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Efficient synthesis of highly enantiopure β-lactam derivatives from biocatalytic transformations of amides and nitriles

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ABSTRACT

Catalyzed by *Rhodococcus erythropolis* AJ270, a nitrile hydratase and amidase containing microbial strain, under mild conditions, kinetic resolution of racemic amides provides an efficient and scalable route to highly enantioenriched *R*-4-carboxymethyl-β-lactams and *S*-4-carbamoylmethyl-β-lactams despite the substrates contain a stereogenic center that is β-positioned to amide functionality. Synthetic potential of the method was demonstrated by the constructions of novel β-lactam-fused heterocyclic compounds through convenient and practical chemical transformations.

1. Introduction

Because of high biocatalytic efficiency, excellent selectivity and environmentally benign conditions, biotransformations have become important methods in organic synthesis. Biotransformations of nitriles[1] through either the nitrilase-catalyzed direct conversion of nitriles into carboxylic acids or the nitrile hydratase-catalyzed hydration of nitriles followed by the amidase-effected amide hydrolysis complement the existing asymmetric chemical and enzymatic methods for the synthesis of chiral carboxylic acids and their derivatives.[2] In comparison with enzymatic ester hydrolysis, biocatalysis of nitrile and amide generates straightforwardly valuable enantiopure amides in addition to the formation of enantiopure carboxylic acids.[1–3] One of the widely used nitrile-hydrolyzing biocatalysts is *Rhodococcus erythropolis* AJ270, which is a nitrile hydratase and amidase containing microbial strain.[4] The versatility of this whole cell biocatalyst has been shown by enantioselective biotransformations of a large number of structurally diverse racemic nitriles including functionalized nitriles and (hetero)cyclic nitriles to produce enantioenriched carboxylic acid and amide products.[5] It has been also reported that while the nitrile hydratase exhibits low or virtually no enantioselectivity, the amidase is highly enantioselective.[5] The amidase has been therefore utilized recently to achieve enantioselective desymmetrization of prochiral[6] and *meso* dicarboxamides.[7] Although the amidase displays high enantioselectivity towards many types of amides,[5] the level of enantioselection of the enzyme decreases dramatically when the stereogenic center of racemic amide substrates moves away from α- to β- or γ-position of amido functionality.[8–12]

Functionalized β-lactams provide an important platform for the construction of antibiotics.[13] 4-Carboxymethyl-β-lactams, for example, serve as the key precursors to carbapenems.[14] In addition, functionalized β-lactams are useful intermediates in organic synthesis.[15] Although the synthesis of 4-carboxymethyl-β-lactams and their derivatives has been extensively studied, development of simple and efficient synthetic methods for enantiopure compounds is still of great importance.[16] We have previously discovered that biotransformations of nitriles and amides are very powerful in the preparation of enantiopure cyclopropane-,[17] oxirane-,[18] aziridine-,[19] azetidine-[20] and azetidinone-carboxylic acids and their amide derivatives.[21] Our continuous interest in synthetic biocatalysis of nitriles and amides led us to investigate the enantioselective biotransformations of 4-cyanomethyl- and 4-carbamoyl-β-lactams, the substrates contain a stereogenic center β-positioned to cyano and amido functional groups, respectively. We report herein highly efficient whole cell-catalyzed synthesis of functionalized β-lactam derivatives with enantiomeric excess values up to >99.5%.

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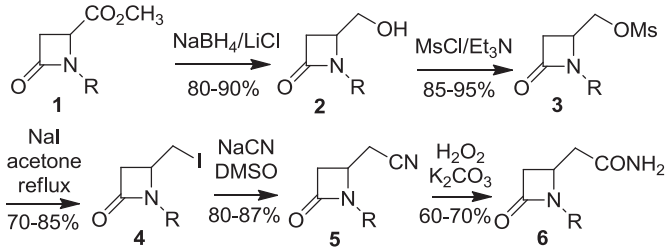
2. Results and discussion

The starting racemic substrates were synthesized from known compounds by means of simple and practical transformations. As illustrated in Scheme 1, for example, 4-methoxycarbonyl-β-lactames **1**[21] were reduced efficiently with $NaBH_4$ in the presence of LiCl to give alcohol derivatives **2**. Reaction of **2** with MsCl in the presence of triethylamine as an acid scavenger produced intermediates **3** in good yields. Treatment of **3** with NaI in refluxing acetone followed by efficient aliphatic substitution reaction with NaCN in DMSO led to the formation of desired nitrile substrates **5**. Subsequent chemical hydration reaction furnished the target amides **6**.

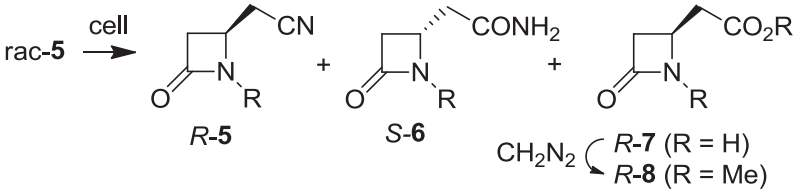


Scheme 1. Preparation of racemic 4-cyanomethyl-β-lactams **5** and **4**-carbamoylmethyl-β-lactams **6**.

The biotransformations of racemic 1-benzyl-4-cyanomethyl-β-lactam **5a** were first examined under standard conditions.[4] Thus, nitrile **5a** was incubated with *Rh. erythropolis* AJ270 whole cells at 30 °C in neutral phosphate buffer. To increase the solubility of nitrile in aqueous buffer, a small amount of acetone (0.5 mL) was added. The biocatalytic hydration of **5a** proceeded effectively, with nearly a half of the substrate being converted into amide **6a** within 2 h. Unfortunately, both amide product isolated and, particularly, the nitrile substrate recovered gave appallingly low enantiomeric excess values (entry 1, Table 1). It should be noted that the low enantioselectivity of the nitrile hydratase involved in the microbial cell towards 4-cyanomethyl-β-lactam **5a** was however not unexpected. As many studies have revealed, the nitrile hydratase exhibit generally very low enantioselectivity in the biotransformation of racemic nitriles. The move of a stereogenic center from α- to β-position within the nitrile substrates further decreases the level of enantioselection of the enzymes.[8–12] Since the enantioselectivity of nitrile hydration reaction was not satisfactory, we deliberately increased the reaction time, envisioning the amidase-catalyzed highly enantioselective transformation of the resulting racemic amide **6a**. To facilitate the separation of products, acid was converted quantitatively into methyl ester using excess CH_2N_2 after biotransformations. Although the complete hydration of nitrile **5a** was slow, an elongated reaction time (28 h) did lead to the formation of amide and acid in good yields. More pleasingly, excellent enantiomeric excess values were obtained for amide *S*-**6a** and ester *R*-**8a** (entry 2, Table 1). Encouraged by the observation of enantioselective biotransformations of nitrile **5a**, substrates that contain a brominated benzyl group on nitrogen were subjected to the whole cell-catalyzed transformations. Surprisingly, introduction of a bromo substituent into any position of the benzene ring resulted in the sluggish hydration reaction of cyano group. After one week's interaction with biocatalyst, for instance, a substantial amount of starting nitriles with low ee values were recovered along with the isolation of enantioenriched amide and acid products in low yields (entries 3–5, Table 1). Noticeably, when an *N*-arylmethyl group was replaced by an *N*-allyl moiety, biotransformations of **5i** proceeded efficiently under the identical conditions (entries 6–8, Table 1). The catalysis of the nitrile hydratase and the amidase in tandem afforded good yields and ee values of products *S*-**6i** and *R*-**8i** within 3 h (entry 8, Table 1). Although the effect of bromine substituent on the catalytic efficiency of the nitrile hydratase remains not clear, it is most probably due to the change of steric bulkiness of or altered hydrophobicity of the substrate that decrease the effective interaction between the enzyme and substrates.

