Advanced Synthesis & Catalysis

Accepted Article

Title: Biocatalytic Desymmetrization of Prochiral 3-Aryl and 3-Arylmethyl Glutaramides: Different Remote Substituent Effect on Catalytic Efficiency and Enantioselectivity

Authors: Yu-Fei Ao, Li-Bin Zhang, Qi-Qiang Wang, De-Xian Wang, and Mei-Xiang Wang

To be cited as: *Adv. Synth. Catal.* 10.1002/adsc.201800956

Link to VoR: http://dx.doi.org/10.1002/adsc.201800956

10.1002/adsc.201800956

FULL PAPER

DOI: 10.1002/adsc.201((will be filled in by the editorial staff))

Biocatalytic Desymmetrization of Prochiral 3-Aryl and 3-Arylmethyl Glutaramides: Different Remote Substituent Effect on Catalytic Efficiency and Enantioselectivity

Yu-Fei Ao,[a]* Li-Bin Zhang,[a] Qi-Qiang Wang,[a,c] De-Xian Wang,[a,c] Mei-Xiang Wang[b]

[a] Beijing National Laboratory for Molecular Sciences, CAS Key Laboratory of Molecular Recognition and Function, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, China
aoyufe@iccas.ac.cn
[b] MOE Key Laboratory of Bioorganic Phosphorus Chemistry & Chemical Biology, Department of Chemistry, Tsinghua University, Beijing 100084, China
[c] University of Chinese Academy of Sciences, Beijing 100049, China

Received: ((will be filled in by the editorial staff))

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/adsc.201######.((Please delete if not appropriate))

Abstract. Catalyzed by an amidase-containing *Rhodococcus erythropolis* AJ270 microbial whole cell catalyst in neutral phosphate buffer at 30 °C, desymmetric hydrolysis of a series of prochiral 3-aryl and 3-arylmethylglutaramides efficiently afforded 3-substituted glutaric acid monoamides in up to 95% yield and >99.5% ee. Even far away from the reaction site, the substituents on the aryl still have a significant effect on the catalytic activity and enantioselectivity and different remote substituent effect was observed for the two types of substrates. The synthetic application of biocatalytic desymmetrization was demonstrated by the facile transformation of the obtained enantiopure (*R*)-3-substituted 4-carbamoylbutanoic acid products to chiral dihydroquinolinone and *δ*-lactone compounds.

Keywords: Amidase; Biotransformations; Desymmetrization; Prochiral Glutaramide; Remote Substituent Effect

Introduction

Owing to the high efficiency, excellent selectivity and environmentally benign reaction conditions, biocatalysis and biotransformation have become an important and powerful strategy in organic synthesis.[1] Along with kinetic resolution processes, which have been widely explored and used in academic laboratories and in industry, biocatalytic desymmetrizations of meso and prochiral compounds are gaining popularity as they enable the convenient and quantitative generation of enantiopure compounds.[2] Hydrolytic enzymes[3] including lipases, esterases and acylases for example have been reported to catalyze enantioselective desymmetrization reactions to produce functionalized chiral products.[4] In addition to extensively used biocatalytic hydrolysis to form C-O bonds, several reports on biocatalytic desymmetric hydrolysis of prochiral dinitriles by nitrile-hydrolyzing biocatalysts have also been published.[5] Catalyzed by the nitrilase [E.C. 3.5.5.1], prochiral 3-hydroxy-glutaronitriles,[6a-c] 3-isobutylglutaronitrile,[6d] and 3-(4'-chlorophenyl)glutaronitrile[6d] undergo efficient hydrolysis to produce the corresponding 4-cyanobutyric acids with excellent enantioselectivity. On the contrary, the biocatalytic hydrolysis of the prochiral glutaronitriles in the presence of nitrile hydratase [E.C. 4.2.1.84] / amidase [E.C. 3.5.1.4]-containing whole cell catalysts such as *Rhodococcus rhodochrous* IFO 15564,[7] *Rhodococcus* SP 361,[8] *Rhodococcus erythropolis* AJ270,[9] *Rhodococcus erythropolis* NCIMB 11540[10] and *Rhodococcus rhodochrous* ATCC BAA-870,[11] forms 4-cyanobutyric acids generally with moderate enantioselectivity. In most cases, the successive procedures include a desymmetric hydration process catalyzed by nitrile hydratase to produce monocyano amide intermediate and a non-selective hydrolysis process catalyzed by amidase to yield monocyano carboxylic acid. From this routine the chemical yields and enantiomeric excess values obtained for the chiral products are usually not satisfactory for the purpose of synthesis. This is mainly due to the mismatch of the nitrile hydratase and amidase in catalyzing nitrile hydration and amide hydrolysis respectively, in terms of catalytic efficiency and enantioselectivity.

Since the desymmetric hydrolysis of prochiral dinitriles using biocatalytic systems involving nitrile hydratase and amidase enzymes is not useful enough in synthesis, attentions have been paid in recent years to the amidase-catalyzed desymmetrization of

10.1002/adsc.201800956

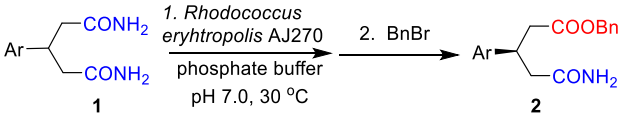
prochiral dicarboxamides. The high enantioselectivity of most of the amidases along with the facile access of various prochiral dicarboxamide substrates would enable a powerful and practical route to the synthesis of invaluable enantioenriched amido-bearing carboxylic acids. Surprisingly, despite extensive studies on kinetic resolution of racemic amides using amidases, by far only limited examples of amidase-catalyzed enantioselective desymmetric hydrolysis of dicarboxamides have been reported.[5] Wu and Li reported in 2003 that in the presence of *Rhodococcus* sp. CGMCC 0497 whole cells prochiral malonamides undergo hydrolysis to give malonamic acids with high ee values and high chemical yields.[12] Nojiri showed recently the enantioselective synthesis of (*R*)-3-(4-chlorophenyl) and (*R*)-3-isobutyl glutaric acids from *Comamonas* sp. KNK-3-7-catalyzed reaction of the corresponding glutaramides.[13] We have demonstrated previously the amidase involved in *Rhodococcus erythropolis* AJ270 is able to transform a number of prochiral malonamides and meso cyclic and heterocyclic dicarboxamides, furnishing highly enantiopure functionalized carboxylic acids in good to excellent yields. For example, we showed that *Rhodococcus erythropolis* AJ270 was able to catalyze the desymmetrization of prochiral amino-substituted malonamides yielding polyfunctionalized carboxylic acid derivatives with ee values up to 99.5%.[14] More remarkably, five-membered *meso*-*N*-heterocyclic and *meso*-cyclopentane dicarboxamides underwent highly efficient and practical desymmetrization reactions to produce functionalized enantiopure pyrrolidine, dihydropyrrole, piperidine derivatives, and cyclopentanecarboxylic acids. [15]

As a continuation of our study on biocatalysis and to further explore the synthetic utility of the amidase in desymmetrization reactions, we undertook the current investigation. Reported herein is a systematic investigation of *Rhodococcus erythropolis* AJ270-catalyzed hydrolysis of prochiral 3-aryl- and 3-arylmethyl-glutaramides, enabling an efficient method in the preparation of enantioenriched 3-aryl- and 3-arylmethyl-glutaric acid monoamide derivatives with ee values up to >99.5%. The remarkable effect of 3-substituents on the efficiency and enantioselectivity of the amidase in desymmetrization reaction is discussed. The synthetic application of the method is highlighted by the conversion of the resulting biocatalytic products into chiral dihydroquinolinone and δ-lactone.

Results and Discussion

We initiated the biotransformation with the reaction of prochiral 3-phenylglutaramide **1a**, which can be easily prepared from commercially available aromatic aldehydes and cyanoacetic acids according to a literature method.[16] (see Supporting Information). Catalyzed by *Rhodococcus erythropolis* AJ270 whole cells in neutral phosphate buffer at 30 °C for 6 days, **1a** (2 mmol) underwent desymmetric hydrolysis to afford the mono-acid product. To facilitate the isolation, the crude acid product was converted into benzyl ester **2a** using benzyl bromide as an alkylation reagent. As shown in Table 1, **2a** was obtained in 77% yield but with only 21% ee (entry 1, Table 1). Decreasing the concentration of **1a** (1 mmol) can facilitate the conversion and increase the isolated yield to 87%, whereas the enantioselectivity was not improved (entry 2, Table 1).

