **1997**, 1099. (b) Meth-Cohn, O.; Wang, M.-X. *J. Chem. Soc., Perkin Trans. 1* **1997**, 3197 and references therein.

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

(13) For reviews, see: (a) Sugai, T.; Yamazaki, T.; Yokoyama, M.; Ohta, H. *Biosci. Biotechnol. Biochem.* **1997**, *61*, 1419 and references therein. (b) Martinkova, L.; Kren, V. *Biocatal. Biotrans.* **2002**, *20*, 73.

(14) For recent examples, see: (a) Wang, M.-X.; Lin, S.-J.; Liu, J.; Zheng, Q.-Y. *Adv. Synth. Catal.* **2004**, *346*, 439. (b) Wang, M.-X.; Lin, S.-J. *J. Org. Chem.* **2002**, *67*, 6542. (c) Wang, M.-X.; Zhao, S.-M. *Tetrahedron Lett.* **2002**, *43*, 6617. (d) Wang, M.-X.; Zhao, S.-M. *Tetrahedron: Asymmetry* **2002**, *13*, 1695. (e) Wang, M.-X.; Li, J.-J.; Ji, G.-J.; Li, J.-S. *J. Mol. Catal. B: Enzymol.* **2001**, *14*, 77. (f) Wang, M.-X.; Lin, S.-J. *Tetrahedron Lett.* **2001**, *42*, 6925. (g) Wang, M.-X.; Liu, C.-S.; Li, J.-S. *Tetrahedron: Asymmetry* **2001**, *12*, 3367. (h) Wang, M.-X.; Lu, G.; Ji, G.-J.; Huang, Z.-T.; Meth-Cohn, O.; Colby, J. *Tetrahedron: Asymmetry* **2000**, *11*, 1123. (i) Wang, M.-X.; Liu, C.-S.; Li, J.-S.; Meth-Cohn, O. *Tetrahedron Lett.* **2000**, *41*, 8549. (j) 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. (k) Wu, Z.-L.; Li, Z.-Y. *Chem. Commun.* **2003**, 386. (l) Effenberger, F.; Osswald, S. *Tetrahedron: Asymmetry* **2001**, *12*, 279. (m) 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. (n) Wu, Z.-L.; Li, Z.-Y. *J. Mol. Catal. B: Enzymol.* **2003**, *22*, 105. (o) Preiml, M.; Hillmayer, K.; Klempier, N. *Tetrahedron Lett.* **2003**, *44*, 5057. (p) Yokoyama, M.; Kashiwagi, M.; Iwasaki, M.; Fushuku, K.; Ohta, H.; Sugai, T. *Tetrahedron: Asymmetry* **2004**, *15*, 2817.

(15) Blakey, A. J.; Colby, J.; Williams, E.; O'Reilly, C. *FEMS Microbiol. Lett.* **1995**, *129*, 57.

(16) (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: Enzymol.* **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.

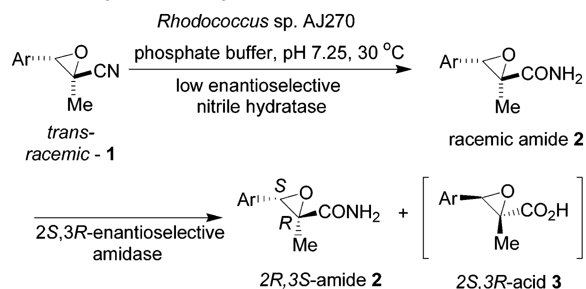
(17) Wang, M.-X.; Lin, S.-J.; Liu, C.-S.; Zheng, Q.-Y.; Li, J.-S. *J. Org. Chem.* **2003**, *68*, 4570.

