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Enantioselective biotransformations of racemic α-substituted phenylacetonitriles and phenylacetamides using *Rhodococcus* sp. AJ270

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Abstract

Rhodococcus sp. AJ270 is an efficient whole-cell system able to catalyze the stereoselective conversions of racemic α-substituted phenylacetonitriles and amides under very mild conditions into enantiopure carboxylic acids and derivatives. The nitrile hydratase involved generally has a broad substrate spectrum against phenylacetonitriles irrespective of the electronic nature of the α-substituent while the amidase is very sensitive to both the electronic and steric factors of the substituent of amides. The overall enantioselectivity of nitrile hydrolysis is mainly determined by the combination of selectivities of nitrile hydratase and of amidase, with the latter being a major contributor. The amidase has high *S*-enantiocontrol against amides while the nitrile hydratase exhibits low *R*-selectivity against nitriles. The scope and limitations of this enantioselective biotransformation process are discussed.

1. Introduction

The past decade has seen tremendous development of biotransformations using microorganisms and isolated enzymes in organic synthesis. Since most of the reactions are highly chemo-, regio- and stereoselective and are performed under very mild conditions, biotransformations offer many opportunities for the syntheses of compounds which are sometimes not readily obtainable by 'conventional' chemical means.[1–3] For example, lipases and esterases have been routinely used for the kinetic resolution of racemic esters and alcohols and for the preparation of homochiral molecules from prochiral and *meso* diesters and diols.[2–4] In contrast to these extensively investigated hydrolytic enzymes, nitrile-hydrolyzing enzymes in organic synthesis have remained largely

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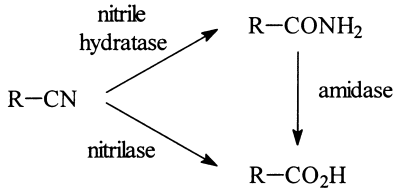
unexplored until recently,[5,6] despite the fact that nitriles are very important compounds[7] and the bioconversion of nitriles into the corresponding carboxylic acids has been known for decades.[8] Enzyme-catalyzed hydrolyses of nitriles have been shown to proceed by either direct transformation to carboxylic acids with a nitrilase[9] or a nitrile hydratase-catalyzed formation of amides which are further hydrolyzed to the acids by an amidase[10] (Scheme 1). A range of nitrile-hydrolyzing micro-organisms has been isolated[11] and some nitrilases, nitrile hydratases and amidases have also been purified.[12] The mechanisms of enzymatic hydrolysis of nitriles using nitrilase and nitrile hydratase have been proposed, and for the latter the hydration stage is believed to involve complexion of the nitrile function to a transition metal such as iron or cobalt.[12] It is particularly worth mentioning that the microbial hydration of acrylonitrile is currently being applied in Japan to produce tens of thousands of tonnes of acrylamide per year.[13]



Scheme 1.

In our previous studies,[11,12] it has been demonstrated that *Rhodococcus* sp. AJ270 is a powerful and robust nitrile hydratase/amidase-containing micro-organism. Compared with other micro-organisms reported recently, it has a very broad activity against almost all types of nitriles including aromatic, heterocyclic and aliphatic ones, and both amides and acids can be produced in high yields from appropriate nitriles.[11] We have also shown that this novel biocatalyst displayed excellent regioselectivity in hydrolyzing aromatic dinitriles and a variety of aliphatic dinitriles bearing a suitably placed second chelating moiety.[12] In order to further explore its potential in organic synthesis, and also to understand the mechanisms of enzymatic hydrolyses of nitriles and amides, we have conducted a systematic study of stereoselective biotransformations by examining the effect of substrate structures on both the stereoselectivity and reactivity of the reactions. Here we wish to report the enantioselective biotransformations of α-substituted phenylacetonitriles and amides using *Rhodococcus* sp. AJ270, a convenient and efficient synthesis of some enantiopure α-arylalkanoic acids and derivatives of pharmaceutical interest.[14]

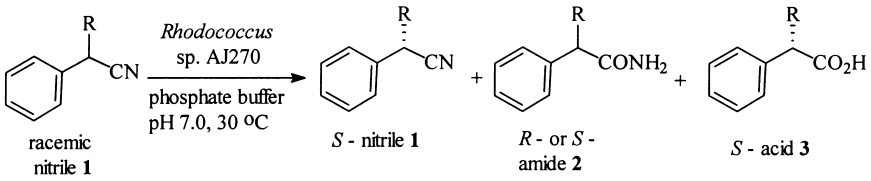
Several studies of enantioselective hydrolysis have been reported and most of them have focused on α-arylpropionitriles, which is not surprising since their hydrolytic product α-arylpropionic acids are related to the non-steroidal anti-inflammatory drugs.[14] Thus, using *Rhodococcus rhodochrous* IFO 15564 (formerly classified as *Rhodococcus butanica* ATCC21197), a micro-organism containing nitrilase, nitrile hydratase and amidase, Kakeya and co-workers[15] and others[16] obtained good enantiomeric excesses of both amide and acid with opposite configuration from the hydrolysis of nitrile. Turner's group[17] used an immobilized whole-cell *Rhodococcus* SP361, a nitrile hydratase/amidase system developed by the Novo Industry of Denmark, and found that the amidase involved is highly *S*-selective towards amide substrates while nitrile hydration is non-selective or poorly *R*- or *S*-selective depending upon the substituent attached to the *para* position of the phenyl ring of the α-arylpropionitrile. Similar stereoselectivity has also been observed with other nitrile hydratase/amidase containing micro-organisms, including