Table 1. Biocatalytic Hydrolysis of 3-Arylglutaramides **1**[a)]



Entry	**1**	Ar	t (h)	**2** (%)[b)] (ee%)[c)]
1[d)]	**1a**	C_6H_5	144	**2a** (77) (21)
2	**1a**	C_6H_5	72	**2a** (87) (21)
3[e)]	**1b**	4-Br-C_6H_4	168	**2b** (43) (3)
4[f)]	**1c**	4-Cl-C_6H_4	168	**2c** (55) (1)
5	**1d**	4-CH_3-C_6H_4	72	**2d** (93) (77)
6	**1e**	3-Br-C_6H_4	120	**2e** (91) (41)
7	**1f**	3-CH_3-C_6H_4	96	**2f** (87) (29)
8	**1g**	2-Br-C_6H_4	89	**2g** (92) (>99.5)
9	**1h**	2-Cl-C_6H_4	72	**2h** (95) (>99.5)
10	**1i**	2-CH_3-C_6H_4	80	**2i** (90) (>99.5)
11[g)]	**1j**	2-CH_3O-C_6H_4	168	**2j** (30) (5)

[a)] Substrates **1** (1 mmol) was incubated with *Rhodococcus erythropolis* AJ270 (2 g wet weight) in phosphate buffer (pH 7.0, 0.1 M, 50 mL) at 30 °C. [b)] Isolated yield. [c)] Determined by chiral HPLC analysis. [d)] **1a** (2 mmol) was used. [e)] Starting diamide **1b** was recovered in 45% yield. [f)] Starting diamide **1c** was recovered in 30% yield. [g)] Starting diamide **1j** was recovered in 60% yield.

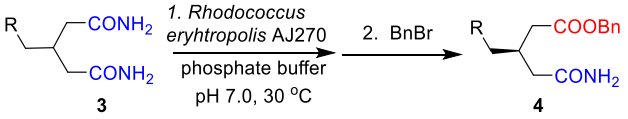
To investigate the effect of substituents attached on the phenyl ring, especially the enantioselectivity-structure relationship of the biotransformation reactions, a number of prochiral 3-substituted glutaramides **1b-1j** were synthesized according to the same method[16] and subjected to biotransformations. As listed in Table 1, all the tested substrates were desymmetrically hydrolyzed under the same conditions, affording the products in 30-95% yields and 1->99.5% ee. Surprisingly, even far away from the reaction site, the substituents still show a significant effect on the catalytic activity and enantioselectivity of the amidase. For example, the introduction of a halogen group such as bromine (**1b**, entry 3, Table 1) or chlorine (**1c**, entry 4, Table 1) at the *para* position of the phenyl ring resulted in sluggish reactions (43% and 55% yields at 168 h) and obtained nearly racemic products (<3% ee). In the case of electron-donating methyl group, the biotransformation reaction was faster and the product

10.1002/adsc.201800956

2d was obtained with much improved yield (93%) and enantioselectivity (77% ee) (entry 5, Table 1). This indicated that electronic donating group is more favorable to the biotransformation. The *meta* position substitution of bromine or methyl group led to moderate reaction rate and enantioselectivity (41% and 29% ee respectively) (entry 6-7, Table 1). To our delight, for the *ortho*-position substitution of bromine, chlorine or methyl group, in all cases the corresponding products **2g**, **2h** and **2i** were obtained in very high yields (≥90%) and excellent ee values (>99.5%) (entry 8-10, Table 1). For methoxyl substitution, however, the biotransformation turned to be very slow and showed very poor enantioselectivity (30% yield and 5% ee) (entry 11, Table 1), probably due to the larger steric hindrance of methoxyl group in comparison to the methyl or halogen group in **2g-i**. These results suggested a significant beneficial effect of appropriate *ortho*-substitution on 3-arylglutaramide on *Rhodococcus erythropolis* AJ270-catalyzed desymmetric hydrolysis.

The above outcomes showed the substituents on the substrates have a significant effect on the catalytic activity and enantioselectivity. It prompted us to extend the scope of substrate to 3-arylmethylglutaramides **3**, which contain more flexible substituents. Substrates **3** were synthesized from from known compounds by the literature method.[17] Remarkably, with a methylene group being introduced, the *Rhodococcus erythropolis* AJ270-catalyzed desymmetrization reactions of prochiral **3** proceeded much more efficiently than that of **1** to afford the products in high yields (>81%) and high enantioselectivity (ee 77-99.5%) (Table 2). For example, the substrate **3a** underwent fast desymmetrization hydrolysis in 7.5 h and furnished the product **4a** in 84% yield and >99.5% ee. The scaled-up biocatalytic reaction proceeded readily, and gram-scale enantiopure **4a** was obtained in 85% yield (entry 2, Table 2). The introduction of different substituents on the benzene ring resulted in generally decreased biocatalytic efficiency (longer reaction period required), however, the excellent enantioselectivity was remained in most cases. The substrate **3b** possessing *ortho*-position substitution of bromine showed slower conversion comparing to the substrates **3c** and **3d** with *meta*- or *para*-substitution (entry 3-5, Table 2). Although the details on the biocatalysis mechanism await further investigation, the lower catalytic efficiency of **3b** can be most probably attributed to the larger steric hindrance for *ortho*-position substitution. This is consistent with the fact that the non-substitution substrate **3a** showed the fastest hydrolysis. The introduction of other electron-withdrawing *para*-substituents such as chloro-, fluoro- and trifluoromethyl group led to similar conversion efficiency, although with a decreased enantioselectivity observed for trifluoromethyl substitution (entry 6-8, Table 2). The substrates bearing electron-donating methyl and methoxyl substituents (**3h** and **3i**) underwent more rapid conversion than those with electron-withdrawing groups (entry 9-10, Table 2). The methoxyl substituent led to a decreased ee value, similar to what observed for trifluoromethyl, reflecting an unfavorable effect of the steric bulkiness group on enantiocontrol. It is worth addressing that the hydrolysis of the substrate **3j** bearing a terminal olefin group proceeded faster than the other substrates although with diminished enantiocontrol (entry 11, Table 2).

Table 2. Biocatalytic Hydrolysis of 3-Arylmethylglutaramides **3**[a)]



Entry	**3**	R	t (h)	**4** (%)[b)] (ee%)[c)]
1	**3a**	C_6H_5	7.5	**4a** (84) (>99.5)
2[d)]	**3a**	C_6H_5	8	**4a** (85) (>99.5)
3	**3b**	2-Br-C_6H_4	65.5	**4b** (88) (>99.5)
4	**3c**	3-Br-C_6H_4	32	**4c** (84) (>99.5)
5	**3d**	4-Br-C_6H_4	24	**4d** (85) (>99.5)
6	**3e**	4-Cl-C_6H_4	24	**4e** (89) (>99.5)
7	**3f**	4-F-C_6H_4	23.5	**4f** (91) (>99.5)
8	**3g**	4-CF_3-C_6H_4	28	**4g** (90) (77)
9	**3h**	4-CH_3-C_6H_4	14	**4h** (92) (>99.5)
10	**3i**	4-CH_3O-C_6H_4	15	**4i** (81) (91)
11	**3j**	CH_2=CH	5	**4j** (81) (77)

[a)] Substrate **4** (2 mmol) was incubated with *Rhodococcus erythropolis* AJ270 (2 g wet weight) in phosphate buffer (pH 7.0, 0.1 M, 50 mL) at 30 °C. [b)] Isolated yield. [c)] Determined by chiral HPLC analysis. [d)] Substrate **4a** (6 mmol), cell (6 g wet weight), and buffer (150 mL) was used.

The different remote substituent effect on the catalytic efficiency and enantioselectivity for the two types of substrates is worth addressing. For 3-aryl substrates **1**, the appropriate *ortho*-substitution on the benzene ring enabled improved catalytic efficiency and excellent enantiocontrol, while the *meta*- or *para*-substitution showed overall sluggish conversion with poor enantioselectivity. These observations indicated that an efficient chiral recognition can be only achieved in the presence of an appropriate *ortho*-substituent. On the contrary, for 3-benzyl substrates **3** the non-substituted substrate **3a** gave highest efficiency. The introduction of substituents on the benzene ring decelerated the conversion but with undisturbed excellent enantiocontrol. Besides, the *ortho*-substitution showed slower conversion than *meta*- or *para*-substitution, implying that a disfavored steric hindrance effect for the remote substitution of substrates **3**. The different remote substituent effect