TABLE 1. Biotransformations of Racemic *trans*-3-Aryl-2-methyloxiranecarbonitriles 1

entry	1	Ar	reaction conditions[a]	**2** yield[b] (%)	**2** ee[c] (%)
1	1a	C_6H_5	2 mmol, 5 min	50[d]	8
2	1a	C_6H_5	2 mmol, 20 min	89	8
3	1a	C_6H_5	2 mmol, 7.5 h	45	>99.5
4	1a	C_6H_5	13.1 mmol, 4 days[e]	46[e]	>99.5
5	1b	4-F-C_6H_4	2 mmol, 10 h	31	99
6	1c	4-Cl-C_6H_4	2 mmol, 7.5 h	49	>99.5
7	1d	3-Cl-C_6H_4	2 mmol, 5.5 days	32	20
8	1d	3-Cl-C_6H_4	2 mmol, acetone (2.5 mL), 6 days	52	41
9	1e	2-Cl-C_6H_4	2 mmol, 11 h[f]	40[f]	<5
10	1e	2-Cl-C_6H_4	2 mmol, 7 days[g]	21[g]	<5
11	1f	4-Br-C_6H_4	2 mmol, acetone (2.5 mL), 8.5 h	48	>99.5
12	1g	4-Me-C_6H_4	2 mmol, 11.5 h	31	>99.5
13	1h	2-Me-C_6H_4	1 mmol, 7 days	44	<5
14	1i	3,4-OCH_2O-C_6H_3	2 mmol, 1 day	32	50
15	1i	3,4-OCH_2O-C_6H_3	2 mmol, acetone (2.5 mL), 1 day	45	32

[a] Biotransformation was carried out in a suspension of *Rhodococcus* sp. AJ270 cells (2 g wet weight) in phosphate buffer (50 mL, pH 7.25) at 30 °C. [b] Isolated yield. [c] Determined by HPLC analysis using a Chiralcel OD or OJ column (see Supporting Information). [d] Nitrile (36%, ee < 5%) was recovered. [e] Nitrile was added portionwise during 4 days, and a small amount of nitrile (394.3 mg) was recovered. [f] A mixture of trans and cis isomers (1:1) was used, and quantitative racemic *cis*-nitrile (50%) was recovered. [g] A mixture of trans and cis isomers (1:1) was used, and almost all racemic *cis*-nitrile (45%) was recovered.

SCHEME 1. Biotransformations of Racemic *trans*-3-Aryl-2-methyloxiranecarbonitriles 1



acid product **3a** was obtained due to its instability under the incubation conditions. The use of acetone (2.5 mL) as a cosolvent to increase the solubility of the amide substrate **2a** in the aqueous buffer gave rise to the improved conversion rate and enantioselectivity of the reaction, yielding enantiopure 2*R*,3*S*-2-methyl-3-phenyloxiranecarboxamide (−)-**2a** (Scheme 2). The aforementioned results indicated clearly that the amidase of *Rhodococcus* sp. AJ270 displays high 2*S*,3*R* enantioselectivity against *trans*-2-methyl-3-phenyloxiranecarboxamide, while the nitrile hydratase shows low enantiocontrol against *trans*-2-methyl-3-phenyloxiranecarbonitrile. The excellent enantioselection of biotransformation of nitrile **1a** originated from the combined effects of enantioselective nitrile hydratase and amidase, with the latter being a dominant force (Scheme 1). It is worth noting that, to prepare enantiomerically pure 2*R*,3*S*-2-methyl-3-phenyloxiranecarboxamide (−)-**2a**, it is advantageous to employ biotransformation of nitrile rather than amide. To demonstrate practical application, the preparative biotransformation was performed under identical conditions by adding substrate (±)-**2a** (13.1 mmol) portionwise during a period of 4 days, and enantiopure (−)-**2a** was obtained on a gram scale (entry 4 in Table 1).