Rhodococcus equi TG328,[18] *Rhodococcus* sp. C3II,[16,19] *Rhodococcus erythropolis* MP50,[16,19] *Agrobacterium tumefaciens* d3[20] and *Pseudomonas putida* NRRL-18668,[21] and with nitrilase-containing micro-organisms such as *Rhodococcus* NCIMB 11216[22] and *Acinetobacter* sp. AK226.[23] Hydrolyses of other racemic substrates such as cyanohydrins[24–27] and their *O*-protected derivatives[28], and amino nitriles[29,30] have been reported to give a range of enantioselectivities varying with the micro-organisms used. It is interesting to note that, catalyzed by *Pseudomonas* sp. B21C9,[31] 2-(4-chlorophenyl)-3-methylbutyronitrile was kinetically resolved into the corresponding *R*-amide and *S*-acid while *Pseudomonas putida* NRRL-18668[21] has been shown very recently to have excellent *S*-nitrile hydratase selectivity, yielding the *S*-amide although it exhibited *S*- and *R*-selectivities of amidase and nitrile hydratase, respectively, against α-arylpropionitriles and α-arylpropionamides.

2. Results

2.1. Enantioselective biotransformations of nitriles

To understand the scope and limitations of the *Rhodococcus* sp. AJ270 in enantioselective hydrolyses of nitriles and amides, we first tested a number of racemic α-substituted phenylacetonitriles **1a**–**g** (Scheme 2). The reaction was carried out conveniently by using whole cells (2 g wet weight) at 30°C in an aqueous phosphate buffer (pH 7.0). The configurations of the amides **2** and acids **3** were obtained by comparing the direction of specific rotations with those of authentic samples. Since optically active α-methylthiophenylacetic acid **3g** and its amide **2g** have not been reported in the literature, we assigned their configurations on the basis of specific rotation assuming that the correlationship between the specific rotation and absolute configuration for α-methoxyphenylacetic acid holds similar to that for α-methylthiophenylacetic acid.[32] The configurations of the recovered nitriles **1c** and **1e** were determined by measuring the optical rotations of the corresponding amides which were obtained from the chemical hydrolysis although partial racemerization occurred during the course of chemical transformation.



R = Me, Et, *n*-Pr, *i*-Pr, *n*-Bu, MeO, MeS

Scheme 2.

The results summarized in Table 1 demonstrate clearly that *Rhodococcus* sp. AJ270 can hydrolyze phenylacetonitriles bearing a variety of substituents at the α-position. The reaction outcome, however, was strongly influenced by the nature of the α-substituent, both steric and electronic factors dramatically affecting the reactivity and, more importantly, the enantioselectivity (Table 1). For the nitriles, having a sterically small group such as methyl **1a** and ethyl **1b**, the biotransformation was very efficient (Table 1, entries 1–4). Effective hydrolysis was also

Table 1
Enantioselective biotransformations of nitriles

Entry	Reactant 1	R	time (h)	Recov. 1 (%)	Config. e.e. (%)	Amide 2 (%)	Config. e.e. (%)	Acid 3 (%)	Config. e.e. (%)
1	**a**	Me	10	-	-	42	*R*, >99	48	*S*, 90
2	**a**	Me	13.5	-	-	36	*R*, >99	58	*S*, 67
3	**b**	Et	70	-	-	58	*R*, 35	39	*S*, >99
4	**b**	Et	96	-	-	34	*R*, 96	40	*S*, >99
5	**c**	*n*-Pr	150	55	*S*, 24	27	*S*, 41	8	*S*, >99
6	**c**	*n*-Pr	214	33	*S*, 28	40	*S*, 13	13	*S*, >99
7	**d**	*i*-Pr	120	-	-	47	*R*, >99	46	*S*, >99
8	**e**	*n*-Bu	300	36	*S*, 36	34	*S*, 20	23	*S*, 98
9	**f**	MeO	46	-	-	78	0	-	-
10	**f**	MeO	72	-	-	56	0	-	-
11	**g**	MeS	120	-	-	64	*R*, 15	10	*S*, 96

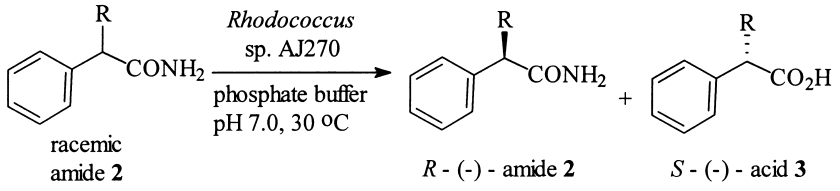
observed when α-isopropylphenylacetonitrile **1d** was used as a substrate (Table 1, entry 7). Only when a large and conformationally flexible group such as *n*-propyl and *n*-butyl was introduced did the hydrolysis of **1c** and **1e** proceed slowly (Table 1, entries 6 and 8). Introduction of a polar moiety such as a methoxy or methylthio group instead of an alkyl at the α-position had no effect on the rate of hydration, both **1f** and **1g** being transformed into the corresponding amides effectively. Surprisingly, however, only a small amount of α-methythiophenylacetic acid **3g** was obtained after a long incubation time. In the case of α-methoxyphenylacetonitrile **1f**, no corresponding acid at all was isolated although prolonged reaction time caused a decrease in chemical yield of amide **2f** (Table 1, entries 9–11).