10.1002/adsc.201800956

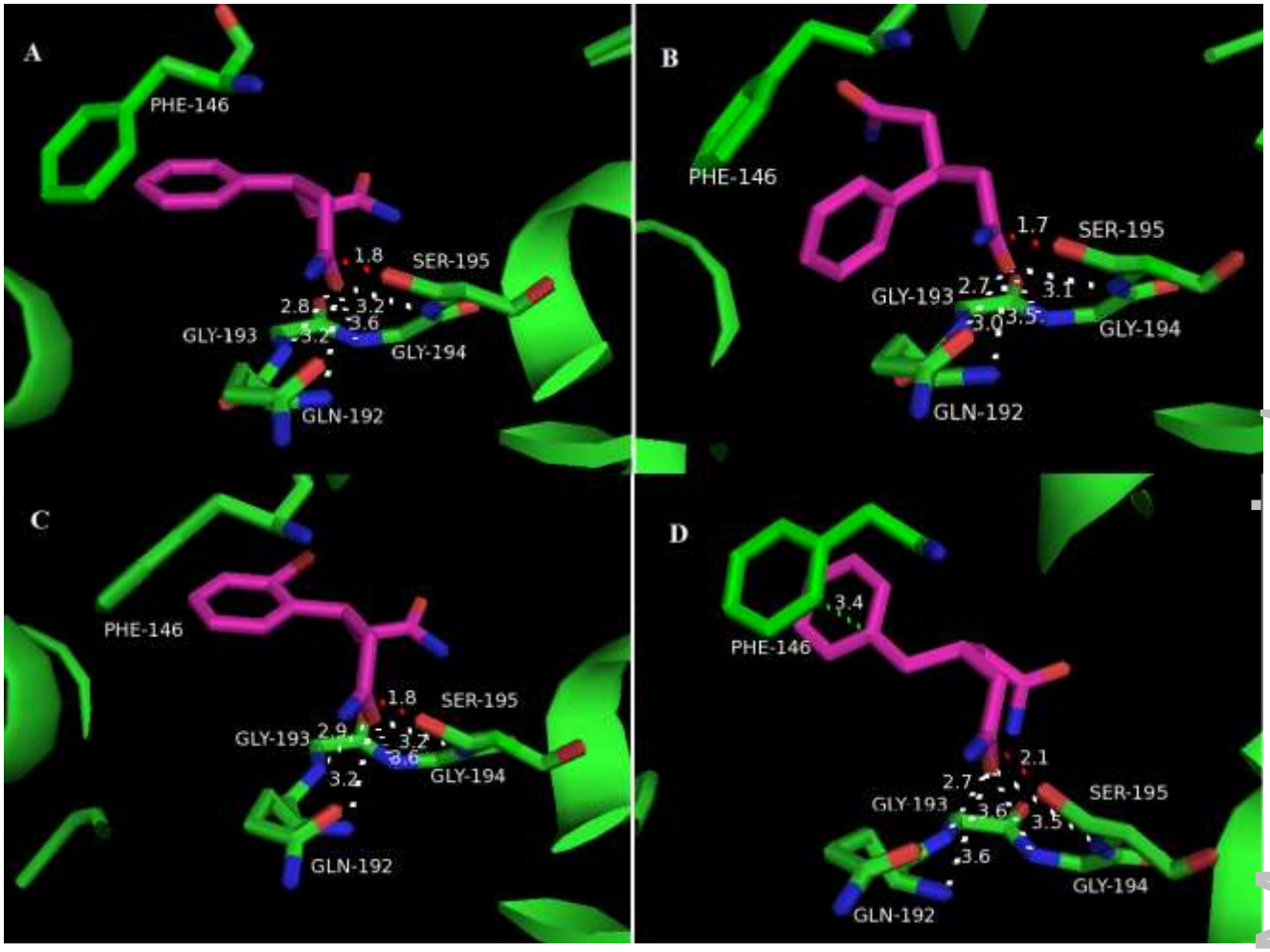


Figure 1. Molecular docking of **1a** (**A**, **B**), **1g** (**C**) and **3a** (**D**) into the active site of amidase of *Rhodococcus erythropolis* AJ270. The protein was drawn as a green cartoon. The oxygen and nitrogen in the active pocket residues and substrates were colored red and blue, respectively. The carbon and bromine of substrates were colored purple and dark red, respectively. The hydrogen bonds were represented by white dashed lines. The π-π interaction was represented by green dashed line. The distances between the serine Oγ atom and the carbonyl carbon were represented by red dashed lines. The Dockings of substrates were performed using available tools in AutoDock 4.2.6. Phe146 and Ser195 were assigned as Flexible Residues and the optimal configuration of Phe146 was displayed in the Figures. The Figures were generated using PyMOL.

can be attributed to a more efficient recognition and chiral discrimination ability of the amidase toward the less bulky and more flexible substrates **3** because of the introduction of the methylene group.

To understand the enantioselectivity of amidase, we tried to model substrates into the binding site of amidase. To our delight, we found the amidase from *Rhodococcus erythropolis* AJ270 is the same as that from *Rhodococcus* sp. N-771 whose crystal structure was solved in the year 2010 (100% identities).[18] It was proposed from the crystal structure of amidase that the active site is narrowly surrounded by hydrophobic residues Phe146, Ile227, Trp328, Leu447 and Ile450. Substrate could be included within the pocket of active sites through non-covalent interactions. The side-chain oxygen atom of Ser195 and oxyanion hole formed with Gln192, Gly193, Gly194 and Ser195 serve as nucleophilic attacker and intermediate stabilizer, respectively.[18, 19] Based on such background, three representative substrates **1a**, **1g** and **3a** were docked into the active site of amidase. As shown in Figure 1, the substrates could form hydrogen bonds with the amide hydrogen of the oxyanion hole. The short distance between Oγ of Ser195 and the carbonyl carbon of substrates (1.7-2.1 Å) suggests the presence of the covalent substrate-enzyme intermediate. Some non-covalent interaction details are worth addressing. For example, the oxyanion hole could form hydrogen bonds with both pro-*R* and pro-*S* amide group of **1a** (Figure 1, A and B), but it selectively interacts with pro-*R* amide group of **1g**. Such selectivity for **1g** enantiomers is probably due to the steric hindrance of pro-*S* enantiomer disfavors the intermolecular interaction (Figure 1, C). In the case of **3a**, π-π stacking between benzene rings of pro-*R* substrate and Phe146 is observed (Figure 1,

10.1002/adsc.201800956

D), which renders the preferential binding of pro-*R* enantiomer and therefore could explain the excellent catalytic enantioselectivity with this type of substrates.

The structure of all products was established based on spectroscopic data and microanalyses. To assign the absolute configuration of the product **2**, single crystals of **2g** in good quality was obtained by slow evaporation of a solution in a mixture of hexane and DCM. X-ray diffraction analysis revealed unambiguously that the molecular structure of **2g** has 3*R*-configured stereogenic center (Figure 2) [20].

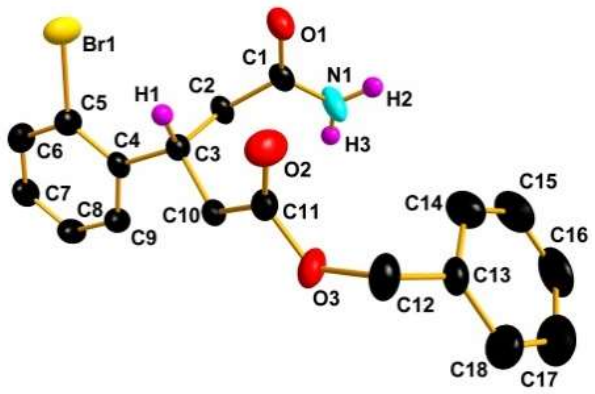
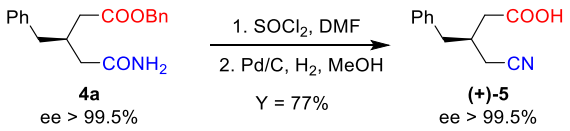


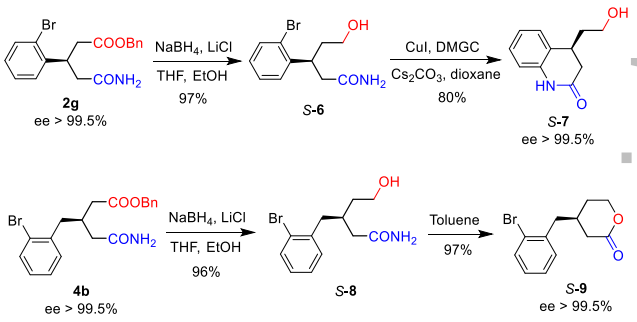
Figure 2. X-ray crystal structure of **2g.**

To assign the absolute configuration of the product **4**, configuration-persistent dehydration followed by catalytic hydrogenation reaction was performed to convert **4a** into 3-benzyl-4-cyanobutanoic acid **5** (scheme 1). Comparison of the optical rotation of the product **5** with that reported in the literature[7] allowed us to assign the absolute configuration of *R*-**5** and therefore of *R*-**4a**. The formation of *R*-configured products with both the two types of substrates indicated convincingly the 3*R*-enantioselectivity of the amidase involved in *Rhodococcus erythropolis* AJ270.



Scheme 1. Synthesis of *R*-3-benzyl-4-cyanobutanoic acid **5**

The obtained (*R*)-3-substituted-4-carbamoylbutanoic acids are conceivably valuable chiral intermediates in synthetic organic chemistry. The multi-functionalized structure renders them as unique building blocks in the synthesis of various natural products and bioactive compounds.[21] To demonstrate the application of the efficient biotransformation method, the synthesis of chiral dihydroquinolinone **7** and *δ*-lactone **9** was achieved from biocatalytic desymmetrization products by two-step simple transformations. As shown in Scheme 2, reduction of ***R*-2g** and ***R*-4b** led to the formation of hydroxyl products ***S*-6** and ***S*-8** in 97% and 96% yields respectively. The subsequent intramolecular cross-coupling reaction of ***S*-6** catalyzed by CuI/*N*,*N*-dimethylglycine (DMGC) under basic conditions furnished the dihydroquinolinone ***S*-7** in a good yield. Heating of ***S*-8** in toluene at reflux afforded *δ*-lactone ***S*-9** via intramolecular cyclization. Notably, no racemization was observed in all the transformations and the two products were obtained in high enantiopurity (>99.5% ee).