To examine the scope of the reaction and the influence of substituent on the efficiency and enantioselectivity of biotransformations, a number of racemic *trans*-3-aryl-2-methyloxiranecarbonitriles **1** were prepared[18] and subjected to incubation with *Rhodococcus* sp. AJ270 (Scheme 1). In all cases, the nitrile hydratase catalyzed hydration reaction proceeded very rapidly; all nitriles tested were found to undergo a complete but low enantioselective hydration reaction within a few hours. The amide hydrolysis, however, was strongly dependent upon the structure of the substrate. More noticeably, it is the substitution pattern rather than the nature of the substituent on the benzene ring of the substrate that plays a crucial role in determining both the rate and the enantioselectivity of an amidase-catalyzed reaction, and therefore the overall reaction. As illustrated in Table 1, nitrile **1a** and all its para-substituted analogues **1b**, **1c**, **1f**, and **1g** underwent a rapid hydrolysis to give enantiomerically pure amide products in excellent yields (entries 3, 5, 6, 11, and 12). With a substituent at the meta position, substrate **1d** took a long incubation time to effect ca.50% conversion of amide, giving optically active amide (−)-**2d** with low enantiomeric excess (entry 7 in Table 1). The hydrolysis of **1i**, a substrate derived from piperonal, proceeded rapidly to afford amide (−)-**2i** with 50% ee (entry 14 in Table 1). The slowest reaction and lowest enantioselection were observed for the reaction of substrates **1e** and **1h**, which contain an ortho-substituted benzene ring. In both cases, a long incubation time such as 7 days and a lower substrate concentration were required to achieve 50% conversion of the amide (entries 10 and 13 in Table 1). Unlike the desymmetrization of 3-susbtituted glutaronitriles,[14g] addition of acetone (2.5 mL) as an additive or a cosolvent did not lead to the improvement of enantioselectivity of biotransformations of nitriles (entries 8 and 15 in Table 1).

In contrast to the racemic *trans*-nitrile and amide isomers **1a** and **2a**, the biotransformations of racemic *cis*-2-methyl-3-phenyloxiranecarbonitrile substrate **4a** and of amide **5a** proceeded sluggishly. Under same reaction conditions as those for **1a**, for example, oxiranecarbonitrile (±)-**4a** could not be completely hydrated after 7 days interaction with the whole-cell catalyst, with 66% of the optically inactive nitrile **4a** being recovered. The subsequent amide hydrolysis was also very slow, and only a small amount of the resulting amide (<9%) was

(18) Svoboda, J.; Kocfeldová, Z.; Paleček, J. *Collect. Czech. Chem. Commun.* **1988**, *53*, 822.

SCHEME 2. Kinetic Resolution of Racemic *trans*-2-Methyl-3-phenyloxiranecarboxamide 2a

trans-*racemic* - **2a** → *Rhodococcus* sp. AJ270, phosphate buffer, pH 7.25 with or without acetone (2.5 ml) additive, 30 °C → 2*R*,3*S*-amide **2a** (chemical yield 41 - 44%, ee 81 - >99.5%) + [2*S*,3*R*-acid **3a**]

SCHEME 3. Biotransformations of Racemic *cis*-2-Methyl-3-phenyloxiranecarbonitrile 4a

cis-*racemic* - **4a** → *Rhodococcus* sp. AJ270, phosphate buffer, pH 7.25, 30 °C, 7 d → *cis*-nitrile **4a** (chemical yield 66%, e.e. 0%) + (-) -amide **5a** (chemical yield 25%, e.e. 28%) + [acid]

consumed by the amidase, leaving 25% of amide (−)-**5a** in low enantiomeric purity (28% ee) (Scheme 3).