In order to synthesize enantiomeric pure β-lactam-bearing acetamides and acetic acids, we then turned our attention to the biotransformation of amide taking the advantage of active and enantioselective amidase involved within the microbial cells. To our delight, under very mild conditions, *Rh. erythropolis* AJ270 displayed excellent activity and enantioselectivity in catalyzing the hydrolysis of racemic 4-carbamoylmethyl-β-lactams **6**. In stark contrast to biotransformations of racemic 1-arylmethyl-4-cyanomethyl-β-lactams **5**, results summarized in Table 2 reveal that all racemic amide substrates tested underwent very rapid reaction. For example, kinetic resolution of **6a** was achieved within 3.25 h (entry 1, Table 2). The presence of a bromo substituent on benzene ring, irrespective of the substitution pattern, did not slow down the reaction at all, with kinetic resolution of **6b–c** going completion in 2 h–3 h (entries 2–4, Table 2). Introduction of other

Table 1
Biotransformations of racemic 4-cyanomethyl-β-lactams **5**[a]



Ent.	(±)-**5**	Time	*R*-**5** (%)[b] (ee %)[c]	*S*-**6** (%)[b] (ee %)[c]	*R*-**8** (%)[b] (ee %)[c]
1	**5a** (R=Bn)	3.5 h	*R*-**5a** (40) (<3)	*S*-**6a** (40) (10.2)	—
2	**5a** (R=Bn)	28 h	—	*S*-**6a** (45) (94.4)	*R*-**8a** (43) (>99.5)
3	**5b** (R=*o*-$BrC_6H_4CH_2$)	7 d	*R*-**5b** (40) (21.0)	*S*-**6b** (29) (>99.5)	*R*-**8b** (30) (74.8)
4	**5c** (R=*m*-$BrC_6H_4CH_2$)	7 d	*R*-**5c** (75) (<3)	*S*-**6c** (10) (>99.5)	*R*-**8c** (13) (70.6)
5	**5d** (R=*p*-$BrC_6H_4CH_2$)	7 d	*R*-**5d** (40) (12.2)	*S*-**6d** (17) (>99.5)	*R*-**8d** (38) (67.4)
6	**5i** (R=Allyl)	0.7 h	*R*-**5i** (23) (8.0)	*S*-**6i** (67) (8.0)	*R*-**8i** (8) (>99.5)
7	**5i** (R=Allyl)	2 h	*R*-**5i** (8) (24.8)	*S*-**6i** (59) (43.0)	*R*-**8i** (25) (>99.5)
8	**5i** (R=Allyl)	3 h	—	*S*-**6i** (44) (87.0)	*R*-**8i** (48) (75.4)

[a] A mixture of substrate **5** (1 mmol), *Rhodococcus erythropolis* AJ270 (2 g wet weight) in phosphate buffer (0.1 M 50 mL) and acetone (0.5 mL) was incubated at 30 °C. The acid products **7** were converted directly into methyl esters **8** after biotransformations.
[b] Isolated yield.
[c] Determined by chiral HPLC analysis.

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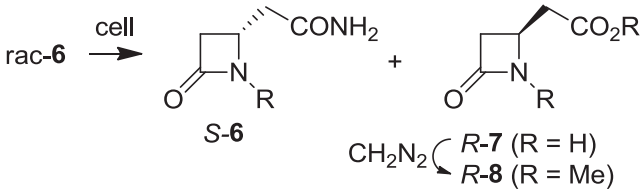
Table 2
Biotransformations of racemic nitriles **6**[a]



Ent.	Substrate	*t* (h)	*S*-**6** (%)[b] (ee %)[c]	*R*-**8** (%)[b] (ee %)[c]	*E*[d]
1	(±)-**6a** (R=Bn)	3.25	*S*-**6a** (41) (>99.5)	*R*-**8a** (46) (>99.5)	>200
2	(±)-**6b** (R=*o*-$BrC_6H_4CH_2$)	2.5	*S*-**6b** (44) (>99.5)	*R*-**8b** (45) (>99.5)	>200
3	(±)-**6c** (R=*m*-$BrC_6H_4CH_2$)	2	*S*-**6c** (43) (>99.5)	*R*-**8c** (49) (87.6)	81
4	(±)-**6d** (R=*p*-$BrC_6H_4CH_2$)	3	*S*-**6d** (47) (>99.5)	*R*-**8d** (46) (>99.5)	>200
5	(±)-**6e** (R=*o*-$ClC_6H_4CH_2$)	2.5	*S*-**6e** (44) (>99.5)	*R*-**8e** (45) (>99.5)	>200
6	(±)-**6f** (R=*o*-$FC_6H_4CH_2$)	2	*S*-**6f** (46) (94.4%)	*R*-**8f** (44) (94.2)	115
7	(±)-**6g** (R=*o*-$MeC_6H_4CH_2$)	2.5	*S*-**6g** (44) (>99.5)	*R*-**8g** (45) (>99.5)	>200
8	(±)-**6h** (R=*o*-$MeOC_6H_4CH_2$)	6	*S*-**6h** (46) (>99.5)	*R*-**8h** (45) (>99.5)	>200
9	(±)-**6i** (R=Allyl)	3	*S*-**6i** (42) (98.6)	*R*-**8i** (49) (89.1)	89
10	(±)-**6d** (R=*p*-$BrC_6H_4CH_2$)	33	*S*-**6d** (39) (>99.5)	*R*-**8d** (51) (71.2)	29
11[e]	(±)-**6h** (R=*o*-$MeOC_6H_4CH_2$)	24	*S*-**6h** (45) (>99.5)	*R*-**8h** (46) (>99.5)	>200

[a] Substrate **6** (1 mmol) was incubated with *Rhodococcus erythropolis* AJ270 (2 g wet weight) in phosphate buffer (0.1 M 50 mL) and acetone (0.5 mL) at 30 °C. The acid products **7** were converted directly into methyl esters **8** after biotransformations.
[b] Isolated yield.
[c] Determined by chiral HPLC analysis.
[d] *E* values were calculated based enantiomeric excess of products following a literature method.[22]
[e] 10 mmol of (±)-**6h**, 4 g wet weight of microbial cells were used.

substituents, such as chloro (**6e**), fluoro (**6f**) and methyl (**6g**) into the benzene ring led to equally efficient conversion of amide function group into acid within 2.5 (entries 5–7, Table 2). Only in the case of 4-carbamoylmethyl-β-lactam **6h** that contains an *N*-1-(2-methoxyphenyl)methyl group, an elongated time (6 h) was required (entry 8, Table 2) due to probably the increased steric hindrance of the substrate. Efficient amidase-catalyzed hydrolysis was expectedly observed for racemic 1-allyl-4-carbamoylmethyl-β-lactam **6i** (entry 9, Table 2). It is noteworthy that, in addition to very fast reaction velocity, the amidase-catalyzed kinetic resolution gave excellent enantioselectivity. This has been exemplified by high enantioselection values (*E*)[22] calculated from the enantiomeric excess values of resulting *S*-**6** and *R*-**8** of the reactions that ranged from 81 to >200 (Table 2). In many cases, enantiopure *S*-4-carbamoylmethyl-β-lactams *S*-**6** and *R*-4-methoxcarbonylmethyl-β-lactams *R*-**8** were obtained in high yields. It should be noted that careful monitoring of the amidase-catalyzed kinetic resolution process appeared important as a longer incubation time could cause more than 50% conversion of an amide substrate, leading, as a consequence, to the decrease of ee of the acid product (entry 10, Table 2). It is also worth mentioning that although the C–N bond of β-lactam is more reactive than that of other amides because of the ring strain, in all *Rh. erythropolis* AJ270-catalyzed biotransformations of nitriles **5** and amides **6**, no β-lactam ring opening reaction was observed, indicating the amidase in microbial cells acts chemo-specifically on the C–N bond of primary carboxamides.

The structures of all products were supported by spectroscopic data and elemental analysis. To assign the absolute configuration of the products, single crystals of amide **6a** and ester **8d** obtained from biocatalysis were cultivated from the slow diffusion of petroleum ether into ethyl acetate solution. X-ray molecular structures, which are depicted in Fig. 1 and 2, show unambiguously the *S*- and *R*-configuration of **6a** and **8d**, respectively. The formation of *R*-configured acid and *S*-configured amide products indicated convincingly the *R*-enantioselectivity of the amidase involved in *Rhodococcus erythropolis* AJ270.

The high enantioselectivity of the amidase towards amide substrates **6** that have a β-stereogenic center is astonishing. Previous studies have revealed the dependence of enantioselectivity of the

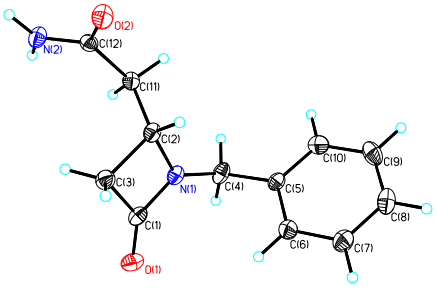


Fig. 1. X-ray molecular structure of *S*-**6a**.

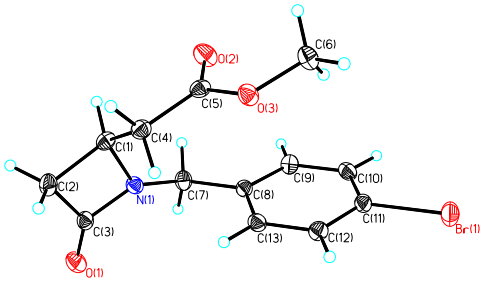


Fig. 2. X-ray molecular structure of *R*-**8d**.

amidase on the structure of the substrates. While either *S*- or *R*-enantiomers of racemic amides bearing an α-stereogentic center are effectively transformed by the enantioselective enzyme,[5] the chiral recognition of the amidase with amides that contain a stereogenic center β-positioned to amide function group is rather weak leading to a dramatically decreased level of enantiocontrol.[8–12] Only in the case of racemic substrates functionalized with a carbon–carbon unsaturated bond, do the amidase-catalyzed reactions afford high enantioselection.[23] Although further studies are badly needed to elucidate a detailed mechanism of enantioselective amidase

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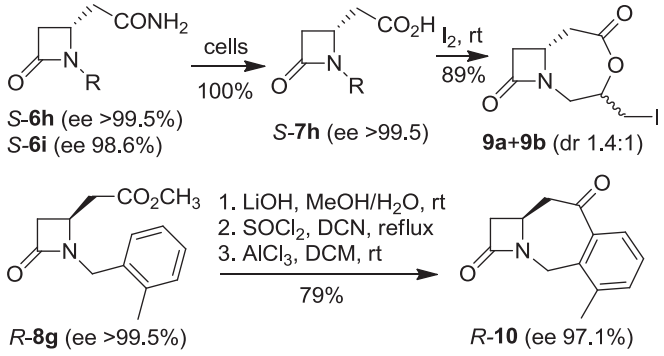
involved in *Rh. erythropolis* AJ270, highly efficient and enantioselective biotransformation of β-lactam-bearing acetamides aforementioned implies that the amidase may comprise most likely a enantioselective binding site for β-lactam moiety.