Scheme 2. Synthesis of Dihydroquinolinone **7** and *δ*-Lactone **9**

Conclusion

In summary, we have shown a highly efficient and enantioselective biocatalytic desymmetrization using the *Rhodococcus erythropolis* AJ270 whole cell catalyst under mild conditions. The *R*-enantioselective amidase-catalyzed hydrolysis of a number of prochiral 3-substituted glutaramides provides a straightforward synthetic route to (*R*)-3-substituted-4-carbamoylbutanoic acids with high yields and excellent enantiomeric excesses. The catalytic efficiency and enantioselectivity of amidase showed close dependence on the structure of substrates. The resulting chiral products, which are not easily accessible by other means, are useful synthetic intermediates and their applications are demonstrated by the construction of densely functionalized heterocyclic compounds.

Experimental Section

General Information

TLC analysis was performed on pre-coated, glass-backed silica gel plates and visualized with UV light and iodine vapor. Column chromatography was performed on silica gel (200-300 mesh). ^{1}H and ^{13}C NMR spectra were recorded on 300 MHz, 400 MHz or 500 MHz NMR spectrometers. Chemical shifts are reported in ppm with either tetramethylsilane or the residual solvent resonance used as an internal standard. Abbreviations were used in the description of NMR data as follows: chemical shift (δ,

ppm), multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet), coupling constant (*J*, Hz). Optical rotations were performed on Rudolph Autopl VI. ESI Mass spectra were determined on a Thermo Fisher Exactive Mass Spectrometer. High performance liquid chromatography (HPLC) was performed on Shimadzu SCL-10AVP. Elemental analysis was recorded on Thermo Quest CE Instruments flash EA1112 analyser. Infrared spectra were recorded using a Nicolet-6700 FT-IR spectrometer with KBr pellets in the 4000-400 cm^{-1} region. Melting points are uncorrected. Anhydrous solvents were obtained following standard procedures. substrates 3-arylglutaramides **1** were prepared according to the literature method.[16] 3-arylmethylglutaramides **3** were synthesized from from known compounds by the literature method.[17] All other commercial chemicals were used without further purification.

General procedure for the biotransformations of 3-arylglutaramides 1a-j.

In an Erlenmeyer flask (150 mL) with a screw cap a suspension of *Rhodococcus erythropolis* AJ270 cells (2 g wet weight) in aqueous phosphate buffer (pH 7.0, 0.1 M, 50 mL) was activated at 30 ºC for 0.5 h. Diamide substrate **1** (1 mmol or 2 mmol) was added in one portion and it was dissolved completely in buffer without any co-solvent, and the resulting mixture was incubated at 30 ºC with orbital shaking (200 rpm). The reaction process was monitored using TLC method. After a period of time, the reaction was quenched by removing microbial cells through a celite pad filtration. For the biocatalytic desymmetrization of meso diamides, the filtration cake was washed consecutively with water (15 mL × 3) and methanol (15 mL × 3). The combined filtrate was heated at 50 ºC under vacuum to remove solvent, giving a waxy solid which is a mixture of acid product and salt. Acid product was dissolved in methanol by washing the waxy solid three times with methanol (15 mL × 3). After the solvent was removed under vacuum, the residue was dissolved in DMF (5 mL) followed by the addition of K_2CO_3 (138 mg, 1 mmol) and benzyl bromide (0.24 mL, 2 mmol). The mixture was stirred overnight, and the reaction was then quenched by adding water (20 mL). Extraction with ethyl acetate (15 mL × 3) followed by silica gel chromatography using acetone as mobile phase to give product **2**. All products were fully characterized by means of spectroscopic data. Enantiomeric excess values were obtained from HPLC analysis using columns coated with chiral stationary phases. In the case of biotransformations of **1b**, **1c** and **1j**, the unreacted meso diamide was recovered from aqueous phase by removing water under vacuum and subsequent silica gel column chromatography eluted with a mixture of acetone and methanol (v:v = 5:1), and **1b**, **1c** and **1j** were recovered in 45%, 30%, 60% yield, respectively.

Benzyl (*R*)-4-carbamoyl-3-phenylbutanoate (2a): white solid (258 mg, 87%); mp: 115 - 116 ºC; $[\alpha]^{25}_D$ +1.1º (c 2.9, $CHCl_3$); ee = 21%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 - 7.26 (m, 5H), 7.24 - 7.18 (m, 5H), 5.36 (brs, 2H), 5.05 - 4.98 (m, 2H), 3.67 - 3.64 (m, 1H), 2.86 - 2.70 (m, 2H), 2.67 - 2.50 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.1, 171.6, 142.4, 135.7, 128.8, 128.5, 128.2, 127.3, 127.1, 66.3, 42.1, 40.5, 38.8; IR (KBr) ν 3395, 3177, 1728, 1652 cm^{-1}; HRMS (FT-MS-ESI): *m/z* calcd. for $C_{18}H_{19}NO_3Na$: 320.1257 $[M+Na]^+$; Found: 320.1261.

Benzyl 3-(4-bromophenyl)-4-carbamoylbutanoate (2b): white solid (162 mg, 43%); mp: 138 - 140 ºC; $[\alpha]^{25}_D$ 0º (c 2.5, $CHCl_3$); ee = 3%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (d, *J* = 8.4 Hz, 2H), 7.33 - 7.31 (m, 3H), 7.19 - 7.16 (m, 2H), 7.08 (d, *J* = 8.4 Hz, 2H), 5.33 (brs, 2H), 5.05 - 4.98 (m, 2H), 3.67 - 3.60 (m, 1H), 2.84 - 2.66 (m, 2H), 2.64 - 2.45 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.6, 171.3, 141.4, 135.6, 131.8, 129.1, 128.5, 128.29, 128.26, 120.9, 66.4, 41.8, 40.2, 38.2; IR (KBr) ν 3398, 3189, 1726, 1652 cm^{-1}; HRMS (FT-MS-ESI): *m/z* calcd. for $C_{18}H_{18}BrNO_3Na$: 398.0362 $[M+Na]^+$ (79Br); Found: 398.0362.

Benzyl 4-carbamoyl-3-(4-chlorophenyl)butanoate (2c): white solid (171 mg, 55%); mp: 131 - 132 ºC; $[\alpha]^{25}_D$ 0º (c 2.2, $CHCl_3$); ee = 1%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 - 7.31 (m, 3H), 7.24 - 7.13 (m, 6H), 5.32 - 5.26 (m, 2H), 5.05 - 4.98 (m, 2H), 3.69 - 3.61 (m, 1H), 2.85 - 2.66 (m, 2H), 2.65 - 2.45 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.5, 171.3, 140.9, 135.6, 132.8, 128.86, 128.69, 128.53, 128.28, 128.25, 66.4, 41.9, 40.3, 38.2; IR (KBr) ν 3345, 3192, 1729, 1665 cm^{-1}; HRMS (FT-MS-ESI): *m/z* calcd. for $C_{18}H_{18}ClNO_3Na$: 354.0867 $[M+Na]^+$; Found: 354.0870.

Benzyl (*R*)-4-carbamoyl-3-(4-methylphenyl)butanoate (2d): white solid (289 mg, 93%); mp: 122 - 123 ºC; $[\alpha]^{25}_D$ +0.9º (c 2.3, $CHCl_3$); ee = 77%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 - 7.29 (m, 3H), 7.19 - 7.17 (m, 2H), 7.11 - 7.06 (m, 4H), 5.49 - 5.40 (m, 2H), 5.04 - 4.97 (m, 2H), 3.64 - 3.57 (m, 1H), 2.83 - 2.66 (m, 2H), 2.63 - 2.47 (m, 2H), 2.30 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.3, 171.7, 139.4, 136.6, 135.8, 129.4, 128.5, 128.18, 128.15, 127.1, 66.3, 42.3, 40.6, 38.5, 21.1; IR (KBr) ν 3399, 3182, 1729, 1648 cm^{-1}; HRMS (FT-MS-ESI): *m/z* calcd. for $C_{19}H_{21}NO_3Na$: 334.1414 $[M+Na]^+$; Found: 334.1416.

Benzyl (*R*)-3-(3-bromophenyl)-4-carbamoylbutanoate (2e): white solid (342 mg, 91%); mp: 93 - 94 ºC; $[\alpha]^{25}_D$ +0.5º (c 3.8, $CHCl_3$); ee = 41%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 - 7.31 (m, 5H), 7.22 - 7.19 (m, 2H), 7.14 - 7.13 (m, 2H), 5.55 - 5.49 (m, 2H), 5.05 - 4.98 (m, 2H), 3.67 - 3.60 (m, 1H), 2.84 - 2.67 (m, 2H), 2.63 - 2.46 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.7, 171.3, 144.9, 135.6, 130.31, 130.29, 130.22, 128.6, 128.28, 128.23, 126.2, 122.7, 66.5, 41.7, 40.1, 38.4; IR (KBr) ν 3345, 3191, 1730, 1668 cm^{-1}; HRMS (FT-MS-ESI): *m/z* calcd. for $C_{18}H_{18}BrNO_3Na$: 398.0362 $[M+Na]^+$ (79Br); Found: 398.0364.