In our previous studies of the biotransformations of cyclopropanecarbonitriles[16] and oxiranecarbonitriles,[17] we have found that the highly efficient and enantioselective biotransformations of nitriles originate from the combined effects of the enantioselective nitrile hydratase and amidase, which in turn are strongly dependent upon the substrate structures. The match of the steric bulkiness of the substituents on the three-membered ring can lead to efficient biocatalysis of both trans- and cis-configured cyclopropanecarbonitriles and amides with remarkable enantiocontrol. For racemic *trans*-3-aryloxiranecarbonitrile analogues, the nitrile hydratase of *Rhodococcus* sp. AJ270 is very active against both enantiomers of racemic trans isomers but not cis-isomeric analogues. On the other hand, the amidase displays high activity with varied enantioselectivity against *trans*-3-aryloxiranecarboxamides, depending on the substitution pattern of a substituent on the benzene ring. Our current study revealed that the biotransformations of racemic 3-aryl-2-methyloxiranecarbonitriles and amides followed the same general reaction pattern as that of 3-aryloxiranecarbonitriles and amides. The introduction of one more methyl group into the oxirane ring, however, resulted in a more salient steric effect on the reaction efficiency and enantioselectivity. For example, biocatalytic hydrolysis of all of the methylated 3-aryloxiranecarbonitriles **1** and amides **2** proceeded slower than their nonmethylated analogues, 3-aryloxiranecarbonitriles and amides.[17] More noticeably, in the case of 3-aryl-2-methyloxiranecarboxamides, the amidase exhibited much more pronounced enantio-discriminating power over the substitution pattern of the substituent on the benzene ring of the a substrate. This was exemplified by the observation of enantioselectivity ranging from excellent to low and to eventually naught with a substituent on the benzene ring of the substrate **2** moving from para to meta and to ortho position. The outcomes of our study were in agreement with the conclusions that the nitrile hydratase in *Rhodococcus* sp. AJ270 is an efficient and less enantioselective biocatalyst for many nitriles except for sterically heavily hindered or folded ones, while the amidase is more size-limited and is more sensitive toward the steric effect of the substrates even when a substituent is remote from the amido functional group.[16,17]

SCHEME 4. Biotransformations of a Mixture of Racemic trans- and *cis*-3-Aryloxiranecarbonitriles

racemic-*trans*-**1a** : *cis*-**4a** = 1:1 → *Rhodococcus* sp. AJ270, phosphate buffer, pH 7.25, 30 °C, 5h → 2*R*,3*S*-amide **2a** (23% yield, >99% ee) + *racemic*-**4a** (48% yield)

SCHEME 5. Biotransformations of 2,3-Dimethyl-3-phenyloxiranecarbonitriles 6 and 7

racemic - **6** (R^1 = Ph, R^2 = Me) / *racemic* - **7** (R^1 = Me, R^2 = Ph) → *Rhodococcus* sp. AJ270, phosphate buffer, pH 7.25, 30 °C → **8** (R^1 = Ph, R^2 = Me) / **9** (R^1 = Me, R^2 = Ph)

To take advantage of a huge difference in hydration reaction rate between *trans*- and *cis*-nitrile isomers, a mixture of *trans*- and *cis*-2-methyl-3-phenyloxiranecarbonitriles (±)-**1a** and (±)-**4a**, obtained directly from the Darzens reaction, was subjected to biocatalysis, with the hope of achieving asymmetric synthesis of enantiopure oxiranecarboxamide and the easy separation of pure *cis*-nitrile in one operation. Gratifyingly, biotransformation did produce optically active amide (−)-**2a** in both excellent chemical yield (92% based on theoretical yield) and enantiomeric purity (ee > 99.5%) after quenching of the reaction in 5 h. Pure *cis*-nitrile **4a** was also readily isolated in high yield (96% based on theoretical yield) (Scheme 4). The successful reaction allowed us to prepare enantiomerically pure 2*R*,3*S*-amides (−)-**2** while avoiding somewhat the tedious chromatographic separation[18] of trans and cis isomers of nitriles prior to biotransformations.

Biotransformations of racemic 3-aryl-3-methyloxiranecarbonitriles[18] were attempted. The substrates were found to be unstable, however, undergoing simultaneous decomposition under the reaction conditions. We then studied the biocatalytic hydrolysis of 2,3-dimethyl-3-phenyloxiranecarbonitriles **6** and **7** (Scheme 5). While *trans*-2,3-dimethyl-3-phenyloxiranecarbonitrile **6** underwent a rapid hydration reaction, the biocatalytic hydration of *cis*-2,3-dimethyl-3-phenyloxiranecarbonitrile **7** appeared to be extremely slow. The enantiomeric excess values for both nitriles recovered and amides formed were very disappointing, reflecting again the low enantioselectivity of the nitrile hydratase against oxiranecarbonitriles (Table 2). Hydrolysis of both amides **8** and **9** were found to be inefficient, most probably due to the increased steric hindrance of the substrates.