To demonstrate the practical utility of the method, a multigram scale biotransformation was implemented. Thus, under the identical conditions using 4 g wet weight *Rh. erythropolis* AJ270, 10 mmol (2.48 g) of racemic **6h** were successfully resolved into enantiopure *S*-**6h** (1.12 g, ee >99.5%) and *R*-**7h** (1.21 g, ee >99.5%) in almost quantitative yields in 24 h (entry 11, Table 2). Coupled with further biocatalytic hydrolysis of *S*-amides, *S*-configured acid products were also obtained. For example, catalyzed by the *Rh. erythropolis* AJ270 whole cell catalyst, enantiopure amide *S*-**6h** underwent complete hydrolysis to afford acid *S*-**7h** without losing enantiomeric purity (Scheme 2). It is worth emphasizing that chemical hydrolysis of amide *S*-**6h** led to the decomposition of β-lactam. Both amide and acid products obtained from biocatalysis are conceivably valuable chiral intermediates in organic synthesis.[15] They can act as unique precursors to carbapenam antibiotics.[14] The functional groups presented within the molecules also provide handles for further chemical manipulations. To illustrate the synthetic potential of the method, synthesis of β-lactam-fused heterocycles was performed. As shown in Scheme 2, iodolactonization of *S*-**7i** that was readily prepared from biocatalytic hydrolysis of *S*-**6i**, gave straightforwardly a mixture of bicyclic products **9** in 89% yield with a diastereomeric ratio being 1.4:1. A β-lactam-fused benzoazepinone product *R*-**10** was synthesized conveniently in a good yield from the saponification of *R*-**8g** with LiOH followed consecutively by the reaction with $SOCl_2$ and an intramolecular Friedel–Crafts acylation reaction.



Scheme 2. Synthetic applications.

3. Conclusion

In summary, we have shown that the amidase involved in *Rh. erythropolis* AJ270 is able to catalyze enantioselective hydrolysis of β-lactam-bearing acetamides while the nitrile hydratase exhibits low enzymatic activity with virtually no enantiocontrol towards β-lactam-bearing acetonitrile substrates. The microbial whole cell-catalyzed kinetic resolution provides an efficient and scalable route to highly enantioenriched *R*-4-carboxymethyl-β-lactams and *S*-4-carbamoylmethyl-β-lactams under mild conditions despite the substrates contain a stereogenic center that is β-positioned to amide functionality. The resulting functionalized β-lactam derivatives that are not easily accessible by other means, are useful chemical entities and their applications are demonstrated by the constructions of novel β-lactam-fused heterocyclic compounds through convenient and practical chemical transformations.

4. Experimental section

To a solution of reactant **1** (50 mmol) in THF (70 mL) was added LiCl (0.1 mol, 6.05 g), $NaBH_4$ (0.1 mol, 3.8 g) and ethanol (140 mL). The mixture was kept stirring overnight at room temperature. The reaction was quenched by adding water (100 mL) at 0 °C, and the mixture was extracted with ethyl acetate (3×100 mL). The combined organic layer was dried over anhydrous Na_2SO_4. After filtration and removal of solvent, crude product **2** was obtained in 80%–90% yield. Without further purification, the crude product **2** was mixed with triethylamine (0.1 mol, 13.9 mL) in DCM (200 mL) at 0 °C. Methylsulfonyl chloride (75 mmol, 5.8 mL) was added slowly, and the resulting mixture was stirred for another one hour at ambient temperature. After mixing with water (400 mL), organic phase was separated and washed with brine (2×100 mL) and dried over anhydrous Na_2SO_4. Solvent was removed under vacuum, and the residue was chromatographied on a silica gel column using a mixture of petroleum ether and ethyl acetate (*v*:*v*=1:1) as mobile phase to give crude product **3** in 85%–95% yield. The crude product **3** was refluxed overnight with NaI (0.16 mol, 24.0 g) in acetone (200 mL). Water (200 mL) was added and the mixture was extracted with DCM (3×100 mL). After drying over anhydrous Na_2SO_4, organic solvent was removed under vacuum to afford crude product **4** in 70%–85% yields. The alkyl iodide **4** obtained was stirred with NaCN powder (0.16 mol, 7.8 g) in DMF (80 mL) at room temperature for 24 h. Water (150 mL) was added and the mixture was extracted with ethyl acetate (4×50 mL). The combined organic layer was dried over anhydrous Na_2SO_4. After removal of solvent under vacuum, the residue was chromatographed on a silica gel column using a mixture of petroleum ether and ethyl acetate (*v*:*v*=1:1) as mobile phase to give pure product **5**.

4.1. (±)-5a as colorless oil (4.67 g, 23.3 mmol)

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.27–7.38 (m, 5H), 4.58 (d, *J*=15.0 Hz, 1H), 4.30 (d, *J*=15.0 Hz, 1H), 3.75 (ddd, *J*=7.8, 5.4, 2.4 Hz, 1H), 3.21 (dd, *J*=15.0, 5.1 Hz, 1H), 2.84 (dd, *J*=15.0, 2.4 Hz, 1H), 2.56 (dd, *J*=16.8, 5.1 Hz, 1H), 2.48 (dd, *J*=17.1, 5.7 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.4, 134.9, 129.1, 128.5, 128.3, 115.9, 46.9, 45.4, 42.8, 21.9; IR (KBr) ν 2251, 1751 cm^{-1}; MS (EI) *m*/*z* (%) 201 $[M+H]^+$, 223 $[M+Na]^+$. HRMS (TOF-MS-EI) Anal. Calcd for $C_{12}H_{12}N_2O$: 200.0950 $[M]^+$. Found: 200.0952 $[M]^+$.

4.2. (±)-5b as colorless oil (3.30 g, 11.8 mmol)

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57 (d, *J*=8.1 Hz, 1H), 7.34–7.39 (m, 2H), 7.21–7.28 (m, 1H), 4.70 (d, *J*=15.0 Hz, 1H), 4.45 (d, *J*=15.0 Hz, 1H), 3.81 (ddd, *J*=6.6, 4.8, 2.4 Hz, 1H), 3.24 (dd, *J*=15.0, 5.1 Hz, 1H), 2.90 (dd, *J*=15.0, 2.4 Hz, 1H), 2.69 (dd, *J*=17.1, 4.5 Hz, 1H), 2.60 (dd, *J*=16.8, 6.0 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.4, 134.2, 133.2, 131.2, 130.1, 128.3, 123.7, 115.6, 47.3, 45.3, 42.8, 21.8; IR (KBr) ν 2251, 1754 cm^{-1}; MS (ESI) *m*/*z* (%) 301 $[M+Na]^+$ (79Br), 303 $[M+Na]^+$ (81Br). Anal. Calcd for $C_{12}H_{12}BrN_2O$: C, 51.63; H, 3.97; N, 10.04. Found: C, 51.51; H, 4.02; N, 9.92.

4.3. (±)-5c as colorless oil (3.60 g, 12.9 mmol)

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45–7.46 (m, 2H), 7.22–7.29 (m, 2H), 4.56 (d, *J*=15.3 Hz, 1H), 4.26 (d, *J*=15.3 Hz, 1H), 3.79 (ddd, *J*=7.8, 5.4, 2.4 Hz, 1H), 3.23 (dd, *J*=15.0, 5.1 Hz, 1H), 2.86 (dd, *J*=15.0, 2.1 Hz, 1H), 2.59 (d, *J*=5.4 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.4, 137.4, 131.4, 131.3, 130.7, 127.0, 123.0, 115.9, 47.1, 44.6, 43.0, 21.9; IR (KBr) ν 2251, 1752 cm^{-1}; MS (ESI) *m*/*z* (%) 301 $[M+Na]^+$ (79Br), 303 $[M+Na]^+$ (81Br). Anal. Calcd for $C_{12}H_{12}BrN_2O$: C, 51.63; H, 3.97; N, 10.04. Found: C, 51.59; H, 4.00; N, 9.81.