Benzyl (*R*)-4-carbamoyl-3-(3-methylphenyl)butanoate (2f): white solid (271 mg, 87%); mp: 96 - 97 ºC; $[\alpha]^{25}_D$ +1.0º (c 1.0, $CHCl_3$); ee = 29%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 - 7.29 (m, 3H), 7.19 - 7.14 (m, 3H), 7.03 - 6.99 (m, 3H), 5.64 - 5.46 (m, 2H), 5.04 - 4.97 (m, 2H), 3.64 - 3.56 (m, 1H), 2.83 - 2.67 (m, 2H), 2.62 - 2.48 (m, 2H), 2.29 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.4, 171.7, 142.4, 138.3, 135.8, 128.63, 128.50, 128.17, 128.14, 128.11, 127.8, 124.2, 66.3, 42.2, 40.5, 38.8, 21.5; IR (KBr)

ν 3345, 3192, 1732, 1667 cm^{-1}; HRMS (FT-MS-ESI): *m/z* calcd. for $C_{19}H_{21}NO_3Na$: 334.1414 $[M+Na]^+$; Found: 334.1415.

Benzyl (*R*)-3-(2-bromophenyl)-4-carbamoylbutanoate (2g): white solid (346 mg, 92%); mp: 88 - 89 ºC; $[\alpha]^{25}_D$ +11.8º (c 0.9, $CHCl_3$); ee > 99.5%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (d, *J* = 8.0 Hz, 1H), 7.30 - 7.28 (m, 3H), 7.22 - 7.18 (m, 4H), 7.07 - 7.05 (m, 1H), 5.80 - 5.65 (m, 2H), 5.05 - 4.98 (m, 2H), 4.15 - 4.08 (m, 1H), 2.94 - 2.78 (m, 2H), 2.60 - 2.58 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.0, 171.4, 141.2, 135.7, 133.4, 128.50, 128.45, 128.18, 128.16, 127.8, 124.5, 66.4, 40.5, 38.5, 37.6; IR (KBr) ν 3345, 3193, 1732, 1668 cm^{-1}; HRMS (FT-MS-ESI): *m/z* calcd. for $C_{18}H_{18}BrNO_3Na$: 398.0362 $[M+Na]^+$ (79Br); Found: 398.0363.

Benzyl (*R*)-4-carbamoyl-3-(2-chlorophenyl)butanoate (2h): white solid (315 mg, 95%); mp: 104 - 105 ºC; $[\alpha]^{25}_D$ +10.8º (c 2.4, $CHCl_3$); ee > 99.5%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 - 7.29 (m, 4H), 7.24 - 7.14 (m, 5H), 5.59 (brs, 2H), 5.05 - 4.99 (m, 2H), 4.17 - 4.10 (m, 1H), 2.95 - 2.80 (m, 2H), 2.62 (d, *J* = 7.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.0, 171.5, 139.5, 135.7, 133.7, 130.1, 128.5, 128.20, 128.18, 128.0, 127.2, 66.4, 40.2, 38.4, 35.2; IR (KBr) ν 3402, 3191, 1729, 1653 cm^{-1}; HRMS (FT-MS-ESI): *m/z* calcd. for $C_{18}H_{18}ClNO_3Na$: 354.0867 $[M+Na]^+$; Found: 354.0869.

Benzyl (*R*)-4-carbamoyl-3-(2-methylphenyl)butanoate (2i): white solid (280 mg, 90%); mp: 73 - 74 ºC; $[\alpha]^{25}_D$ +16.7º (c 1.5, $CHCl_3$); ee > 99.5%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 - 7.27 (m, 3H), 7.15 - 7.09 (m, 6H), 5.66 (brs, 1H), 5.43 (brs, 1H), 5.01 - 4.95 (m, 2H), 3.98 - 3.90 (m, 1H), 2.83 - 2.68 (m, 2H), 2.57 - 2.44 (m, 2H), 2.35 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.5, 171.7, 140.9, 136.2, 135.7, 130.8, 128.5, 128.15, 128.12, 126.7, 126.4, 125.3, 66.3, 41.9, 40.1, 33.6, 19.6; IR (KBr) ν 3338, 3191, 1731, 1666 cm^{-1}; HRMS (FT-MS-ESI): *m/z* calcd. for $C_{19}H_{21}NO_3Na$: 334.1414 $[M+Na]^+$; Found: 334.1416.

Benzyl 4-carbamoyl-3-(2-methoxyphenyl)butanoate (2j): white solid (98 mg, 30%); mp: 121 - 122 ºC; $[\alpha]^{25}_D$ 0º (c 2.6, $CHCl_3$); ee = 5%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 - 7.29 (m, 3H), 7.23 - 7.19 (m, 3H), 7.16 - 7.13 (m, 1H), 6.90 - 6.85 (m, 2H), 5.61 (brs, 1H), 5.34 (brs, 1H), 5.05 - 4.99 (m, 2H), 3.97 - 3.89 (m, 1H), 3.81 (s, 3H), 2.85 - 2.82 (m, 2H), 2.67 - 2.65 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.8, 172.1, 157.0, 135.9, 130.2, 128.48, 128.38, 128.14, 128.11, 120.9, 110.9, 66.2, 55.4, 40.5, 38.7, 33.8; IR (KBr) ν 3351, 3191, 1730, 1665 cm^{-1}; HRMS (FT-MS-ESI): *m/z* calcd. for $C_{19}H_{21}NO_4K$: 350.1363 $[M+Na]^+$; Found: 350.1365.

General procedure for the biotransformations of 3-arylmethylglutaramides 3a-j.

3-arylmethylglutaramide **3** was treated following the aforementioned biotransformations procedure and gave product **4**. All products were fully characterized by means of spectroscopic data. Enantiomeric excess values were obtained from HPLC analysis using columns coated with chiral stationary phases.

Benzyl (*R*)-3-benzyl-4-carbamoylbutanoate (4a): white solid (522 mg, 84%); mp: 87 - 88 ºC; $[\alpha]^{25}_D$ -11.2º (c 1.3, $CHCl_3$); ee > 99.5%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 - 7.33 (m, 5H), 7.29 - 7.25 (m, 2H), 7.22 - 7.14 (m, 3H), 5.49 (brs, 1H), 5.22 (brs, 1H), 5.15 - 5.08 (m, 2H), 2.74 - 2.60 (m, 3H), 2.44 (d, *J* = 6.4 Hz, 2H), 2.26 - 2.24 (m, 2H); ^{13}C NMR (75MHz, $CDCl_3$) δ 174.2, 172.6, 139.3, 135.8, 129.3, 128.6, 128.44, 128.38, 126.4, 66.3, 40.0, 39.2, 37.4, 34.6; IR (KBr) ν 3391, 3210, 1730, 1639, 1613 cm^{-1}; HRMS (FT-MS-ESI): *m/z* calcd. for $C_{19}H_{21}NO_3Na$: 334.1414 $[M+Na]^+$; Found: 334.1418. In the scaled-up biocatalytic reaction, 1579 mg **9a** was obtained in 85% yield and >99% ee (entry 2, Table 2).

Benzyl (*R*)-3-(2-bromobenzyl)-4-carbamoylbutanoate (4b): white solid (686 mg, 88%); mp: 100 - 101 ºC; $[\alpha]^{25}_D$ -6.7º (c 0.9, $CHCl_3$); ee > 99.5%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51 (d, *J* = 7.9 Hz, 1H), 7.36 - 7.28 (m, 5H), 7.22 - 7.17 (m, 2H), 7.10 - 7.03 (m, 1H), 5.59 (brs, 2H), 5.14 - 5.06 (m, 2H), 2.93- 2.65 (m, 3H), 2.55 - 2.42 (m, 2H), 2.38 - 2.22 (m, 2H); ^{13}C NMR (75MHz, $CDCl_3$) δ 173.9, 172.5, 138.9, 135.8, 133.0, 131.6, 128.6, 128.39, 128.35, 128.16, 127.4, 125.0, 66.4, 39.9, 39.0, 37.4, 33.4; IR (KBr) ν 3380, 3187, 1720, 1655 cm^{-1}; MS (ESI) *m/z* 414 $[M+Na]^+$ (81Br); Anal. Calcd. for $C_{19}H_{20}NO_3Br$: C, 58.47; H, 5.17; N, 3.59. Found: C, 58.73; H, 5.20; N, 3.50.

Benzyl (*R*)-3-(3-bromobenzyl)-4-carbamoylbutanoate (4c): white solid (655 mg, 84%); mp: 65 - 66 ºC; $[\alpha]^{25}_D$ -10.0º (c 1.2, $CHCl_3$); ee > 99.5%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 - 7.32 (m, 7H), 7.16 - 7.07 (m, 2H), 5.55 (brs, 2H), 5.16 - 5.07 (m, 2H), 2.75- 2.55 (m, 3H), 2.43 - 2.41 (m, 2H), 2.31 - 2.17 (m, 2H); ^{13}C NMR (75MHz, $CDCl_3$) δ 173.7, 172.3, 141.7, 135.8, 132.3, 130.0, 129.6, 128.6, 128.4, 128.0, 122.5, 66.4, 39.5, 38.9, 37.2, 34.5; IR (KBr) ν 3449, 3206, 1718, 1651 cm^{-1}; MS (ESI) *m/z* 390 $[M+H]^+$ (79Br); Anal. Calcd. for $C_{19}H_{20}NO_3Br$: C, 58.47; H, 5.17; N, 3.59. Found: C, 58.49; H, 5.20; N, 3.47.