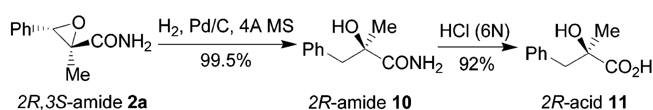
Optically active 3-aryl-2-methyloxiranecarboxamides (−)-**2** are valuable intermediates for the synthesis of a

TABLE 2. Biotransformations of 2,3-Dimethyl-3-phenyloxiranecarbonitriles 6 and 7

entry	substrate	reaction conditions[a]	**6** or **7** yield[b] (%)	**6** or **7** ee[c] (%)	**8** or **9** yield[b] (%)	**8** or **9** ee[c] (%)
1	**6** (trans)	2 mmol, 8 min	**6** (48)	**6** (14)	**8** (44)	**8** (9)
2	**6** (trans)	2 mmol, 7 days			**8** (92)	**8** (<5)
3	**6** (trans)	0.2 mmol, 7 days			**8** (64)	**8** (35)
4	**7** (cis)	2 mmol, 7 days	**7** (71)	**7** (5)	**9** (15)	**9** (19)
5	**7** (cis)	1 mmol, 7 days	7 (65)	7 (11)	**9** (35)	**9** (17)
6	**7** (cis)	0.5 mmol, 5 days	**7** (35)	**7** (35)	**9** (63)	**9** (17)
7	**7** (cis)	0.2 mmol, 7 days			**9** (88)	**9** (<5)

[a] Biotransformation was carried out in a suspension of *Rhodococcus* sp. AJ270 cells (2 g wet weight) in phosphate buffer (50 mL, pH 7.25) at 30 °C. [b] Isolated yield. [c] Determined by HPLC analysis using a Chiralcel OJ column (see Supporting Information). Absolute configurations of the products were not determined.

SCHEME 6. Synthesis of 2*R*-2-Hydroxy-2-methyl-3-phenylpropionic Acid 11 and Amide 10



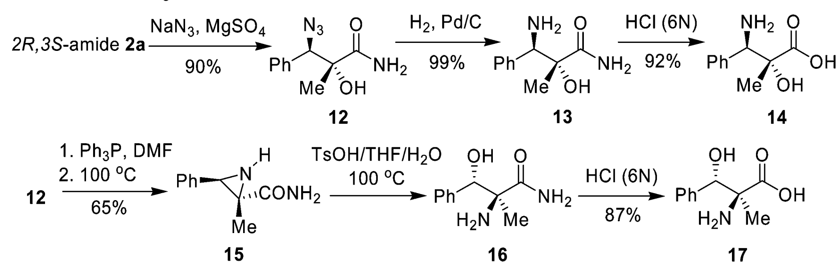
range of diversified chiral compounds. To demonstrate its versatility in organic synthesis, and also to determine the absolute configuration of biocatalytic products (−)-**2**, we first converted 2*R*,3*S*-2-methyl-3-phenyloxiranecarboxamide (−)-**2a** into *R*-(+)-2-hydroxy-2-methyl-3-phenylpropionic acid, a useful building block in natural products synthesis. Thus, regiospecific hydrogenation of **2a** catalyzed by Pd/C in the presence of molecular sieves (4 Å) gave *R*-(+)-2-hydroxy-2-methyl-3-phenylpropionamide **10** in an almost quantitative yield. Chemical hydrolysis of amide **10** in refluxing hydrochloric acid (6 N) furnished *R*-(+)-2-hydroxy-2-methyl-3-phenylpropionic acid **11** in 92% yield (Scheme 6). The optical rotation of **11** is identical to that of an authentic *R*-(+)-2-hydroxy-2-methyl-3-phenylpropionic acid sample,[19] suggesting that the configuration of **11** is *R*, and therefore the biotransformation product (−)-**2a** is 2*R*,3*S* configured. Optically active 2-hydroxy-2-methyl-3-phenylpropionic acid had been obtained from either a tedious optical resolution[20] or lengthy multistep syntheses using chiral auxiliaries.[19,21]