Although the electronic nature of the α-substituent of nitrile **1** did not greatly influence the efficiency of biohydrolysis of nitrile, interestingly it led to a totally different stereochemistry. For example, α-alkylphenylacetonitriles **1a**, **1b** and **1d** underwent highly stereoselective

biotransformations to give optically active *R*-amides and *S*-acids with enantiomeric excesses higher than 99% (Table 1, entries 1, 4 and 7). Slow and incomplete biotransformations of the sterically more bulky nitriles **1c** and **1e** gave rise predominantly to *S*-enantiomers of the corresponding acids **3c** and **3e**, albeit in very low chemical yields. However, It is noteworthy that both amides **2c** and **2e** and untransformed nitriles **1c** and **1e** isolated from the reaction were also *S*-enantiomers in excess although the optical yields were low (Table 1, entries 5, 6 and 8). This seems very strange, as an enantioselective nitrile hydratase should give amide and nitrile with opposite configurations. The same *S*-enantioselectivity of amidase was observed for the biotransformation of methylthiophenylacetonitrile **1g**, which gave a 96% enantiomeric excess of acid **3g** (Table 1, entry 11). In sharp contrast, no stereoselectivity at all was found for the reaction of α-methoxyphenylacetonitrile **1f** (Table 1, entries 9 and 10). It was considered that a phenylacetonitrile substrate substituted at the α-position by an even more polar group might lead to high and reversed enantioselectivity. We, therefore, examined the biotransformations of α-acetoxyphenylacetonitrile **1h** (*O*-acetylmandelonitrile) and α-benzoyloxyphenylacetonitrile **1i** (*O*-benzoylmandelonitrile) under the same reaction conditions. The reaction of **1h** was rapid and efficient while **1i** could not be fully transformed, with more than half being recovered after 6 days' reaction. Unfortunately, in both cases, no corresponding amide and acid were obtained and, instead, *S*-mandelic acid in ca. 30% ee was isolated along with benzoic acid.

2.2. Enantioselective biotransformations of amides

To shed further light on these stereoselective reactions some racemic amides were prepared and subjected to the *Rhodococcus* sp. AJ270-catalyzed hydrolysis (Scheme 3). The results listed in Table 2 show high activity and enantioselectivity of amidase against some α-alkylphenylacetamides. For example, both the unchanged amide **2b** and the product acid **3b** were obtained in excellent chemical yields and enantiomeric excesses if the reaction was quenched at the right stage (Table 2, entry 4). As with the nitrile hydratase, the amidase was also sensitive to steric hindrance. The presence of a larger alkyl group such as *n*-propyl and *n*-butyl within the substrate therefore greatly inhibited the hydrolysis, with only a small proportion of amide being transformed after more than ten days (Table 2, entries 5–8). More noticeably, however, the enantioselectivity was almost lost for amide **2e** after prolonged incubation (Table 2, entries 7 and 8). This result is surprising since the biotransformation using the corresponding nitrile **1e** as a substrate afforded the acid **3e** in an enantiomeric excess of 98% (Table 1, entry 8). It is very important to note that in all cases the amide obtained was *R*-enantiomer in excess, the stereochemistry being different from that of the reaction using nitriles as the substrate (Table 1, entries 5, 6 and 8).



R = Me, Et, *n*-Pr, *i*-Pr, *n*-Bu

Scheme 3.

Table 2
Enantioselective biotransformations of amides

Entry	Reactant	R	time (h)	*R*-amide	e.e. (%)	*S*-acid	e.e. (%)
	2			**2** (%)		**3** (%)	
1	**a**	Me	8	44	>99	51	56
2	**a**	Me	27	22	>99	78	49
3	**b**	Et	48	72	29	20	>99
4	**b**	Et	120	47	>99	51	>99
5	**c**	*n*-Pr	256	89	15	8	95
6	**c**	*n*-Pr	325	84	20	15	>99
7	**e**	*n*-Bu	256	60	3	27	12
8	**e**	*n*-Bu	325	57	6	25	14

3. Discussion

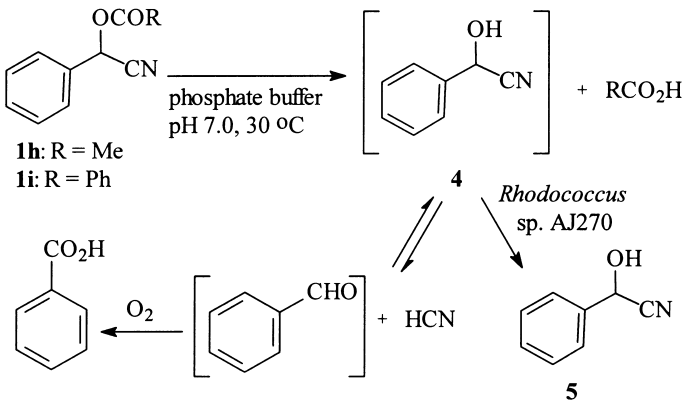
The rapid and efficient conversion of most of the nitriles tested in this study into the corresponding amides indicates clearly that the nitrile hydratase involved in *Rhodococcus* sp. AJ270 has a broader substrate spectrum irrespective of the electronic nature of the substituent of the nitriles. This is in agreement with the conclusion we reached from our previous studies.[11] In contrast to the nitrile hydratase, the amidase of *Rhodococcus* sp. AJ270, however, shows both electronic and steric limitations. The presence of a polar α-substituent such as methoxy or methylthio in the molecule strongly inhibits the hydrolysis of amides. The introduction of a bulky and flexible alkyl group including *n*-propyl and *n*-butyl also has a detrimental effect towards the amidase action.