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4.4. (±)-5d as white solid (3.45 g, 12.4 mmol)

Mp 39.0–40.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.49 (d, J=8.4 Hz, 2H), 7.20 (d, J=8.4 Hz, 2H), 4.52 (d, J=15.3 Hz, 1H), 4.25 (d, J=15.3 Hz, 1H), 3.77 (ddd, J=7.5, 5.4, 2.4 Hz, 1H), 3.19 (dd, J=15.0, 5.1 Hz, 1H), 2.83 (dd, J=14.7, 2.1 Hz, 1H), 2.59 (d, J=5.4 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.3, 134.1, 130.0, 122.0, 122.6, 115.9, 46.9, 44.5, 42.7, 21.7; IR (KBr) ν 2251, 1752 cm^{-1}; MS (ESI) m/z (%) 301 $[M+Na]^+$ (79Br), 303 $[M+Na]^+$ (81Br). Anal. Calcd for $C_{12}H_{11}BrN_2O$: C, 51.63; H, 3.97; N, 10.04. Found: C, 51.28; H, 4.13; N, 9.85.

4.5. (±)-5e as white solid (3.14 g, 13.4 mmol)

Mp 63.0–64.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43–7.38 (m, 2H), 7.33–7.29 (m, 2H), 4.68 (d, J=15.2 Hz, 1H), 4.42 (d, J=15.2 Hz, 1H), 3.81–3.75 (m, 1H), 3.22 (dd, J=14.9, 5.1 Hz, 1H), 2.88 (dd, J=14.9, 2.2 Hz, 1H), 2.71–2.55 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.3, 133.7, 132.5, 131.3, 129.9, 127.7, 115.6, 47.3, 42.9, 42.8, 21.8; IR (KBr) ν 2251, 1751 cm^{-1}; MS (ESI) m/z (%) 257 $[M+Na]^+$. HRMS (FT-MS-ESI) Anal. Calcd for $C_{12}H_{11}N_2OCl$: 257.0452 $[M+Na]^+$. Found: 257.0449 $[M+Na]^+$.

4.6. (±)-5f as colorless oil (4.02 g, 18.4 mmol)

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37–7.30 (m, 2H), 7.20–7.07 (m, 2H), 4.61 (d, J=15.2 Hz, 1H), 4.32 (d, J=15.2 Hz, 1H), 3.81–3.75 (m, 1H), 3.19 (dd, J=14.9, 5.1 Hz, 1H), 2.86 (dd, J=14.9, 2.2 Hz, 1H), 2.68–2.58 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.2, 162.5, 159.3, 131.2, 131.2, 130.4, 130.3, 125.0, 124.9, 122.1, 121.9, 115.9, 115.6, 47.1, 42.9, 38.8, 38.7, 21.7; IR (KBr) ν 2251, 1755 cm^{-1}; MS (ESI) m/z (%) 219 $[M+H]^+$. HRMS (FT-MS-ESI) Anal. Calcd for $C_{12}H_{11}N_2OF$: 219.0928 $[M+H]^+$. Found: 219.0928 $[M+H]^+$.

4.7. (±)-5g as white solid (3.42 g, 16.0 mmol)

Mp 85.0–86.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26–7.20 (m, 4H), 4.46 (dd, J=44.9, 14.9 Hz, 2H), 3.71–3.65 (m, 1H), 3.20 (dd, J=14.9, 5.1 Hz, 1H), 2.87–2.82 (m, 1H), 2.53–2.35 (m, 2H), 2.37 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.1, 137.0, 132.4, 131.2, 129.6, 128.7, 126.5, 115.6, 47.0, 43.7, 42.7, 21.8, 19.2; IR (KBr) ν 2250, 1750 cm^{-1}; MS (ESI) m/z (%) 237 $[M+Na]^+$. HRMS (FT-MS-ESI) Anal. Calcd for $C_{13}H_{14}N_2O$: 237.0998 $[M+Na]^+$. Found: 237.0996 $[M+Na]^+$.

4.8. (±)-5h as colorless oil (5.76 g, 25.0 mmol)

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35–7.26 (m, 2H), 6.99–6.90 (m, 2H), 4.53 (d, J=14.6 Hz, 1H), 4.29 (d, J=14.6 Hz, 1H), 3.87 (s, 3H), 3.73–3.67 (m, 1H), 3.14 (dd, J=14.8, 5.0 Hz, 1H), 2.84–2.78 (m, 1H), 2.71–2.51 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.2, 157.4, 130.9, 129.9, 123.0, 121.1, 115.9, 110.6, 55.4, 47.1, 42.7, 40.2, 21.6; IR (KBr) ν 2250, 1750 cm^{-1}; MS (ESI) m/z (%) 253 $[M+Na]^+$. HRMS (FT-MS-ESI) Anal. Calcd for $C_{13}H_{14}N_2O_2$: 253.0948 $[M+Na]^+$. Found: 253.0946 $[M+Na]^+$.

4.9. (±)-5i as colorless oil (1.81 g, 12.1 mmol)

^{1}H NMR (300 MHz, $CDCl_3$) δ 5.74–5.87 (m, 1H), 5.26–5.34 (m, 2H), 4.49 (dd, J=15.6, 6.0 Hz, 1H), 3.89 (ddd, J=7.5, 5.4, 2.4 Hz, 1H), 3.79 (dd, J=15.6, 6.6 Hz, 1H), 3.21 (dd, J=15.0, 5.1 Hz, 1H), 2.83 (dd, J=15.0, 2.1 Hz, 1H), 2.74 (d, J=5.4 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.2, 131.4, 119.6, 115.9, 47.1, 43.9, 42.7, 22.0; IR (KBr) ν 2250, 1750 cm^{-1}; MS (EI) m/z (%) 151 $[M+H]^+$, 173 $[M+Na]^+$. HRMS (TOF-MS-EI) Anal. Calcd for $C_8H_{10}N_2O$: 150.0793. Found: 150.0791.

4.10. General procedure for the synthesis of racemic amides 6

To a solution of racemic **5** (10 mmol) in ethanol (40 mL) was added anhydrous K_2CO_3 powder (300 mg) while stirring. Aqueous H_2O_2 (30%, 7.5 mL) was then added slowly at 0 °C, and the resulting mixture was stirred overnight. A saturated aqueous solution of sodium thiosulfate ($Na_2S_2O_3$) (50 mL) was added, and the mixture was extracted with ethyl acetate (3×20 mL). Organic phase was dried over anhydrous Na_2SO_4. After removal of solvent, the residue was chromatographed on a silica gel column eluted with a mixture of ethyl acetate and acetone (v:v=1:1) to give pure amide products **6.**

*4.10.1. (±)-**6a** as white solid (1.42 g, 65%).* Mp 116.0–117.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.27–7.37 (m, 5H), 5.35 (br s, 1H), 5.30 (br s, 1H), 4.44 (d, J=15.0 Hz, 1H), 4.33 (d, J=15.0 Hz, 1H), 3.99 (ddd, J=8.7, 6.6, 2.1 Hz, 1H), 3.19 (dd, J=14.7, 5.1 Hz, 1H), 2.71 (dd, J=14.7, 2.1 Hz, 1H), 2.46 (dd, J=15.6, 6.6 Hz, 1H), 2.37 (dd, J=15.3, 6.9 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.2, 167.0, 136.1, 128.8, 128.2, 127.8, 48.4, 45.1, 42.8, 39.3; IR (KBr) ν 3365, 3188, 1739, 1658, 1622 cm^{-1}; MS (ESI) m/z (%) 219 $[M+H]^+$, 241 $[M+Na]^+$; Anal. Calcd for $C_{12}H_{14}N_2O_2$: C, 66.04; H, 6.47; N, 12.84. Found: C, 66.18; H, 6.34; N, 12.85.

*4.10.2. (±)-**6b** as white solid (1.75 g, 59%).* Mp 109.0–110.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56 (d, J=8.1 Hz, 1H), 7.28–7.35 (m, 2H), 7.15–7.20 (m, 1H), 5.77 (br s, 1H), 5.68 (br s, 1H), 4.61 (d, J=15.6 Hz, 1H), 4.40 (d, J=15.6 Hz, 1H), 3.96 (ddd, J=7.8, 5.4, 2.4 Hz, 1H), 3.21 (dd, J=14.7, 5.1 Hz, 1H), 2.76 (dd, J=14.7, 2.1 Hz, 1H), 2.63 (dd, J=15.0, 5.4 Hz, 1H), 2.36 (dd, J=15.0, 8.1 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.6, 167.0, 135.1, 133.1, 130.4, 129.6, 128.0, 123.5, 48.8, 45.2, 43.2, 39.1; IR (KBr) ν 3381, 3202, 1752, 1647, 1614 cm^{-1}; MS (ESI) m/z (%) 319 $[M+Na]^+$ (79Br), 321 $[M+Na]^+$ (81Br). Anal. Calcd for $C_{12}H_{13}BrN_2O_2$: C, 48.50; H, 4.41; N, 9.43. Found: C, 48.39; H, 4.42; N, 9.11.

*4.10.3. (±)-**6c** as white solid (1.57 g, 53%).* Mp 109.0–110.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40–7.43 (m, 2H), 7.18–7.26 (m, 2H), 5.38 (br s, 1H), 5.33 (br s, 1H), 4.41 (d, J=15.3 Hz, 1H), 4.31 (d, J=15.3 Hz, 1H), 4.02 (ddd, J=9.0, 6.9, 2.4 Hz, 1H), 3.20 (dd, J=15.0, 5.1 Hz, 1H), 2.71 (dd, J=14.7, 2.4 Hz, 1H), 2.45 (d, J=6.6 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.8, 167.0, 138.8, 131.2, 130.8, 130.4, 126.9, 122.7, 48.5, 44.6, 42.9, 39.5; IR (KBr) ν 3361, 3186, 1741, 1657 cm^{-1}; MS (ESI) m/z (%) 319 $[M+Na]^+$ (79Br), 321 $[M+Na]^+$ (81Br). Anal. Calcd for $C_{12}H_{13}BrN_2O_2$: C, 48.50; H, 4.41; N, 9.43. Found: C, 48.35; H, 4.48; N, 9.10.