Ring opening reaction of 2*R*,3*S*-2-methyl-3-phenyloxiranecarboxamide (−)-**2a** by sodium azide proceeded very efficiently and diastereospecifically under mild conditions to afford exclusively 2*R*,3*R*-(−)-3-azido-2-hydroxy-2-methyl-3-phenylpropionamide **12** in 90% yield. Catalytic hydrogenation of azide **12** led to the formation of 2*R*,3*R*-(−)-3-amino-2-hydroxy-2-methyl-3-phenylpropionamide **13**, which was readily hydrolyzed in hydrochloric acid (6 N) to produce 2*R*,3*R*-(−)-3-amino-2-hydroxy-2-methyl-3-phenylpropionic acid **14**, an α-methylated isoserine derivative, in excellent yield (Scheme 7). An attempt was made to open the epoxide ring of **2a** at the 2-position using a number of nitrogen nucleophiles under different conditions.[22] Unfortunately, no satisfactory results were obtained. In almost all cases, the ring opening reaction preferentially occurred at the 3-position (data not shown). Although the strong tendency of the ring opening reaction at the 3-position of 2*R*,3*S*-2-methyl-3-phenyloxiranecarboxamide **2a** requires detailed investigation, it might be most probably due to the electronic effect of the phenyl and amido substituents that lead the 3-carbon to be more electron positive than the 2-carbon and therefore more susceptible to nucleophilic attack. The steric hindrance of the quaternary carbon might pose further inhibition of substitution reaction at the 2-carbon. To synthesize α-methylated serine derivative, an alternative approach was tried. It was reported by Zwanenburg and co-workers[23] that diethyl 3-azido-2-hydroxysuccinate can undergo intramolecular aziridination reaction upon the treatment of Ph_3P in DMF to afford diethyl aziridine-2,3-dicarboxylate, while Davis and Zhou[24] showed an efficient hydrolytic ring opening reaction of 3-arylaziridine-2-methanol using *p*-toluenesulfonic acid (TsOH) under very mild conditions. Thus, on the treatment of PPh_3 followed by heating, azide **12** was transformed into aziridine 2*S*,3*R*-**15** in 65% yield. Following Davis and Zhou's procedure,[24] however, no effective hydrolytic ring opening reaction of **13** was observed. Only at an elevated temperature (100 °C) did the reaction proceed. Subsequent hydrolysis of the resulting amide **16** under acidic conditions furnished enantiopure 2*S*,3*S*-(+)-2-amino-3-hydroxy-2-methyl-3-phenylpropionic acid **17**[25] in good yield (Scheme 7). Without isolation of **15**, the overall yield of **17** was improved to 79.5%.

Conclusion

In summary, we have shown that *Rhodococcus* sp. AJ270 whole cells can catalyze the hydrolysis of a number of differently substituted and configured oxiranecarbonitriles under very mild conditions. Both the efficiency

SCHEME 7. Synthesis of α-Methylated Serine and Isoserine Derivatives



and enantioselectivity of biocatalysis, however, were strongly dependent upon the structures of both nitrile and amide substrates. While almost all trans-configured 3-aryl-2-methyl-oxiranecarbonitriles and *trans*-2,3-dimethyl-3-phenyloxiranecarbonitrile were efficiently hydrated with the aid of the low enantioselective nitrile hydratase, the amidase exhibited excellent enantioselectivity against 2*S*,3*R*-2-methyl-3-(para-substituted phenyl)oxiranecarboxamides. The biocatalytic reaction of oxiranecarbonitriles has provided a highly efficient and practical synthesis of enantiopure 2*R*,3*S*-(−)-3-aryl-2-methyloxiranecarboxamides, electrophilic epoxides with tertiary and quaternary stereocenters. The resulting 2*R*,3*S*-(−)-3-aryl-2-methyloxiranecarboxamides, which are hard to prepare by other methods, can serve as versatile chiral synthons in the synthesis of polyfunctionalized chiral molecules bearing a quaternary carbon. This has been exemplified by a straightforward synthesis of *R*-(+)-2-hydroxy-2-methyl-3-phenylpropionic acid through the regio- and stereospecific hydrogenation of the epoxide ring of 2*R*,3*S*-(−)-2-methyl-3-phenyloxiranecarboxamide followed by hydrolysis of amide. We have also demonstrated that 2*R*,3*S*-(−)-2-methyl-3-phenyloxiranecarboxamide is able to undergo regio- and diastereospecific ring opening reaction with sodium azide to afford exclusively 2*R*,3*R*-(−)-3-azido-2-hydroxy-2-methyl-3-phenylpropionamide, an powerful intermediate that has been further transformed readily into 2*R*,3*R*-(−)-3-amino-2-hydroxy-2-methyl-3-phenylpropionic acid and 2*S*,3*S*-(+)-2-amino-3-hydroxy-2-methyl-3-phenylpropionic acid, the enantiomerically pure α-methylated isoserine and serine derivatives, respectively.