The results of the biotransformations of nitriles and of amides have shown strong evidence that the enantioselectivity of the biotransformations of nitriles is the outcome of a combination of selectivities of the nitrile hydratase and of the amidase, with the latter being a major and important contributor. The formation of the *S*-carboxylic acid in almost all cases either from the biotransformation of nitrile or from the biotransformation of amide demonstrated the strict *S*-enantioselectivity of the amidase. The results obtained also suggest that the amidase in *Rhodococcus* sp. AJ270 seems to have the ability to recognize substituents of different sizes and of marginally different polarities. The replacement of the alkyl substituent such as methyl and ethyl

by a polar methoxy or methylthio, for instance, led to a mismatch in the recognition between the amidase and substrate, and the hydrolysis of amides **2f** and **2g**, therefore, was less effective. Increase in the length of the alkyl substituent to more than three methylenes, on the other hand, also resulted greatly in the inhibition of amidase function. It should be noted that the reason for the huge difference in the enantioselective formation of *S*-α-*n*-butylphenylacetic acid **3e** between using nitrile and using amide as the substrate (Table 1, entry 8 and Table 2, entries 7 and 8) is not clear. One may suggest that a specific racemase against the acid was stimulated under the reaction conditions when using the amide as the substrate. It may also be due to the low solubility of the amide **2e** in the aqueous buffer, which leads to only a very limited amount of both enantiomers of amide in solution available to the amidase. We did find that when a very small amount of nitrile or amide was fed to the organism under the same conditions, the conversion to acid was rapid and usually went to completion with the formation of racemic acid. To prepare optically active carboxylic acids using *Rhodococcus* sp. AJ270, the advantage of employing nitriles as starting materials is apparent.

The difficulty remains at this stage in defining the stereoselectivity of nitrile hydratase. The isolation of both *S*-nitrile and *S*-amide from racemic substrates **1c** and **1e** makes rationalization even more difficult. Nevertheless, although the nitrile hydratase also suffers from steric limitations, it has a relatively broader substrate spectrum against both enantiomers of nitriles tested and therefore the enantioselective control appears less effective than amidase. The low enantiomeric excess of the *S*-nitriles **1c** and **1e** recovered from the reaction showed that the nitrile hydratase in *Rhodococcus* sp. AJ270 has poor *R*-selectivity against these racemic nitriles. It should be noted that if the enantioselectivities of the biotransformation of nitrile were affected only by the combination of a highly selective *S*-amidase and a low *R*-selective nitrile hydratase, the amide formed during the course of the reaction would be *R*-configured. The fact that an excess of *S*-amide was obtained in these cases suggested the involvement of a racemase that most probably acted specifically against α-*n*-propyl- and α-*n*-butylphenylacetamides. The proposal of a specific amide racemase rather than a nitrile racemase was further supported by the observation of an extremely poor enantiomeric excess of amide **2c** from the biotransformation of racemic amide **2c** while the acid **3c** was obtained in excellent enantiopurity (Table 2, entries 5 and 6).

The isolation of mandelic acid **5** and benzoic acid from the hydrolysis of acylated mandelonitriles revealed for the first time that *Rhodococcus* sp. AJ270 has esterase activity.[24] The rapid hydrolysis of a relatively bulky acylated mandelonitrile **1h** also strongly suggested that ester hydrolysis precedes nitrile attack, since we have already established that bulky α-substituents inhibit nitrile and amide conversion. As a consequence, the interaction of α-acetoxyphenylacetonitrile **1h** with *Rhodococcus* sp. AJ270 first resulted in the hydrolysis of the ester to afford mandelonitrile **4**, which was then converted into mandelic acid **5** by the action of nitrile hydratase and amidase. The isolation of a small amount of benzoic acid ($<10\%$) from this reaction provided further evidence of the formation of mandelonitrile, as mandelonitrile coexisted in an equilibrium with HCN and benzaldehyde and the latter was oxidized by air to form benzoic acid (Scheme 4). Due to the steric hindrance of the phenyl group, the ester hydrolysis of *O*-benzoylmandelonitrile **1i** was less effective and the resulting mandelonitrile underwent similar hydrolysis. The fact that the chemical yield of benzoic acid was slightly higher than theoretical in this case again indicated that oxidation of benzaldehyde occurred during the course of the reaction. It is hard to determine at this stage whether it is the nitrile hydratase/amidase or the esterase that leads to the low enantioselectivity of the reaction. It is noteworthy that the esterase involved in *Rhodococcus* sp. AJ270 seems somewhat specific against esters derived from mandelonitrile,



Scheme 4.

especially *O*-acetylmandelonitrile **1h**, as our earlier study showed that methyl benzoate was not attacked by the organism.[11] Both the specificity and stereoselectivity of the esterase of *Rhodococcus* sp. AJ270 await further study.

4. Conclusion

Rhodococcus sp. AJ270 is an efficient biocatalytic system, very useful for the preparation of enantiopure carboxylic acids and derivatives through stereoselective hydrolyses of racemic α-substituted phenylacetonitriles and amides. The nitrile hydratase involved generally has a broad substrate spectrum against phenylacetonitriles irrespective of the electronic nature of the α-substituent while the amidase is very sensitive to both electronic and steric factors of the substituent of amides. The overall enantioselectivity of nitrile hydrolysis is mainly determined by the combination of selectivities of nitrile hydratase and of amidase, with the latter being a major contributor. The amidase has high *S*-enantiocontrol against amides while the nitrile hydratase exhibits lower *R*-selectivity against nitriles. *Rhodococcus* sp. AJ270 may probably contain an amide racemase acting specifically on α-*n*-propyl- and α-*n*-butylphenylacetamides. The biotransformations of these amides and the corresponding nitriles therefore led to very low enantiomeric excesses of *R*- or even *S*-amide. *Rhodococcus* sp. AJ270 has been shown to have specific esterase activity against acylated mandelonitriles, especially *O*-acetylmandelonitrile.