Benzyl (*R*)-3-(4-bromobenzyl)-4-carbamoylbutanoate (4d): white solid (663 mg, 85%); mp: 96 - 97 ºC; $[\alpha]^{25}_D$ -6.0º (c 1.0, $CHCl_3$); ee > 99.5%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 - 7.36 (m, 7H), 7.03 (d, *J* = 8.4 Hz, 2H), 5.48 (brs, 1H), 5.24 (brs, 1H), 5.14 - 5.07 (m, 2H), 2.72 - 2.54 (m, 3H), 2.42 (d, *J* = 6.0 Hz, 2H), 2.29 - 2.18 (m, 2H); ^{13}C NMR (75MHz, $CDCl_3$) δ 173.8, 172.3, 138.3, 135.8, 131.5, 131.1, 128.6, 128.4, 120.2, 66.4, 39.3, 38.9, 37.2 34.4; IR (KBr) ν 3390, 3196, 1732, 1652 cm^{-1}; MS (ESI) *m/z* 392 $[M+H]^+$ (81Br); Anal. Calcd. for $C_{19}H_{20}NO_3Br$: C, 58.47; H, 5.17; N, 3.59. Found: C, 57.98; H, 5.09; N, 3.50.

Benzyl (*R*)-4-carbamoyl-3-(4-chlorobenzyl)butanoate (4e): white solid (615 mg, 89%); mp: 81 - 82 ºC; $[\alpha]^{25}_D$ -10.0º (c 0.8, $CHCl_3$); ee > 99.5%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 - 7.34 (m, 5H), 7.24 - 7.24 (m, 2H), 7.09 - 7.07 (m, 3H), 5.49 (brs, 1H), 5.31 (brs, 1H), 5.14 - 5.07 (m, 2H), 2.70 - 2.58 (m, 3H), 2.42 (d, *J* = 6.4 Hz, 2H), 2.25 - 2.22 (m, 2H); ^{13}C NMR (125MHz, $CDCl_3$) δ 174.1, 172.4, 137.8, 135.7, 132.1, 130.6, 128.60, 128.50, 128.37, 128.36,

66.3, 39.2, 39.0, 37.3, 34.4; IR (KBr) ν 3394, 3197, 1732, 1652 cm^{-1}; MS (ESI) *m/z* 368 $[M+Na]^+$ (35Cl); Anal. Calcd. for $C_{19}H_{20}NO_3Cl$: C, 65.99; H, 5.83; N, 4.05. Found: C, 66.07; H, 5.85; N, 4.17.

Benzyl (*R*)-4-carbamoyl-3-(4-fluorobenzyl)butanoate (4f): white solid (599 mg, 91%); mp: 67 - 68 °C; $[\alpha]^{25}_D$ -18.4° (c 2.6, $CHCl_3$); ee > 99.5%; ^1H NMR (400 MHz, $CDCl_3$) δ 7.39 - 7.33 (m, 5H), 7.12 - 7.09 (m, 2H), 6.96 - 6.92 (m, 2H), 5.61 (brs, 1H), 5.48 (brs, 1H), 5.14 - 5.07 (m, 2H), 2.72 - 2.56 (m, 3H), 2.42 (d, *J* = 4.9 Hz, 2H), 2.29 - 2.20 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.9, 172.5, 161.6 (d, *J* = 242.8 Hz), 135.8, 134.8 (d, *J* = 3.2 Hz), 130.7 (d, *J* = 7.9 Hz), 128.7, 128.4, 115.2 (d, *J* = 21.0 Hz), 66.4, 39.1, 38.9, 37.2, 34.7; IR (KBr) ν 3402, 3205, 1725, 1643 cm^{-1}; HRMS (FT-MS-ESI): *m/z* calcd. for $C_{19}H_{20}FNO_3K$: 368.1059 $[M+K]^+$; Found: 368.1060.

Benzyl (*R*)-4-carbamoyl-3-[4-(Trifluoromethyl)benzyl] butanoate (4g): white solid (682 mg, 90%); mp: 74 - 75 °C; $[\alpha]^{25}_D$ -6.8° (c 2.1, $CHCl_3$); ee = 77%; ^1H NMR (400 MHz, $CDCl_3$) δ 7.51 (d, *J* = 8.0 Hz, 2H), 7.36 - 7.35 (m, 5H), 7.27 (d, *J* = 8.0 Hz, 2H), 5.76 (brs, 1H), 5.62 (brs, 1H), 5.14 - 5.07 (m, 2H), 2.82 - 2.62 (m, 3H), 2.42 (d, *J* = 6.4 Hz, 2H), 2.30 - 2.18 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.7, 172.3, 143.5, 135.8, 129.6, 128.77 (q, *J* = 32.1 Hz), 128.64, 128.44, 128.41, 125.35 (q, *J* = 3.7 Hz), 124.3 (q, *J* = 270.2 Hz), 66.4, 39.6, 38.9, 37.3, 34.4; IR (KBr) ν 3396, 3199, 1729, 1659 cm^{-1}; HRMS (FT-MS-ESI): *m/z* calcd. for $C_{20}H_{20}F_3NO_3Na$: 402.1288 $[M+Na]^+$; Found: 402.1292.

Benzyl (*R*)-4-carbamoyl-3-(4-methylbenzyl)butanoate (4h): white solid (598 mg, 92%); mp: 84 - 85 °C; $[\alpha]^{25}_D$ -10.6° (c 2.6, $CHCl_3$); ee > 99.5%; ^1H NMR (400 MHz, $CDCl_3$) δ 7.35 - 7.33 (m, 5H), 7.04 (q, *J* = 8.0 Hz, 4H), 5.88 (brs, 1H), 5.68 (brs, 1H), 5.12 - 5.05 (m, 2H), 2.67 - 2.57 (m, 3H), 2.41 (d, *J* = 5.6 Hz, 2H), 2.29 (s, 3H), 2.22 (d, *J* = 5.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.3, 172.6, 136.2, 135.9, 135.8, 129.2, 129.1, 128.6, 128.36, 128.33, 66.3, 39.6, 39.2, 37.5, 34.6, 21.0; IR (KBr) ν 3394, 3198, 1727, 1652 cm^{-1}; HRMS (FT-MS-ESI): *m/z* calcd. for $C_{20}H_{23}NO_3Na$: 348.1570 $[M+Na]^+$; Found: 348.1573.

Benzyl (*R*)-4-carbamoyl-3-(4-methoxybenzyl)butanoate (4i): white solid (552 mg, 81%); mp: 106 - 107 °C; $[\alpha]^{25}_D$ -7.0° (c 1.2, $CHCl_3$); ee = 91%; ^1H NMR (300 MHz, $CDCl_3$) δ 7.39 - 7.29 (m, 5H), 7.06 (d, *J* = 8.4 Hz, 2H), 6.80 (d, *J* = 8.4 Hz, 2H), 5.61 (brs, 2H), 5.10 - 5.09 (m, 2H), 3.77 (s, 3H), 2.69 - 2.50 (m, 3H), 2.42 (d, *J* = 5.7 Hz, 2H), 2.29 - 2.16 (m, 2H); ^{13}C NMR (75MHz, $CDCl_3$) δ 174.1, 172.6, 158.2, 135.9, 131.3, 130.3, 128.6, 128.4, 113.8, 66.3, 55.2, 39.14, 39.10, 37.4, 34.8; IR (KBr) ν 3436, 3212, 1719, 1652 cm^{-1}; MS (ESI) *m/z* 364 $[M+Na]^+$; Anal. Calcd. for $C_{20}H_{23}NO_4$: C, 70.36; H, 6.79; N, 4.10. Found: C, 70.27; H, 6.80; N, 4.14.

Benzyl (*R*)-3-allyl-4-carbamoylbutanoate (4j): white solid (423 mg, 81%); mp: 70 - 71 °C; $[\alpha]^{25}_D$ -4.1° (c 1.5, $CHCl_3$); ee = 77%; ^1H NMR (300 MHz, $CDCl_3$) δ 7.36 - 7.27 (m, 5H), 5.81 - 5.65 (m, 3H), 5.12 (s, 2H), 5.07 - 5.01 (m, 2H), 2.49 - 2.38 (m, 3H), 2.26 - 2.10 (m, 4H); ^{13}C NMR (75MHz, $CDCl_3$) δ 174.1, 172.6, 135.8, 135.5, 128.6, 128.3, 117.7, 66.3, 39.4, 38.2, 37.7, 32.4; IR (KBr) ν 3384, 3211, 1718, 1643, 1614 cm^{-1}; MS (ESI) *m/z* 284 $[M+Na]^+$; Anal. Calcd. for $C_{15}H_{19}NO_3$: C, 68.94; H, 7.33; N, 5.36. Found: C, 69.07; H, 7.23; N, 5.51.

General procedure for the Preparation of Racemic Esters rac-2 and rac-4.