*4.10.4. (±)-**6d** as white solid (1.87 g, 63%).* Mp 129.0–130.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45 (d, J=8.1 Hz, 2H), 7.17 (d, J=8.1 Hz, 2H), 5.70 (br s, 1H), 5.61 (br s, 1H), 4.40 (d, J=15.3 Hz, 1H), 4.25 (d, J=15.3 Hz, 1H), 3.98 (d, J=5.7 Hz, 1H), 3.16 (dd, J=14.7, 5.1 Hz, 1H), 2.69 (d, J=14.4 Hz, 1H), 2.44 (d, J=6.6 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.6, 166.9, 135.5, 131.9, 130.1, 121.7, 48.3, 44.7, 42.9, 39.6; IR (KBr) ν 3384, 3204, 1738, 1667, 1622 cm^{-1}; MS (ESI) m/z (%) 319 $[M+Na]^+$ (79Br), 321 $[M+Na]^+$ (81Br). Anal. Calcd for $C_{12}H_{13}BrN_2O_2$: C, 48.50; H, 4.41; N, 9.43. Found: C, 48.28; H, 4.37; N, 9.24.

*4.10.5. (±)-**6e** as white solid (1.46 g, 58%).* Mp 99.0–100.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39–7.35 (m, 2H), 7.29–7.24 (m, 2H), 5.39 (br s, 2H), 4.61 (d, J=15.5 Hz, 1H), 4.42 (d, J=15.5 Hz, 1H), 4.00–3.92 (m, 1H), 3.22 (dd, J=14.9, 5.1 Hz, 1H), 2.75 (dd, J=14.9, 2.2 Hz, 1H), 2.61 (dd, J=15.2, 5.4 Hz, 1H), 2.35 (dd, J=15.2, 8.0 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.6, 167.0, 133.4, 133.4, 130.4, 129.8, 129.3, 127.3, 48.7, 43.2, 42.6, 39.1; IR (KBr) ν 3341, 3195, 1736, 1668 cm^{-1}; MS (ESI) m/z (%) 275 $[M+Na]^+$. Anal. Calcd for

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$C_{12}H_{13}ClN_2O_2$: C, 57.04; H, 5.19; N, 11.09. Found: C, 56.92; H, 5.18; N, 11.19.

*4.10.6. (±)-**6f** as white solid (1.58 g, 67%).* Mp 84.0–85.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36–7.25 (m, 2H), 7.16–7.03 (m, 2H), 5.63 (br s, 2H), 4.54 (d, J=15.3 Hz, 1H), 4.32 (d, J=15.3 Hz, 1H), 3.98–3.91 (m, 1H), 3.17 (dd, J=14.9, 5.1 Hz, 1H), 2.76–2.70 (m, 1H), 2.66–2.59 (m, 1H), 2.38 (dd, J=15.2, 7.7 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.5, 166.9, 162.4, 159.1, 130.8, 130.7, 129.9, 129.7, 124.6, 124.6, 123.1, 122.9, 115.7, 115.5, 48.5, 43.1, 39.0, 38.6, 38.5; IR (KBr) ν 3351, 3196, 1736, 1671 cm^{-1}; MS (ESI) m/z (%) 259 $[M+Na]^+$. Anal. Calcd for $C_{12}H_{13}FN_2O_2$: C, 61.01; H, 5.55; N, 11.86. Found: C, 61.22; H, 5.70; N, 11.98.

*4.10.7. (±)-**6g** as white solid (1.62 g, 70%).* Mp 131.0–132.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.21–7.17 (m, 4H), 5.42 (br s, 1H), 5.33 (br s, 1H), 4.40 (q, J=15.2 Hz, 2H), 3.93–3.86 (m, 1H), 3.18 (dd, J=14.8, 5.1 Hz, 1H), 2.76–2.70 (m, 1H), 2.47–2.25 (m, 2H), 2.34 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4, 166.7, 136.6, 133.7, 130.7, 129.2, 128.1, 126.2, 48.6, 43.3, 43.0, 39.1, 19.1; IR (KBr) ν 3390, 3199, 1732, 1671 cm^{-1}; MS (ESI) m/z (%) 255 $[M+Na]^+$. Anal. Calcd for $C_{13}H_{16}N_2O_2$: C, 67.22; H, 6.94; N, 12.06. Found: C, 67.20; H, 6.99; N, 12.05.

*4.10.8. (±)-**6h** as white solid (1.83 g, 74%).* Mp 121.0–122.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34–7.24 (m, 2H), 7.01–6.92 (m, 2H), 4.84 (s, 2H), 4.53 (d, J=15.0 Hz, 1H), 4.26 (d, J=15.0 Hz, 1H), 3.89–3.83 (m, 1H), 3.87 (s, 3H), 3.07 (dd, J=14.7, 4.9 Hz, 1H), 2.75–2.64 (m, 2H), 2.39 (dd, J=14.6, 8.1 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.0, 169.4, 158.9, 131.1, 130.5, 125.0, 121.7, 111.8, 55.9, 50.3, 43.0, 41.1, 39.6; IR (KBr) ν 3350, 3195, 1732, 1669 cm^{-1}; MS (ESI) m/z (%) 271 $[M+Na]^+$. Anal. Calcd for $C_{13}H_{16}N_2O_3$: C, 62.89; H, 6.50; N, 11.28. Found: C, 63.00; H, 6.68; N, 11.44.

*4.10.9. (±)-**6i** as white solid (0.77 g, 46%).* Mp 67.0–68.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.70–5.85 (m, 1H), 5.70 (s, 1H), 5.18–5.26 (m, 2H), 4.55 (s, 1H), 4.05 (ddd, J=8.7, 6.9, 2.1 Hz, 1H), 3.94 (dd, J=15.6, 5.7 Hz, 1H), 3.71 (dd, J=11.1, 6.6 Hz, 1H), 3.18 (dd, J=14.7, 5.1 Hz, 1H), 2.69 (dd, J=15.0, 2.7 Hz, 1H), 2.67 (dd, J=15.3, 6.6 Hz, 1H), 2.47 (dd, J=15.3, 7.2 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.2, 167.0, 132.0, 118.4, 48.4, 43.7, 42.7, 39.3; IR (KBr) ν 3324, 3198, 1734, 1671 cm^{-1}; MS (ESI) m/z (%) 169 $[M+H]^+$, 191 $[M+Na]^+$. HRMS (FT-ICRMS) Anal. Calcd for $C_8H_{12}N_2O_2$: 169.0972 $[M+H]^+$. Found: 169.0968 $[M+H]^+$.

4.11. General procedure for biotransformations of amides 6 and nitriles 5

In an Erlenmeyer flask (150 mL) with a screw cap a suspension of *Rh. erythropolis* AJ270 cells[16] (2 g wet weight) in aqueous phosphate buffer (pH 7.0, 0.1 M, 50 mL) was activated at 30 °C for 0.5 h. A solution of amides **6** or nitriles **5** (1 mmol) in acetone (0.5 mL) was then added in one portion, and the resulting mixture was incubated at 30 °C with orbital shaking (200 rpm). The reaction process was monitored using TLC method. After a period of time (see Tables 1 and 2), the reaction was quenched by removing microbial cells through a Celite pad filtration. The filtration cake was washed with water (3×15 mL) and ethyl acetate (3×15 mL). The aqueous layer was extracted with ethyl acetate. The combined organic phase was dried over anhydrous Na_2SO_4. After removal of solvent, the residue was chromatographed on a silica gel column using a mixture of petroleum ether and acetone (v:v=1:1) to afford pure nitriles **5** and amides **6**. The aqueous solution that contains acid product was freeze-dried, and residue was dissolved in methanol (5 mL) and then treated with CH_2N_2 solution in ether (5 mL) at −15 °C. The mixture was kept stirring at room temperature for 1 h, and then mixed with water (10 mL) and extracted with ethyl acetate (3×5 mL). The organic phase was combined and dried over anhydrous Na_2SO_4. Ester products **8a**–**d** were obtained after removal of solvent. In the case of the biotransformations of **6e**–**i**, the residue was column chromatographed on a silica gel column eluted first with ethyl acetate to give ester products **8e**–**i** then with ethyl acetate and acetone (v:v=1:1) to give additional amount of amides **6e**–**i**. Enantioenriched amide products have the identical spectroscopic data as that of racemic samples.

*4.11.1. (S)-(−)-**6a** as white solid (89 mg, 41%).* Mp 116.0–117.0 °C; $[\alpha]_D^{25}$ −37.5° (c 0.5, CH_3OH); ee 95.0% (HPLC analysis using a Chiralcel ADH column). Single crystal was obtained from recrystallization from a mixture of petroleum ether and ethyl acetate.