5. Experimental

5.1. General

Both melting points, which were determined using a Reichert Kofler hot-stage apparatus, and boiling points are uncorrected. IR spectra were obtained on a Perkin–Elmer 782 instrument as liquid films or KBr discs. NMR spectra were recorded on Varian Unity 200 and Bruker AM 300 spectrometers. Chemical shifts are reported in ppm and coupling constants are given in hertz. Mass spectra were measured on an AEI MS-50 mass spectrometer and microanalyses were carried out by the Analytical Laboratory of the Institute.

Polarimetry was carried out using an Optical Activity AA-10R polarimeter and the measurements were made at the sodium D-line with a 5 cm pathlength cell. Concentrations (c) are given in g/100 ml. The enantiomeric excess (ee) values of all products were obtained by means of chiral HPLC and chiral GC analyses using racemic samples as references. The enantiomeric excesses of all amides were obtained with a Shimadzu LC-10AVP HPLC system using a Chiracel OD column at a flow rate of 0.8 ml/min, with 2-propanol:hexane:H_3PO_4 [90:10:0.1] as the mobile phase. The enantiomeric excesses of α-methoxy-, α-methylthio- and α-hydroxyphenylacetic acids were analyzed using the same chiral HPLC conditions but at a flow rate of 0.4 ml/min. The chiral analysis of nitriles was carried using a Chiral BSA-RP column with phosphate buffer (0.1 M, pH 7.4):acetonitrile:1-butanol [88:10:2] as the mobile phase at a flow rate of 1 ml/min. To obtain good chiral separation, α-alkylphenylacetic acids were converted to their methyl esters using diazomethane.[33] The methyl esters of α-methyl- and α-ethylphenylacetic acids were well separated on a Chiracel OJ column with hexane:2-propanol [180:1] as the solvent at a flow rate of 0.8 and 0.4 ml/min, respectively. The separations of enantiomers of methyl α-*n*-propylphenylacetic acetate and of methyl α-*n*-butylphenylacetic acetate were achieved using a chiral GC CP-Chirasil-Dex CB column at the column temperature of 110 and 115°C, respectively.

Except for **1a** and **1b**, which were purchased from Aldrich Chemical Co., nitriles **1c**–**e**,[34] **1f**,[35] **1g**,[36] **1h**[37] and **1i**[37] were prepared according to the literature. Racemic amides[38] and acids[39] were obtained from chemical hydrolysis of nitriles following the literature methods.

5.2. General procedure for the biotransformations of nitriles and amides

To an Erlenmeyer flask (250 ml) with a screw cap was added *Rhodococcus* sp. AJ270 cells (2 g wet weight) and potassium phosphate buffer (0.1 M, pH 7.0, 50 ml) and the resting cells were activated at 30°C for 0.5 h with orbital shaking. Nitriles (3 mmol) or amides (2 mmol) were added in one portion to the flask and the mixture was incubated at 30°C using an orbital shaker (200 rpm). The reaction, monitored by TLC, was quenched after a period of time (see Tables 1 and 2) by removing the biomass through a Celite pad filtration. The resulting aqueous solution was basified to pH 12 with aqueous NaOH (2 M). Extraction with diethyl ether gave, after drying ($MgSO_4$) and concentration, the amide and unconverted nitrile. Separation of amide and nitrile was effected by column chromatography. The aqueous solution was then acidified using aqueous HCl (2 M) to pH 2 and extracted with diethyl ether. Acid was obtained after removal of the solvent. All products were characterized by their spectra data and comparison of the melting points and optical rotary power with the known compounds, which are listed below, or by full characterization.

5.2.1. Enzymic hydrolysis of (±)-2-phenylpropionitrile **1a**

(*R*)-(−)-2-Phenylpropionamide **2a**: 10 h (42%) $[\alpha]_D^{25}$ −56.8 (c 4.31, $CHCl_3$), ee >99%; 13.5 h (36%) $[\alpha]_D^{25}$ −53.4 (c 4.31, $CHCl_3$) [lit.[40] $[\alpha]_D^{25}$ +46 (EtOH), (*S*)-(+)-2-phenylpropionamide], ee >99% (chiral HPLC). Mp: 90–92°C [lit.[40] 101–102°C, (*S*)-(+)-2-phenylpropionamide]; ν_{max} (KBr)/cm^{-1} 3345, 3170 (NH_2), 1650 (C=O); δ_H 7.23–7.38 (m, 5H, ArH), 5.81 (br s, 1H, NHH), 5.37 (br s, 1H, NHH), 3.59 (q, J = 7.0, 1H, CH), 1.52 (d, J = 7.0, 3H, CH_3).

(*S*)-(+)-2-Phenylpropanoic acid **3a**: 10 h (48%) $[\alpha]_D^{25}$ +61.8 (c 4.27, $CHCl_3$), ee 90%; 13.5 h (58%) $[\alpha]_D^{25}$ +57.8 (c 5.52, $CHCl_3$) [lit.[40] $[\alpha]_D^{20}$ +81.1 (EtOH)], ee 67% (chiral HPLC on the corresponding methyl ester). Oil; ν_{max} (film)/cm^{-1} 2870–3113 (COOH), 1700 (C=O); δ_H 8.32 (br s, 1H, COOH), 7.20–7.40 (m, 5H, ArH), 3.72 (q, J = 7.2, 1H, CH), 1.51 d, J = 7.2, 3H, CH_3).