Experimental Section

General Procedure for the Biotransformations of Nitriles or Amides. To an Erlenmeyer flask (150 mL) with a screw cap was added *Rhodococcus* sp. AJ270 cells[15] (2 g wet weight) and potassium phosphate buffer (0.1 M, pH 7.25, 50 mL), and the resting cells were activated at 30 °C for 0.5 h with orbital shaking. Racemic nitrile **1**, **4**, **6**, or **7** or amide **2a** as fine powder was 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 and text) by removing the biomass through a Celite pad filtration. The resulting aqueous solution was extracted with ethyl acetate (60 mL × 3). After drying ($MgSO_4$) and removing solvent under vacuum, the residue was chromatographed on a silica gel column using a mixture of petroleum ether and ethyl acetate (from 2:1 to 1:5) as the mobile phase to give pure product. All products were characterized by their spectra data and comparison of the melting points and optical rotary power with that of the known compounds, or by full characterization (see Supporting Information). Enantiomeric excess values were obtained from HPLC analysis (see Supporting Information).

Acknowledgment. We thank the State Major Fundamental Research Program (2003CB716005, G2000077506), the Ministry of Science and Technology (2002CCA03100), the National Natural Science Foundation of China, and the Chinese Academy of Sciences for financial support.

Supporting Information Available: General experimental. Spectroscopic data of all compounds prepared. ^1H and ^{13}C NMR spectra of **2**, **1a**, **4a**, **4e**, **5a**, **6**−**15**, and **17**. HPLC analysis of all chiral products. This material is available free of charge via the Internet at http://pubs.acs.org.

JO0482615

(19) Jew, S.-s.; Terashima, S.; Koga, K. *Tetrahedron* **1979**, *35*, 2337 and references therein.
(20) Davies, A. G.; Ebeid, F. M.; Kenyon, J. *J. Chem. Soc.* **1957**, 3154.
(21) Frye, S. V.; Eliel, E. L. *Tetrahedron Lett.* **1985**, *26*, 3907.
(22) For various examples of ring opening reactions of electrophilic epoxides by nitrogen nucleophiles, see: (a) Chong, J. M.; Sharpless, K. B. *J. Org. Chem.* **1985**, *50*, 1560. (b) Behrens, C. H.; Sharpless, K. B. *J. Org. Chem.* **1985**, *50*, 5696. (c) Saito, S.; Takahashi, N.; Ishikawa, T.; Moriwake, T. *Tetrahedron Lett.* **1991**, *32*, 667. (d) Cacciola, J.; Alexander, R. S.; Fevig, J. M.; Stouten, P. F. W. *Tetrahedron Lett.* **1997**, *38*, 5741. (e) Fringuelli, F.; Pizzo, F.; Rucci, M.; Vaccaro, L. *J. Org. Chem.* **2003**, *68*, 7041 and references therein.
(23) Legters, J.; Thijs, L.; Zwanenburg, B. *Tetrahedron* **1991**, *47*, 5287.
(24) Davis, F.; Zhou, P. *Tetrahedron Lett.* **1994**, *35*, 7525.
(25) Avenoza, A.; Cativiela, C.; Corzana, F.; Peregrina, J. M.; Zurbano, M. M. *Tetrahedron: Asymmetry* **2000**, *11*, 2195.
(26) Jew, S.-s.; Kim, H.-a.; Kim, J.-h. *Heterocycles*, **1997**, *46*, 65.