*4.11.2. (S)-(−)-**6b** as white solid (131 mg, 44%).* Mp 109.0–110.0 °C; $[\alpha]_D^{25}$ −12.1° (c 0.7, CH_3OH); ee>99.5% (HPLC analysis using a Chiralcel ADH column).

*4.11.3. (S)-(−)-**6c** as white solid (128 mg, 43%).* Mp 109.0–110.0 °C; $[\alpha]_D^{25}$ −6.7° (c 5.4, CH_3OH); ee>99.5% (HPLC analysis using a Chiralcel ODH column).

*4.11.4. (S)-(−)-**6d** as white solid (139 mg, 47%).* Mp 129.0–130.0 °C; $[\alpha]_D^{25}$ −9.3° (c 0.7, CH_3OH); ee>99.5% (HPLC analysis using a Chiralcel ADH column).

*4.11.5. (S)-(+)-**6e** as white solid (111 mg, 44%).* Mp 100.0–101.0 °C; $[\alpha]_D^{25}$ +4.0° (c 1.5, CH_3OH); ee>99.5% (HPLC analysis using a Chiralcel ADH column).

*4.11.6. (S)-(+)-**6f** as white solid (108 mg, 46%).* Mp 86.0–87.0 °C; $[\alpha]_D^{25}$ +1.3° (c 1.5, CH_3OH); ee=94.5% (HPLC analysis using a Chiralcel ADH column).

*4.11.7. (S)-(+)-**6g** as white solid (102 mg, 44%).* Mp 131.0–132.0 °C; $[\alpha]_D^{25}$ +9.6° (c 1.7, CH_3OH); ee>99.5% (HPLC analysis using a Chiralcel ADH column).

*4.11.8. (S)-(−)-**6h** as white solid (114 mg, 46%).* Mp 122.0–123.0 °C; $[\alpha]_D^{25}$ −15.3° (c 1.7, CH_3OH); ee>99.5% (HPLC analysis using a Chiralcel ADH column).

*4.11.9. (S)-(+)-**6i** as white solid (71 mg, 42%).* Mp 67.0–68.0 °C; $[\alpha]_D^{25}$ +2.0° (c 3.0, CH_3OH); ee 85.8% (HPLC analysis using a Chiralcel ADH column).

*4.11.10. (R)-(+)-**8a** as colorless oil (107 mg, 46%).* $[\alpha]_D^{25}$ +23.3° (c 0.6, benzene), ee>99.5% (HPLC analysis using a Chiralcel OJH column); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25–7.37 (m, 5H), 4.50 (d, J=15.3 Hz, 1H), 4.25 (d, J=15.3 Hz, 1H), 3.89 (ddd, J=8.7, 6.9, 2.4 Hz, 1H), 3.60 (s, 3H), 3.17 (dd, J=14.7, 5.1 Hz, 1H), 2.72 (dd, J=14.7, 2.1 Hz, 1H), 2.59 (dd, J=16.2, 6.3 Hz, 1H), 2.48 (dd, J=16.5, 7.2 Hz, 1H); ^{13}C NMR(75 MHz, $CDCl_3$) δ 170.8, 166.6, 136.1, 128.8, 128.2, 127.7, 51.9, 47.7, 45.1, 43.0, 38.1; IR (KBr) ν 1746 cm^{-1}; MS (ESI) m/z (%) 234 $[M+H]^+$, 256 $[M+Na]^+$. HRMS (TOF-MSEI) Anal. Calcd for $C_{13}H_{15}NO_3$. 233.1052. Found: 233.1056.

*4.11.11. (R)-(+)-**8b** as colorless oil (140 mg, 45%).* $[\alpha]_D^{25}$ +18.0° (c 1.0, diethyl ether); ee>99.5% HPLC analysis using a Chiralcel OJH column); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55 (d, J=8.1 Hz, 1H), 7.29–7.35 (m, 2H), 7.14–7.20 (m, 1H), 4.61 (d, J=15.6 Hz, 1H), 4.38 (d, J=15.6 Hz, 1H), 3.90 (ddd, J=7.5, 5.1, 2.1 Hz, 1H), 3.61 (s, 3H),

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3.20 (dd, *J*=14.7, 5.1 Hz, 1H), 2.75 (dd, *J*=14.7, 2.1 Hz, 1H), 2.71 (dd, *J*=15.9, 5.4 Hz, 1H), 2.49 (dd, *J*=16.2, 8.1 Hz, 1H); ^{13}C NMR(75 MHz, $CDCl_3$) δ 170.5, 166.7, 135.0, 133.0, 130.3, 129.5, 127.9, 123.4, 51.9, 48.2, 45.1, 43.2, 37.8; IR (KBr) ν 1752 cm^{-1}; MS (ESI) *m*/*z* (%) 334 $[M+Na]^+$ (79Br), 336 $[M+Na]^+$ (81Br). Anal. Calcd for $C_{13}H_{14}BrNO_3$: C, 50.02; H, 4.52; N, 4.49. Found: C, 51.01; H, 4.62; N, 4.33.

*4.11.12. (R)-(+)-**8c** as colorless oil (153 mg, 49%).* $[\alpha]_D^{25}$ +3.8° (*c* 1.6, diethyl ether); ee 87.6% (HPLC analysis using a Chiralcel ADH column); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40–7.43 (m, 2H), 7.21–7.23 (m, 2H), 4.44 (d, *J*=15.3 Hz, 1H), 4.25 (d, *J*=15.3 Hz, 1H), 3.94 (ddd, *J*=9.0, 6.9, 2.4 Hz, 1H), 3.62 (s, 3H), 3.19 (dd, *J*=14.7, 5.1 Hz, 1H), 2.73 (dd, *J*=14.7, 2.4 Hz, 1H), 2.57 (d, *J*=1.5 Hz, 1H), 2.55 (d, *J*=0.9 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.8, 166.7, 138.6, 131.1, 130.9, 130.4, 126.8, 122.7, 52.0, 48.0, 44.5, 43.0, 38.2; IR (KBr) ν 1744 cm^{-1}; MS (ESI) *m*/*z* (%) 334 $[M+Na]^+$ (79Br), 336 $[M+Na]^+$(81Br). HRMS (TOF-MS-EI) Anal. Calcd for $C_{13}H_{14}BrNO_3$: 311.0157 (79Br), 313.0137 (81Br). Found: 311.0160 (79Br), 313.0141 (81Br).

*4.11.13. (R)-(+)-**8d** as white solid (143 mg, 46%).* Mp 37.0–38.0 °C; $[\alpha]_D^{25}$ +7.6° (*c* 1.85, diethyl ether); ee>99.5% (HPLC analysis using a Chiralcel ADH column); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (d, *J*=8.4 Hz, 2H), 7.16 (d, *J*=8.4 Hz, 2H), 4.44 (d, *J*=15.6 Hz, 1H), 4.22 (d, *J*=15.6 Hz, 1H), 3.90 (ddd, *J*=8.7, 6.6, 2.1 Hz, 1H), 3.62 (s, 3H), 3.17 (dd, *J*=15.0, 5.1 Hz, 1H), 2.72 (dd, *J*=14.7, 2.1 Hz, 1H), 2.58 (dd, *J*=16.5, 6.6 Hz, 1H), 2.52 (dd, *J*=13.2, 3.3 Hz, 1H); ^{13}C NMR(75 MHz, $CDCl_3$) δ 170.8, 166.7, 135.3, 131.9, 129.9, 121.7, 51.9, 47.8, 44.6, 43.0, 38.2; IR (KBr) ν 1729 cm^{-1}; MS (ESI) *m*/*z* (%) 334 $[M+Na]^+$(79Br), 336 $[M+Na]^+$(81Br). HRMS (TOF-MS-EI) Anal. Calcd for $C_{13}H_{14}BrNO_3$: 311.0157 (79Br); 313.0137 (81Br). Found: 311.0160 (79Br); 313.0140 (81Br). X-ray quality single crystal was obtained from recrystallization from a mixture of petroleum ether and ethyl acetate.

*4.11.14. (R)-(−)-**8e** as colorless oil (120 mg, 45%).* $[\alpha]_D^{25}$ −13.7° (*c* 2.0, $CHCl_3$); ee>99.5% (HPLC analysis using a Chiralcel OJH column); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39–7.33 (m, 2H), 7.29–7.24 (m, 2H), 4.62 (d, *J*=15.6 Hz, 1H), 4.39 (d, *J*=15.6 Hz, 1H), 3.93–3.86 (m, 1H), 3.62 (s, 3H), 3.20 (dd, *J*=14.9, 5.1 Hz, 1H), 2.78–2.67 (m, 2H), 2.48 (dd, *J*=16.0, 7.9 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.5, 166.7, 133.4, 133.4, 130.3, 129.8, 129.3, 127.3, 51.9, 48.2, 43.2, 42.6, 37.8; IR (KBr) ν 2953, 1755 cm^{-1}; MS (ESI) *m*/*z* (%) 290 $[M+Na]^+$. HRMS (FT-MS-ESI) Anal. Calcd for $C_{13}H_{14}NO_3Cl$: 290.0554 $[M+Na]^+$. Found: 290.0551 $[M+Na]^+$.