*5.2.2. Enzymic hydrolysis of (±)-2-phenylbutyronitrile **1b***

(*R*)-(−)-2-Phenylbutyramide **2b**: 70 h (58%) $[\alpha]_D^{25}$ −23.2 (*c* 12.3, $CHCl_3$), ee 35%; 96 h (34%) $[\alpha]_D^{25}$ −56.5 (*c* 6.99, $CHCl_3$) [lit.[40] $[\alpha]_D^{24}$ +57 (EtOH) (*S*)-(+)-2-phenylbutyramide], ee 96% (chiral HPLC). Mp: 74–76°C [lit.[40] 80.5–81°C, (*S*)-(+)-2-phenylbutyramide]; ν_{max} (KBr)/cm^{-1} 3375, 3170 (NH_2), 1649 (C=O); δ_H 7.20–7.39 (m, 5H, ArH), 5.99 (br s, 1H, NHH), 5.49 (br s, 1H, NHH), 3.27 (t, *J* = 7.8, 1H, CH), 1.67–2.26 (m, 2H, CH_2), 0.88 (t, *J* = 7.4, 3H, CH_3).

(*S*)-(+)-2-Phenylbutyric acid **3b**: 70 h (39%) $[\alpha]_D^{25}$ +61.3 (*c* 4.97, $CHCl_3$), ee >99%; 96 h (40%) $[\alpha]_D^{25}$ +63.7 (*c* 5.48 $CHCl_3$) [lit.[40] $[\alpha]_D^{25}$ +78.5 (EtOH)], ee >99% (chiral HPLC on the corresponding methyl ester). Oil; ν_{max} (film)/cm^{-1} 2865–3110 (COOH), 1690 (C=O); δ_H 9.85 (br s, 1H, COOH), 7.25–7.36 (m, 5H, ArH), 3.45 (t, *J* = 7.8, 1H, CH), 1.70–2.21 (m, 2H, CH_2), 0.91 (t, *J* = 7.4, 3H, CH_3).

*5.2.3. Enzymic hydrolysis of (±)-2-phenylvaleronitrile **1c**[34]*

(*S*)-(+)-2-Phenylvaleramide **2c**: 150 h (27%) $[\alpha]_D^{25}$ +39.9 (*c* 5.80, $CHCl_3$), ee 41%; 214 h (40%) $[\alpha]_D^{25}$ 22.8 (*c* 6.86, $CHCl_3$) [lit.[41] $[\alpha]_D^{25}$ −50 (EtOH) (*R*)-(−)-2-phenylvaleramide], ee 13% (chiral HPLC). Mp: 78–79°C [lit.[41] 106–108°C, (*R*)-(−)-2-phenylvaleramide]; ν_{max} (KBr)/cm^{-1} 3420, 3200 (NH_2), 1651 (C=O); δ_H 7.20–7.40 (m, 5H, ArH), 5.70 (br s, 1H, NHH), 5.41 (br s, 1H, NHH), 3.39 (t, *J* = 6.8, 1H, CH), 1.67–2.21 (m, 2H, CH_2), 1.13–1.40 (m, 2H, CH_2), 0.90 (t, *J* = 7.0, 3H, CH_3).

(*S*)-(+)-2-Phenylvaleric acid **3c**: 150 h (8%) $[\alpha]_D^{25}$ +55.0 (*c* 1.09, $CHCl_3$), ee >99%; 214 h (13%) $[\alpha]_D^{25}$ +56.9 (*c* 1.96, $CHCl_3$) [lit.[41] $[\alpha]_D^{25}$ +63.4 (*c* 0.9, EtOH)], ee >99% (chiral GC on the corresponding methyl ester). Oil; ν_{max} (film)/cm^{-1} 2864–3095 (COOH), 1710 (C=O); δ_H 7.33 (s, 5H, ArH), 3.57 (t, *J* = 7.6, 1H, CH), 1.64–2.18 (m, 2H, CH_2), 1.11–1.40 (m, 2H, CH_2), 0.91 (t, *J* = 7.2, 3H, CH_3).

(*S*)-(+)-2-Phenylvaleronitrile **1c**:[34] 150 h (55%) $[\alpha]_D^{25}$ +13.4 (*c* 3.84, $CHCl_3$), ee 24%; 214 h (33%) $[\alpha]_D^{25}$ +21.2 (*c* 2.5, $CHCl_3$), ee 28% (chiral HPLC). Oil; ν_{max} (film)/cm^{-1} 2230 (CN); δ_H 7.30–7.42 (m, 5H, ArH), 3.78 (t, *J* = 6.4, 1H, CH), 1.41–2.04 (m, 4H, CH_2CH_2), 0.97 (t, *J* = 7.2, 3H, CH_3).

*5.2.4. Enzymic hydrolysis of (±)-3-methyl-2-phenybutyronitrile **1d**[34]*

(*R*)-(−)-3-Methyl-2-phenylbutyramide **2d**: 120 h (47%) $[\alpha]_D^{25}$ −72.9 (*c* 1.32, $CHCl_3$) [lit.[40] $[\alpha]_D^{24}$ +56 (EtOH), (*S*)-(+)-3-methyl-2-phenylbutyramide], ee >99% (chiral HPLC). Mp: 110–112°C [lit.[40] 141–142°C, (*S*)-(+)-3-methyl-2-phenylbutyramide]; ν_{max} (KBr)/cm^{-1} 3395, 3180 (NH_2), 1641 (C=O); δ_H 7.34 (s, 5H, ArH), 6.90 (br s, 1H N*H*H), 5.85 (br s, 1H, NH*H*), 2.99 (d, *J* = 10.6, 1H, CH), 2.39–2.42 (m, 1H, CH), 1.10 (d, *J* = 6.3, 3H, CH_3), 0.75 (d, *J* = 6.3, 3H, CH_3).