Diamide **1** or **3** (0.4 mmol) in hydrochloric acid (6 M in water, 2 mL) was heated at 95 °C until the starting material was consumed. The solution was basified to pH 5.0 with aqueous NaOH solution (1 M). After removal of water, the residue was treated following the aforementioned procedure as that for the esterification of acids from biotransformations. The following racemic benzyl esters were obtained and they gave identical spectroscopic data as their enantioenriched ones. *rac*-**2a**: white solid (77 mg, 65%); mp 114 - 116 °C; *rac*-**2b**: white solid (96 mg, 64%); mp 137 - 139 °C; *rac*-**2c**: white solid (91 mg, 69%); mp 130 - 132 °C; *rac*-**2d**: white solid (78 mg, 63%); mp 122 - 124 °C; *rac*-**2e**: white solid (90 mg, 60%); mp 92 - 94 °C; *rac*-**2f**: white solid (66 mg, 53%); mp 95 - 97 °C; *rac*-**2g**: white solid (89 mg, 59%); mp 87 - 88 °C; *rac*-**2h**: white solid (73 mg, 55%); mp 103 - 105 °C; *rac*-**2i**: white solid (65 mg, 52%); mp 72 - 74 °C; *rac*-**2j**: white solid (64 mg, 49%); mp 120 - 122 °C; *rac*-**4a**: white solid (86 mg, 69%); mp 86 - 88 °C; *rac*-**4b**: white solid (90 mg, 58%); mp 99 - 101 °C; *rac*-**4c**: white solid (95 mg, 61%); mp 64 - 66 °C; *rac*-**4d**: white solid (98 mg, 63%); mp 95 - 97 °C; *rac*-**4e**: white solid (90 mg, 65%); mp 80 - 82 °C; *rac*-**4f**: white solid (83 mg, 63%); mp 66 - 68 °C; *rac*-**4g**: white solid (79 mg, 52%); mp 73 - 75 °C; *rac*-**4h**: white solid (81 mg, 62%); mp 83 - 85 °C; *rac*-**4i**: white solid (80 mg, 59%); mp 104 - 106 °C; *rac*-**4j**: white solid (70 mg, 67%); mp 69 - 71 °C.

Preparation of (*R*)-3-benzyl-4-cyanobutanoic acid 5.

4a (156 mg, 0.5 mmol) was mixed with DMF (5 mL) and $SOCl_2$ (0.5 mL) at 0 °C. After stirring at room temperature for 3 h, water (5 mL) was added, and mixture was extracted with ethyl acetate (5 mL × 3). The combined organic solution was dried over anhydrous $MgSO_4$ and concentrated under vacuum to give acid intermediate. The acid was mixed with Pd/C catalyst (10%, 15 mg) and dry methanol (10 mL) and stirred at room temperature under a hydrogen balloon. After removal of catalyst and solvent, the residue was chromatographed on a silica gel column eluted with a mixture of petroleum ether and ethyl acetate (v:v = 1:1) to afford *R*-(+)-**5**[7]: white solid (78 mg, 77%); $[\alpha]^{25}_D$ +38.7° (c 1.5, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 9.58 (brs, 1H), 7.35 - 7.18 (m, 5H), 2.87 - 2.70 (m, 2H), 2.59 - 2.35 (m, 5H); ^{13}C NMR (75MHz, $CDCl_3$) δ 177.1, 137.7, 129.1, 128.9, 127.0, 117.8, 39.4, 36.9, 33.7, 21.1; IR (KBr) ν 2929, 2243, 1715 cm^{-1}.

General procedure for the Preparation of 6 and 8.

To a solution of ester **2g** or **4b** (0.5 mmol) in tetrahydrofuran (0.5 mL) was added anhydrous lithium chloride (43 mg, 1 mmol) and sodium borohydride (38 mg,

1 mmol). After addition of ethanol (1.5 mL), the mixture was stirred at room temperature overnight. The mixture was cooled with an ice-water bath, and water (1.5 mL) was then added. The mixture was extracted with ethyl acetate (3 × 8 mL). after drying over anhydrous $MgSO_4$ and removing the solvent under vacuum, the residue of the organic phase was chromatographed on a silica gel column using a mixture of petroleum ether and ethyl acetate (v:v = 1:1) to afford **6** or **8**.

(*S*)-3-(2-Bromophenyl)-5-hydroxypentanamide (6): white solid(132 mg, 97%); mp: 79 - 80 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 - 7.55 (m, 1H), 7.30 - 7.24 (m, 2H), 7.11 - 7.06 (m, 1H), 5.50 (brs, 1H), 5.43 (brs, 1H), 3.88 - 3.84 (m, 1H), 3.60 - 3.58 (m, 2H), 2.60 (d, *J* = 7.2 Hz, 2H), 2.13 - 2.11 (m, 1H), 2.02 - 1.97 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.6, 142.8, 133.3, 128.20, 128.06, 127.9, 124.7, 60.3, 41.8, 38.0, 37.4; IR (KBr) ν 3341, 3192, 1668 cm^{-1}; HRMS (FT-MS-ESI): *m/z* calcd. for $C_{11}H_{13}BrNO_2$: 272.0115 $[M-H]^-$ (81Br); Found: 272.0117.

(*S*)-3-(2-Bromobenzyl)-5-hydroxypentanamide (8): white solid (137 mg, 96%); mp: 108 - 109 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.55 - 7.52 (m, 1H), 7.31 - 7.25 (m, 2H), 7.12 - 7.07 (m, 1H), 3.61 (t, *J* = 6.8 Hz, 2H), 2.86 - 2.74 (m, 2H), 2.40 - 2.35 (m, 1H), 2.30 - 2.13 (m, 2H), 1.63 - 1.57 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 176.7, 139.6, 132.6, 131.4, 127.7, 127.1, 124.5, 59.5, 40.3, 39.4, 36.1, 33.1; IR (KBr) ν 3338, 3191, 1661 cm^{-1}; HRMS (FT-MS-ESI): *m/z* calcd. for $C_{12}H_{16}BrNO_2Na$: 308.0257 $[M+Na]^+$ (79Br); Found: 308.0263.

Preparation of (*S*)-4-(2-hydroxyethyl)-3,4-dihydroquinolin-2(1*H*)-one 7.

Under argon protection, a mixture of **6** (82 mg, 0.3 mmol), CuI (29 mg, 0.15 mmol), *N*, *N*-dimethylglycine hydrochloride (42 mg, 0.3 mmol), Cs_2CO_3 (245 mg, 0.75 mmol) and dry 1,4-dioxane (15 mL) was refluxed for 24 h. After cooling, ethyl acetate (30 mL) was added and the resulting mixture was filtered through a short silica gel pad. The filtrate was concentrated and the residue was chromatographed on a silica gel column using a mixture of petroleum ether and ethyl acetate (v:v = 1:1) to afford **7** (46 mg, 80%) as colourless oil; $[\alpha]^{25}_D$ -6.7° (c 0.4, $CHCl_3$); ee > 99.5%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (brs, 1H), 7.22 - 7.17 (m, 2H), 7.04 - 7.00 (m, 1H), 6.75 (d, *J* = 8.0 Hz, 1H), 3.74 - 3.61 (m, 2H), 3.27 - 3.21 (m, 1H), 2.84 - 2.78 (m, 1H), 2.60 - 2.55 (m, 1H), 1.86 - 1.79 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.7, 136.5, 128.3, 127.9, 127.1, 123.4, 115.8, 59.9, 36.5, 36.3, 32.9; IR (KBr) ν 3219, 2926, 1674 cm^{-1}; HRMS (FT-MS-ESI): *m/z* calcd. for $C_{11}H_{12}NO_2$: 190.0873 $[M-H]^-$; Found: 190.0869.

Preparation of (*S*)-4-(2-bromobenzyl)tetrahydro-2*H*-pyran-2-one 9.

A solution of **8** (57 mg, 0.2 mmol) in toluene (10 mL) was refluxed for 24 h. after evaporation, the residue was chromatographed on a silica gel column using a mixture of petroleum ether and ethyl acetate (v:v = 10:1) to afford **9** (52 mg, 97%) as colourless oil; $[\alpha]^{25}_D$ -5.7° (c 0.4, $CHCl_3$); ee > 99.5%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 - 7.55 (m, 1H), 7.28 - 7.24 (m, 1H), 7.18 - 7.16 (m, 1H), 7.13 - 7.09 (m, 1H), 4.47 - 4.42 (m, 1H), 4.27 - 4.20 (m, 1H), 2.79 (d, *J* = 7.2 Hz, 2H), 2.70 - 2.63 (m, 1H), 2.41 - 2.34 (m, 1H), 2.30 - 2.23 (m, 1H), 1.95 - 1.88 (m, 1H), 1.70 - 1.60 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 137.9, 133.2, 131.3, 128.4, 127.5, 124.7, 68.6, 42.2, 36.3, 32.1, 28.6; IR (KBr) ν 1736 cm^{-1}; HRMS (FT-MS-CI): *m/z* calcd. for $C_{12}H_{14}BrO_2$: 269.0172 $[M+H]^+$ (79Br); Found: 269.0169.

Acknowledgements

Financial supports from National Natural Science Foundation of China (21502202, 21521002), MOST (2014CB643601) are gratefully acknowledged.