*4.11.15. (R)-(−)-**8f** as colorless oil (110 mg, 44%).* $[\alpha]_{25}D$ −7.6° (*c* 2.0, $CHCl_3$); ee=94.2% HPLC analysis using a Chiralcel ADH column); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32–7.25 (m, 2H), 7.16–7.03 (m, 2H), 4.55 (d, *J*=15.4 Hz, 1H), 4.30 (d, *J*=15.4 Hz, 1H), 3.92–3.86 (m, 1H), 3.64 (s, 3H), 3.16 (dd, *J*=14.8, 5.1 Hz, 1H), 2.75–2.68 (m, 2H), 2.50 (dd, *J*=16.1, 7.5 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.6, 166.6, 162.4, 159.1, 130.7, 130.6, 129.8, 129.7, 124.6, 124.5, 123.1, 122.9, 115.7, 115.4, 51.9, 48.1, 43.2, 38.4, 38.4, 37.8; IR (KBr) ν 2954, 1752 cm^{-1}; MS (ESI) *m*/*z* (%) 274 $[M+Na]^+$. HRMS (FT-MS-ESI) Anal. Calcd for $C_{13}H_{14}NO_3F$: 274.0850 $[M+Na]^+$. Found: 274.0847 $[M+Na]^+$.

*4.11.16. (R)-2-(−)-**8g** as colorless oil (111 mg, 45%).* $[\alpha]_D^{25}$ −18.5° (*c* 2.0, $CHCl_3$); ee>99.5% (HPLC analysis using a Chiralcel ODH column); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.20–7.16 (m, 4H), 4.52 (d, *J*=15.2 Hz, 1H), 4.39 (d, *J*=15.2 Hz, 1H), 3.84–3.77 (m, 1H), 3.59 (s, 3H), 3.17 (dd, *J*=14.8, 5.1 Hz, 1H), 2.72 (dd, *J*=14.8, 2.1 Hz, 1H), 2.56 (dd, *J*=16.0, 5.4 Hz, 1H), 2.45–2.39 (m, 1H), 2.33 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.5, 166.4, 136.5, 133.4, 130.7, 129.1, 128.1, 126.2, 51.8, 47.9, 43.2, 43.0, 37.8, 19.1; IR (KBr) ν 2953, 1739 cm^{-1}; MS (ESI) *m*/*z* (%) 270 $[M+Na]^+$; HRMS (ESI) Anal. Calcd for $C_{14}H_{17}NO_3$: 270.1101 $[M+Na]^+$, Found: 270.1098 $[M+Na]^+$.

*4.11.17. (R)-(+)-**8h** colorless oil (118 mg, 45%).* $[\alpha]_D^{25}$ +3.0° (c 1.4, $CHCl_3$); ee>99.5% (HPLC analysis using a Chiralcel ODH column); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31–7.22 (m, 2H), 6.95–6.86 (m, 2H), 4.52 (d, *J*=15.0 Hz, 1H), 4.23 (d, *J*=15.0 Hz, 1H), 3.84–3.79 (m, 4H), 3.63 (s, 3H), 3.11 (dd, *J*=14.7, 5.0 Hz, 1H), 2.80–2.66 (m, 2H), 2.43 (dd, *J*=15.8, 8.6 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.8, 166.5, 157.4, 130.2, 129.3, 123.9, 120.8, 110.4, 55.3, 51.8, 48.0, 43.1, 39.7, 37.7; IR (KBr) ν 2952, 1743 cm^{-1}; MS (ESI) *m*/*z* (%) 286 $[M+Na]^+$. HRMS (FT-MS-ESI) Anal. Calcd for $C_{14}H_{17}NO_4$: 286.1050 $[M+Na]^+$. Found: 286.1047 $[M+Na]^+$.

*4.11.18. (R)-(−)-**8i** as colorless oil (90 mg, 49%).* $[\alpha]_D^{25}$ −10.3° (*c* 1.5, diethyl ether); ee=89.1% (HPLC analysis using a Chiralcel OJH column); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.70–5.83 (m, 1H), 5.18–5.24 (m, 2H), 3.97 (ddd, *J*=9.3, 6.9, 2.1 Hz, 1H), 3.91 (d, *J*=5.7 Hz, 1H), 3.66–3.80 (m, 1H), 3.70 (s, 3H), 3.17 (dd, *J*=15.0, 5.1 Hz, 1H), 2.76 (dd, *J*=16.2, 6.3 Hz, 1H), 2.70 (dd, *J*=16.5, 2.1 Hz, 1H), 2.57 (dd, *J*=16.2, 6.9 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.8, 166.5, 132.1, 118.4, 51.9, 47.9, 43.7, 42.9, 38.2; IR (KBr) ν 1739 cm^{-1}; MS (ESI) *m*/*z* (%) 184 $[M+H]^+$, 206 $[M+Na]^+$. HRMS (FT-ICRMS) Anal. Calcd for $C_9H_{13}NO_3$: 184.0968 $[M+H]^+$. Found: 184.0967 $[M+H]^+$.

*4.11.18.1. Procedure for multigram biotransformation of racemic **6h**.* Following the same aforementioned standard procedure for the biotransformation of nitriles and amides, reaction of racemic **6h** (10 mmol, 2.48 g) gave (*S*)-**6h** (1.12 g, 45%, ee>99.5%) and (*R*)-**8h** (1.21 g, 46%, ee>99.5%).

*4.11.18.2. Procedure for the synthesis of S-acid (S)-**7h** from biotransformation of S-amide (S)-**6h**.* Following the general procedure for the biotransformations of racemic nitriles and amides, (*S*)-**6h** (0.5 mmol, 128 mg) was converted completely within 60 h into acid (*S*)-**7h** as colorless oil (120 mg, 97%): $[\alpha]_D^{25}$ −10.0° (c 0.7, $CHCl_3$); ee>99.5% (HPLC analysis of ester using a Chiralcel ADH column); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30–7.23 (m, 2H), 6.98–6.89 (m, 2H), 4.51 (d, *J*=15.0 Hz, 1H), 4.23 (d, *J*=15.0 Hz, 1H), 3.85–3.80 (m, 4H), 3.63 (s, 3H), 3.04 (dd, *J*=14.7, 5.0 Hz, 1H), 2.71–2.64 (m, 2H), 2.24 (dd, *J*=14.8, 9.2 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.4, 170.1, 159.3, 131.4, 130.7, 125.5, 122.0, 112.0, 56.2, 51.4, 43.7, 42.3, 41.1; IR (KBr) ν 3393, 2926, 1733, 1581 cm^{-1}; MS (ESI) *m*/*z* (%) 248 $[M-H]^-$. HRMS (FT-MS-ESI) Anal. Calcd for $C_{13}H_{15}NO_4$: 288.0633 $[M+K]^+$. Found: 288.0634 $[M+K]^+$.

*4.11.18.3. Synthesis of **9**.* After biotransformation of (*S*)-**6i** (0.4 mmol, 67 mg), the acid product (*S*)-**7i** was mixed directly with iodine (1.5 mmol, 190 mg) in acetonitrile (1.5 mL). After reaction at room temperature for 18 h, a saturated aqueous solution of $Na_2S_2O_3$ was added, and the mixture was extracted with ethyl acetate (3×5 mL). Organic phase was dried over anhydrous Na_2SO_4. After removal of solvent under vacuum, the residue was chromatographed on a silica gel column eluted with a mixture of petroleum ether and ethyl acetate (*v*:*v*=1:2) to give products **9a** and **9b**.

9a as white solid (61 mg, 52%): mp 171.0–172.0 °C; $[\alpha]_D^{25}$ −15.0° (*c* 0.5, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.54–4.47 (m, 1H), 4.31 (d, *J*=15.0 Hz, 1H), 3.91–3.85 (m, 1H), 3.37–3.21 (m, 3H), 3.15–3.05 (m, 2H), 2.92–2.72 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.7, 164.7, 78.9, 46.7, 45.3, 44.6, 41.2, 1.0; IR (KBr) ν 1740 cm^{-1}; MS (ESI) *m*/*z* (%) 350 $[M+Na+CH_3OH]^+$. HRMS (FT-MS-ESI) Anal. Calcd for $C_8H_{10}INO_3$: 349.9860 $[M+Na+CH_3OH]^+$. Found: 349.9857 $[M+Na+CH_3OH]^+$.

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9b as colorless oil (44 mg, 37%): $[\alpha]_D^{25}$ +16.7° (*c* 0.5, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 4.73–4.64 (m, 1H), 4.01–3.98 (m, 1H), 3.89–3.81 (m, 1H), 3.44–3.25 (m, 5H), 2.99–2.87 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.8, 165.8, 75.3, 45.2, 45.1, 43.6, 37.0, 0.1; IR (KBr) ν 1739 cm^{-1}; MS (ESI) *m*/*z* (%) 350 $[M+Na+CH_3OH]^+$. HRMS (FT-MS-ESI) Anal. Calcd for $C_8H_{10}INO_3$: 349.9860 $[M+Na+CH_3OH]^+$. Found: 349.9858 $[M+Na+CH_3OH]^+$.