(*S*)-(+)-3-Methyl-2-phenylbutyric acid **3d**: 120 h (46%) $[\alpha]_D^{25}$ +60 (*c* 1.95, $CHCl_3$) [lit.[42] $[\alpha]_D^{25}$ +60.5 ($CHCl_3$)], ee >99% (chiral HPLC on the corresponding methyl ester). Mp: 50–51°C (lit.[42] 49.5–50.5°C); ν_{max} (film)/cm^{-1} 2860–3100 (COOH), 1705 (C=O); δ_H 7.31–7.39 (m, 5H, ArH), 7.11 (br s, 1H, COOH), 3.20 (d, *J* = 10.6, 1H, CH), 2.32–2.45 (m, 1H, CH), 1.13 (d, *J* = 6.5, 3H, CH_3), 0.76 (d, *J* = 6.6, 3H, CH_3).

*5.2.5. Enzymic hydrolysis of (±)-2-phenylhexanenitrile **1e**[34]*

(*S*)-(+)-2-Phenylhexanamide **2e**: 300 h (34%) $[\alpha]_D^{25}$ +20.8 (*c* 4.87, $CHCl_3$) [lit.[41] $[\alpha]_D^{25}$ +41.9 (EtOH)], ee 20% (chiral HPLC). Mp: 98–99°C (lit.[41] 92–93°C); ν_{max} (KBr)/cm^{-1} 3370, 3180 (NH_2), 1650 (C=O); δ_H 7.30 (s, 5H, ArH), 5.83 (br s, 1H, N*H*H), 5.42 (br s, 1H, NH*H*), 3.36 (t, *J* = 7.0, 1H, CH), 1.67–2.22 (m, 2H, CH_2), 1.14–1.40 (m, 4H, CH_2CH_2), 0.86 (t, *J* = 7.0, 3H, CH_3).

(*S*)-(+)-2-Phenylhexanoic acid **3e**: 300 h (23%) $[\alpha]_D^{25}$ +51.1 (*c* 4.34, $CHCl_3$) [lit.[41] $[\alpha]_D^{25}$ +62.4 (*c* 0.967, EtOH)], ee 98% (chiral GC on the corresponding methyl ester); ν_{max} (KBr)/cm^{-1} 2860–3100 (COOH), 1705 (C=O); δ_H 8.62 (br s, 1H, COOH), 7.30 (s, 5H, ArH), 3.52 (t, J=7.6, 1H, CH), 1.66–2.15 (m, 2H, CH_2), 1.17–1.36 (m, 4H, CH_2CH_2), 0.86 (t, J=7.2, 3H, CH_3).

(*S*)-(+)-2-Phenylhexanenitrile **1e**:[34] 300 h (36%) $[\alpha]_D^{25}$ +32.6 (*c* 5.41, $CHCl_3$), ee 36% (chiral HPLC); ν_{max} (KBr)/cm^{-1} 2239 (CN); δ_H 7.29–7.37 (m, 5H, ArH), 3.76 (t, J=6.8, 1H, CH), 1.76–1.99 (m, 2H, CH_2), 1.26–1.57 (m, 4H, CH_2CH_2), 0.91 (t, J=7.4, 3H, CH_3).

5.2.6. Enzymic hydrolysis of (±)-2-methoxy-2-phenylacetonitrile **1f**[35]

(±)-2-Methoxy-2-phenylacetamide **2f**: 37 h (74%) $[\alpha]_D^{25}$ 0 (*c* 3.04, $CHCl_3$), ee 0%. 49 h (56%) $[\alpha]_D^{25}$ 0 (*c* 9.51, $CHCl_3$), ee 0% (chiral HPLC). Mp: 111–112°C (lit.[43] 112–114°C); ν_{max} (KBr)/cm^{-1} 3450, 3175 (NH_2), 1690, 1650 (C=O); δ_H 7.31–7.41 (m, 5H, ArH), 6.62 (br s, 1H, NHH), 6.40 (br s, 1H, NHH), 4.59 (s, 1H, CH), 3.36 (s, 3H, OCH_3).

5.2.7. Enzymic hydrolysis of (±)-2-methylthio-2-phenylacetonitrile **1g**[36]

(−)-2-Methylthio-2-phenylacetamide **2g**: a solution of 2-methylthio-2-phenylacetonitrile **1g** (2 mmol, 326 mg) in acetone (3 ml) was added to the suspension of *Rhodococcus* sp. AJ270 in buffer and the mixture was incubated for 120 h to give, after purification, (−)-2-methylthio-2-phenylacetamide **2g** (64%) $[\alpha]_D^{25}$ −26.0 (*c* 4.16, $CHCl_3$), ee 15% (chiral HPLC). Mp: 141°C. Found: C, 59.36; H, 6.04; N, 7.73. $C_9H_{11}NOS$ requires: C, 59.64; H, 6.12; N, 7.73; ν_{max} (KBr)/cm^{-1} 3390, 3175 (NH_2), 1653 (C=O); δ_H 7.30–7.45 (m, 5H, ArH), 6.60 (br s, 1H, NHH), 5.91 (br s, 1H, NHH), 4.48 (s, 1H, CH), 2.17 (s, 3H, SCH_3); *m*/*z* (EI) 181 (M^+, 3%), 137 (100).

(+)-2-Methylthio-2-phenylacetic acid **3g**: 120 h (15%) $[\alpha]_D^{25}$ +113.7 (*c* 2.34, $CHCl_3$), ee 96% (chiral HPLC). Mp: 73–76°C (lit.[44] 81°C); ν_{max} (KBr)/cm^{-1} 2875–3063 (COOH), 1706 (C=O); δ_H 9.84 (br s, 1H, COOH), 7.32–7.48 (m, 5H, ArH), 4.51 (s, 1H, CH), 2.13 (s, 3H, SCH_3).