References

[1] For overviews, see a) M.-X. Wang, *Top. Catal.* **2005**, *35*, 117-130; b) W.-D. Fessner, N. J. Turner, M.-X. Wang, *Adv. Synth. Catal*. **2011**, *353*, 2189-2190; c) T. Hudlicky, *Chem. Rev.* **2011**, *111*, 3995-3997; d) U. T. Bornscheuer, G. W. Huisman, R. J. Kazlauskas, S. Lutz, J. C. Moore, K. Robins, *Nature* **2012**, *485*, 185-194; e) M.-X. Wang, *Acc. Chem. Res.* **2015**, *48*, 602-611; f) M. Hönig, P. Sondermann, N. J. Turner, E. M. Carreira, *Angew. Chem. Int. Ed.* **2017**, *56*, 8942-8973; g) R. O. M. A. Souza, L. S. M. Miranda, U. T. Bornscheuer, *Chem. Eur. J.* **2017**, *23*, 12040-12063.

[2] For overviews, see a) E. García-Urdiales, I. Alfonso, V. Gotor, *Chem. Rev.* **2005**, *105*, 313-354; b) E. García-Urdiales, I. Alfonso, V. Gotor, *Chem. Rev.* **2011**, *111*, PR110-PR180; c) J. M. Palomo, Z. Cabrera, *Curr. Org. Synth*. **2012**, *9*, 791-805.

[3] For some recent books, see a) K. Faber, *Biotransformations in Organic Chemistry 6th ed.*, Springer, Berlin, **2011**; b) K. Faber, W.-D. Fessner, N. J. Turner, *Biocatalysis in Organic Synthesis 1st ed.*, Thieme, Stuttgart, **2015**; c) A. Goswami, J. D. Stewart, *Organic Synthesis Using Biocatalysis, 1st ed.*, Elsevier, Amsterdam, **2016**.

[4]For selected recent examples, see a) M. Z. Xu, J. Ren, J. S. Gong, W. Y. Dong, Q. Q. Wu, Z. H. Xu, D. M. Zhu, *Chin. J. Biotech*. **2013**, *29*, 31-40; b) B. Skrobo, J. Deska, *Org. Lett.* **2013**, *15*, 5998-6001; c) F. Guo, S. Franzen, L. Ye, J. Gu, H. Yu, *Biotech. Bioengin*. **2014**, *111*, 1729-1739; d) N.-W. Wan, Z.-Q. Liu, F. Xue, Z.-Y. Shen, Y.-G. Zheng, *ChemCatChem*. **2015**, *7*, 2446-2450; e) M. Nojiri, M. Hibi, H. Shizawa, N. Horinouchi, Y. Yasohara, S. Takahashi, J. Ogawa, *Appl. Microbiol. Biotechnol.* **2015**, *99*, 9961-9969; f) A. Pizzilli, R. Zoppi, P. Hoyos, S. Gómez, F. G. Gatti, M. J. Hernáiz, A. R. Alcántara, *Tetrahedron* **2015**, *71*, 9172-9176; g) P.-H. Chan, S.-W. Tsai, *Chem. Engin. Sci.* **2016**, *139*, 41-48; h) H. K. Kotapati, J. D. Robinson, D. R. Lawrence, K. R. Fortner, C. W. Stanford, D. R. Powell, R. Wardenga, U. T. Bornscheuer, D. S. Masterson, *Eur. J. Org. Chem.* **2017**, 3009-3016; i) J. Wu, H. Wang, B.

Yang, W. Song, C. Liang, L. Liu, *RCS Adv*. **2017**, *7*, 38264-38272.

[5] For overview, see: Y.-F. Ao, Q.-Q. Wang, D.-X. Wang, *Chin. J. Org. Chem.* **2016**, *36*, 2333-2343.

[6] a) G. DeSantis, Z. Zhu, W. A. Greenberg, K. Wong, J. Chaplin, S. R. Hanson, B. Farwell, L. W. Nicholson, C. L. Rand, D. P. Weiner, D. E. Robertson, M. J. Bruk, *J. Am. Chem. Soc.* **2002**, *124*, 9024-9025; b) G. DeSantis, K. Wong, B. Farwell, K. Chatman, Z. Zhu, G. Tomlinson, H. Huang, X. Tan, L. Bibbs, P. Chen, K. Kretz, M. J. Burk, *J. Am. Chem. Soc.* **2003**, *125*, 11476-11477; c) S. Bergeron, D. A. Chaplin, J. H. Edwards, B. S. W. Ellis, C. L. Hill, K. Holt-Tiffin, J. R. Knight, T. Mahoney, A. P. Osborne, G. Ruecroft, *Org. Process Res. Dev.* **2006**, *10*, 661-665; d) Y. Duan, P. Yao, J. Ren, C. Han, Q. Li, J. Yuan, J. Feng, Q. Wu, D. Zhu, *Sci. China Chem.* **2014**, *57*, 1164-1171.

[7] H. Kakeya, N. Sakai, A. Sano, M. Yokoyama, T. Sugai, H. Ohta, *Chem. Lett.* **1991**, *20*, 1823-1824.

[8] J. A. Crosby, J. S. Parratt, N. J. Turner, *Tetrahedron: Asymmetry*, **1992**, *3*, 1547-1550.

[9] a) M.-X. Wang, C.-X. Liu, J.-S. Li, O. Meth-Cohn, *Tetrahedron Lett.* **2000**, *41*, 8549-8552. b) M.-X. Wang, C.-X. Liu, J.-S. Li, *Tetrahedron: Asymmetry*, **2001**, *12*, 3367-3373.

[10] M. K. S. Vink, C. A. Schortinghuis, J. Luten, J. H. van Maarseveen, H. E. Schoemaker, H. Hiemstra, F. P. J. T. Rutjes, *J. Org. Chem.* **2002**, *67*, 7869-7871.

[11] H. H. Kinfe, V. Chhiba, J. Frederick, M. L. Bode, K. Mathiba, P. A. Steenkamp, D. Brady, *J. Mol. Catal. B: Enzym.* **2009**, *59*, 231-236.

[12] Z.-L. Wu, Z.-Y. Li, *J. Org. Chem.* **2003**, *68*, 2479-2482.

[13] a) M. Nojiri, K. Uekita, M. Ohnuki, N. Taoka, Y. Yasohara, *J. Appl. Microbiol.* **2013**, *115*, 1127-1133; b) M. Nojiri, S. Yoshida, H. Kanamaru, Y. Yasohara, *J. Appl. Microbiol.* **2016**, *120*, 1542-1551.

[14] a) L.-B. Zhang, D.-X. Wang, M.-X. Wang, *Tetrahedron* **2011**, *67*, 5604-5609; b) L.-B. Zhang, D.-X. Wang, L. Zhao, M.-X. Wang, *J. Org. Chem.* **2012**, *77*, 5584-5591.

[15] a) P. Chen, M. Gao, D.-X. Wang, L. Zhao, M.-X. Wang, *Chem. Commun.* **2012**, *48*, 3482-3484; b) P. Chen, M. Gao, D.-X. Wang, L. Zhao, M.-X. Wang, *J. Org. Chem.* **2012**, *77*, 4063-4072; c) Y.-F. Ao, D.-X. Wang, L. Zhao, M.-X. Wang, *Chem. Asian J.* **2015**, *10*, 938-947.

[16] G. P. Schiemenz, H. Engelhard, *Chem. Ber.* **1962**, *95*, 195-198.

[17] a) E. Samu, P. Huszthy, L. Somogyi, M. Hollósi, *Tetrahedron: Asymmetry* **1999**, *10*, 2775-2795; b) J. Poldy, R. Peakall, R. A. Barrow, *Eur. J. Org. Chem.* **2012**, 5818-5827.

[18] A. Ohtaki, K. Murata, Y. Sato, K. Noguchi, H. Miyatake, N. Dohmae, K. Yamada, M. Yohda, M. Odaka, *BBA-Proteins Proteom.* **2010**, *1804*, 184-192.

[19] For selected recent examples about the catalytic mechanism of amidases, see: a) R.-C. Zheng, J.-Q. Jin, Z.-M. Wu, X.-L. Tang, L.-Q. Jin, Y.-G. Zheng, *Bioorg. Chem.* **2018**, *76*, 81-87; b) H. Li, S. Zhu, G. Zheng, *Bioorg. Med. Chem. Lett.* **2018**, *28*, 1071-1076.

[20] CCDC-1833672 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

[21] a) F. E. Bloom, In: *Goodman and Gilman's The Pharmacological Basis of Therapeutics, 11th ed*., (Eds.: L. L. Brunton, J. S. Lazo, K. L. Parker), McGraw-Hill, New York, **2006**, pp. 317-340; b) P. Ramesh, D. Suman, K. S. N. Reddy, *Synthesis* **2018**, *50*, 211-226.

10.1002/adsc.201800956

FULL PAPER

Biocatalytic Desymmetrization of Prochiral 3-Aryl and 3-Arylmethyl Glutaramides: Different Remote Substituent Effect on Catalytic Efficiency and Enantioselectivity

Adv. Synth. Catal. **Year**, *Volume*, Page – Page

Yu-Fei Ao,* Li-Bin Zhang, Qi-Qiang Wang, De-Xian Wang, Mei-Xiang Wang