4.11.18.4. Synthesis of ***10****.* a solution of (*R*)-**8g** (0.5 mmol, 124 mg) in methanol (6 mL) was added dropwise a solution of LiOH (1 mmol, 24 mg) in water (1.5 mL). After stirring at room temperature overnight, dilute hydrochloric acid (1 M, 1 mL) was added slowly. Solvent was removed under vacuum at 50 °C, and the residue was washed thoroughly with methanol (3×15 mL). Methanol solution was collected and concentrated to give acid product. Without further purification, the acid was mixed with $SOCl_2$ (0.1 mL), DMF (one drop) and dry DCM (6 mL), and the resulting mixture was refluxed for 2 h. After removal of solvent under vacuum, dry DCM (8 mL) and $AlCl_3$ (2 mmol, 267 mg) were added. The reaction mixture was stirred at room temperature for 2 h. Brine (10 mL) was added and the mixture was extracted with DCM (3×10 mL). The combined organic layer was dried over anhydrous Na_2SO_4. Solvent was then removed and the residue was chromatographed on a silica gel column using ethyl acetate as mobile phase to give pure product (*R*)-(−)-**10** as white solid (85 mg, 79%): mp 114.0–115.0 °C; $[\alpha]_D^{25}$ −47.5° (*c* 0.8, $CHCl_3$); ee=97.1% (HPLC analysis using a Chiralcel ADH column); ^1H NMR (300 MHz, $CDCl_3$) δ 7.42–7.39 (m, 1H), 7.29–7.21 (m, 2H), 5.00 (d, *J*=17.6 Hz, 1H), 4.26 (d, *J*=17.6 Hz, 1H), 4.08–4.07 (m, 1H), 3.45 (dd, *J*=13.0, 5.9 Hz, 1H), 3.22 (dd, *J*=14.9, 4.7 Hz, 1H), 3.03–2.90 (m, 2H), 2.34 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.9, 165.8, 139.7, 135.6, 135.2, 133.4, 127.4, 127.3, 47.5, 46.9, 42.3, 42.1, 19.8; IR (KBr) ν 2925, 1751, 1682 cm^{-1}; MS (ESI) *m*/*z* (%) 238 $[M+Na]^+$. Anal. Calcd for $C_{13}H_{13}NO_2$: C, 72.54; H, 6.09; N, 6.51. Found: C, 72.44; H, 6.06; N, 6.50.

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Supplementary data

^1H and ^{13}C NMR spectra of products, X-ray molecular structures of *S*-**6a** and *R*-**8d** (CIFs), HPLC chromatograms of products. Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tet.2014.05.018.

References and notes

1. For an overview, see: Wang, M.-X. *Top. Catal.* **2005**, *35*, 117–130.
2. For reviews, see: (a) Ramteke, P. W.; Maurice, N. G.; Joseph, B.; Wadher, B. J. *Biotechnol. Appl. Biochem.* **2013**, *60*, 459–481; (b) Prasad, S.; Bhalla, T. C. *Biotechnol. Adv.* **2010**, *28*, 725–741; (c) Velankar, H.; Clarke, K. G.; du Preez, R.; Cowan, D. A.; Burton, S. G. *Trends Biotechnol.* **2010**, *28*, 561–569; (d) D'Antona, N.; Morrone, R. In *Biocatalysis: Green Transformations of Nitrile Function in Green Chemistry for Environmental Sustainability*; Sharma, S. K., Mudhoo, A., Eds.; CRC: 2010.
3. (a) Busto, E.; Gotor-Fernández, V.; Gotor, V. *Chem. Rev.* **2011**, *111*, 3998–4035; (b) Ordóñez, M.; Cativiela, C. *Tetrahedron: Asymmetry* **2007**, *18*, 3–99; (c) Cativiela, C.; Díaz-de-Villegas, M. D. *Tetrahedron: Asymmetry* **2007**, *18*, 569–623; (d) Gröger, H. In *Enzyme-catalyzed Asymmetric Synthesis in Catal. Asymmetric Synthesis*; Ojima, I., Ed.; John Wiley & Sons: 2010.
4. (a) Blakey, A. J.; Colby, J.; Williams, E.; O'Reilly, C. *FEMS Microbiol. Lett.* **1995**, *129*, 57–61; (b) O'Mahony, R.; Doran, J.; Coffey, L.; Cahill, O. J.; Black, G. W.; O'Reilly, C. *Antonie van Leeuwenhoek* **2005**, *87*, 221–232; (c) Wang, M.-X.; Lu, G.; Ji, G.-J.; Huang, Z.-T.; Meth-Cohn, O.; Colby, J. *Tetrahedron: Asymmetry* **2000**, *11*, 1123–1135.
5. (a) Wang, M.-X. *Top. Organomet. Chem.* **2011**, *36*, 105–121; (b) Wang, M.-X. *Chimia* **2009**, *63*, 331–333.
6. (a) Zhang, L.-B.; Wang, D.-X.; Zhao, L.; Wang, M.-X. *J. Org. Chem.* **2012**, *77*, 5584–5591; (b) Zhang, L.-B.; Wang, D.-X.; Wang, M.-X. *Tetrahedron* **2011**, *67*, 5604–5609.
7. (a) Chen, P.; Gao, M.; Wang, D.-X.; Zhao, L.; Wang, M.-X. *J. Org. Chem.* **2012**, *77*, 4063–4072; (b) Chen, P.; Gao, M.; Wang, D.-X.; Zhao, L.; Wang, M.-X. *Chem. Commun.* **2012**, 3482–3484.
8. Taylor, S. K.; Chmiel, N. H.; Simons, L. J.; Vyvyan, J. R. *J. Org. Chem.* **1996**, *61*, 9084–9085.
9. Klempier, N.; De Raat, A.; Faber, K.; Griengl, H. *Tetrahedron Lett.* **1991**, *32*, 341–344.
10. Wang, M.-X.; Wu, Y. *Org. Biomol. Chem.* **2003**, *1*, 535–540.
11. Ma, D.-Y.; Wang, D.-X.; Pan, J.; Huang, Z.-T.; Wang, M.-X. *J. Org. Chem.* **2008**, *73*, 4087–4091.
12. Ma, D.-Y.; Wang, D.-X.; Zheng, Q.-Y.; Wang, M.-X. *Tetrahedron: Asymmetry* **2006**, *17*, 2366–2376.
13. Mehta, P. D.; Sengar, N. P. S.; Pathak, A. K. *Eur. J. Med. Chem.* **2010**, *45*, 5541–5560.
14. (a) Salzmann, T. N.; Ratcliffe, R. W.; Christensen, B. G.; Bouffard. *J. Am. Chem. Soc.* **1980**, *102*, 6161–6163; (b) Okano, K.; Izawa, T.; Ohno, M. *Tetrahedron Lett.* **1983**, *24*, 217–220; (c) Bodner, M. J.; Phelan, R. M.; Townsend, C. A. *Org. Lett.* **2009**, *11*, 3606–3609.
15. Alcaide, B.; Almendros, P.; Aragoncillo, C. *Chem. Rev.* **2007**, *107*, 4437–4492.
16. Aranda, M. T.; Perez-Faginas, P.; Gonzalez-Muniz, R. *Curr. Org. Synth.* **2009**, 325–341.
17. (a) Wang, M.-X.; Feng, G.-Q. *J. Org. Chem.* **2003**, *68*, 621–624; (b) Wang, M.-X.; Feng, G.-Q.; Zheng, Q.-Y. *Adv. Synth. Catal.* **2003**, *345*, 695–698; (c) Wang, M.-X.; Feng, G.-Q. *Tetrahedron Lett.* **2000**, *41*, 6501–6505.
18. (a) Wang, M.-X.; Lin, S.-J.; Liu, C.-S.; Zheng, Q.-Y.; Li, J.-S. *J. Org. Chem.* **2003**, *68*, 4570–4573; (b) Wang, M.-X.; Deng, G.; Wang, D.-X.; Zheng, Q.-Y. *J. Org. Chem.* **2005**, *70*, 2439–2444.
19. (a) Wang, J.-Y.; Wang, D.-X.; Zheng, Q.-Y.; Huang, Z.-T.; Wang, M.-X. *J. Org. Chem.* **2007**, *72*, 2040–2045; (b) Wang, J.-Y.; Wang, D.-X.; Pan, J.; Huang, Z.-T.; Wang, M.-X. *J. Org. Chem.* **2007**, *72*, 9391–9394.
20. Leng, D.-H.; Wang, D.-X.; Pan, J.; Huang, Z.-T.; Wang, M.-X. *J. Org. Chem.* **2009**, *74*, 6077–6082.
21. Leng, D.-H.; Wang, D.-X.; Huang, Z.-T.; Wang, M.-X. *Org. Biomol. Chem.* **2010**, *8*, 4736–4743.
22. (a) Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299; (b) Faber, K.; Hoenig, H. Program 'Selectivity', http://www.TUGraz.at/orgc/.
23. Gao, M.; Wang, D.-X.; Zheng, Q.-Y.; Wang, M.-X. *J. Org. Chem.* **2006**, *71*, 9532–9535.