5.2.8. Enzymic hydrolysis of (±)-2-acetoxy-2-phenylacetonitrile **1h**[37]

(±)-2-Acetoxy-2-phenylacetonitrile **1h** (420 mg, 2.4 mmol) yielded, after 62 h, (*S*)-(+)-mandelic acid **5** (208 mg, 57%) and benzoic acid (10 mg, 3%). (*S*)-(+)-Mandelic acid $[\alpha]_D^{25}$ +17.0 (*c* 3.43, CH_3OH) [lit.[43] $[\alpha]_D^{25}$ +156.6 (H_2O)], ee 29% (chiral HPLC). Mp: 115–118°C (lit.[43] 133–138°C); ν_{max} (KBr)/cm^{-1} 3400 (OH), 2630–3090 (COOH), 1719 (sh), 1710 (C=O); δ_H 7.36–7.47 (m, 5H, ArH), 5.36 (br s, 2H, OH, COOH), 5.27 (s, 1H, CH).

5.2.9. Enzymic hydrolysis of (±)-2-benzoyloxy-2-phenylacetonitrile **1i**[37]

(±)-2-Benzoyloxy-2-phenylacetonitrile **1i** (420 mg, 1.77 mmol) yielded, after 96 h, (*S*)-(+)-mandelic acid **5** (50 mg, 19%) $[\alpha]_D^{25}$ +32.7 (*c* 0.80, CH_3OH), ee 35% (chiral HPLC) and benzoic acid (168 mg, 78%). (±)-2-Benzoyloxy-2-phenylacetonitrile (153 mg, 34%) was also recovered. $[\alpha]_D^{25}$ 0 (*c* 2.56, CH_3OH); ν_{max} (KBr)/cm^{-1} 1730, 1724 (C=O); δ_H 7.47–8.11 (m, 10H, ArH), 6.70 (s, 1H, CH).

5.2.10. Enzymic hydrolysis of (±)-2-phenylpropionamide **2a**

(*R*)-(−)-2-Phenylpropionamide **2a**: 8 h (44%) $[\alpha]_D^{25}$ −58.0 (*c* 3.32, $CHCl_3$), ee >99%; 27 h (22%) $[\alpha]_D^{25}$ −58.0 (*c* 2.16, $CHCl_3$), ee >99% (chiral HPLC).

(*S*)-(+)-2-Phenylpropanoic acid **3a**: 8 h (51%) $[\alpha]_D^{25}$ +47.6 (*c* 4.60, $CHCl_3$), ee 56%; 27 h (78%) $[\alpha]_D^{25}$ +20.5 (*c* 5.13, $CHCl_3$), ee 49% (chiral HPLC on the corresponding methyl ester).

5.2.11. Enzymic hydrolysis of (±)-2-phenybutyramide ***2b***

(*R*)-(−)-2-Phenylbutyramide **2b**: 48 h (72%) $[\alpha]_D^{25}$ −23.8 (*c* 4.95, $CHCl_3$), ee 29%; 120 h (47%) $[\alpha]_D^{25}$ −67.1 (*c* 8.02, $CHCl_3$), ee >99% (chiral HPLC).

(*S*)-(+)-2-Phenylbutyric acid **3b**: 48 h (20%) $[\alpha]_D^{25}$ +70.2 (*c* 3.79, $CHCl_3$), ee >99%; 120 h (51%) $[\alpha]_D^{25}$ +68.5 (*c* 10.4, $CHCl_3$), ee >99% (chiral HPLC on the corresponding methyl ester).

5.2.12. Enzymic hydrolysis of (±)-2-phenylvaleramide ***2c***

(*R*)-(−)-2-Phenylvaleramide **2c**: 256 h (89%) $[\alpha]_D^{25}$ −2.6 (*c* 5.90, $CHCl_3$), ee 15%; 325 h (84%) $[\alpha]_D^{25}$ −7.5 (*c* 13.3, $CHCl_3$) [lit.[41] $[\alpha]_D^{25}$ −50 (EtOH), (*R*)-(−)-2-phenylvaleramide] ee 20% (chiral HPLC).

(*S*)-(+)-2-Phenylvaleric acid **3c**: 256 h (8%) $[\alpha]_D^{25}$ +58.2 (*c* 0.74, $CHCl_3$), ee 95%; 325 h (15%) $[\alpha]_D^{25}$ +58.8 (*c* 1.45, $CHCl_3$), ee >99% (chiral GC on the corresponding methyl ester).

5.2.13. Enzymic hydrolysis of (±)-2-phenylhexanamide ***2e***

(*R*)-(−)-2-Phenylhexanamide **2e**: 256 h (60%) $[\alpha]_D^{25}$ −3.6 (*c* 4.89, $CHCl_3$), ee 3%; 325 h (57%) $[\alpha]_D^{25}$ −4.7 (*c* 4.75, $CHCl_3$) [lit.[41] $[\alpha]_D^{25}$ +41.9 (EtOH), (*S*)-(+)-2-phenylhexanamide], ee 6% (chiral HPLC).

(*S*)-(+)-2-Phenylhexanoic acid **3e**: 256 h (27%) $[\alpha]_D^{25}$ +8.2 (*c* 2.45, $CHCl_3$), ee 12%; 325 h (25%) $[\alpha]_D^{25}$ +8.1 (*c* 2.88, $CHCl_3$), ee 14% (chiral GC on the corresponding methyl ester).

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