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Enzymatic desymmetrization of 3-alkyl- and 3-arylglutaronitriles, a simple and convenient approach to optically active 4-amino-3-phenylbutanoic acids

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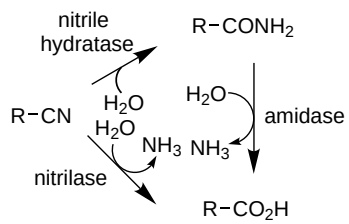
Abstract—The enantioselective hydrolysis of 3-alkyl- and 3-arylglutaronitriles catalyzed by *Rhodococcus* sp. AJ270 cells, afforded the corresponding (*S*)-3-substituted 4-cyanobutanoic acids with low to moderate enantiomeric purities. Additives such as acetone were found to significantly enhance the enantioselectivity of the desymmetrization, giving enantiomeric excesses of up to 95%. The synthetic potential of the homochiral product was also demonstrated by the preparation of optically active (*R*)- and (*S*)-4-amino-3-phenylbutanoic acids.

1. Introduction

The enzyme-catalyzed hydrolysis of nitriles has been shown to proceed through two distinct pathways. *Nitrilase*[1] enzymes convert a nitrile directly into the corresponding carboxylic acid and ammonia, whereas *nitrile hydratases*[2] catalyze the hydration of the nitrile to the amide, which is then further transformed into the acid by the action of an *amidase*[2] (Scheme 1). A large number of microorganisms containing nitrile-hydrolyzing enzymes have been isolated[3] and the microbial hydration of acrylonitrile has been successfully applied to the industrial production of several thousands of tons of acrylamide per annum.[4] In recent years much attention has been given to the stereoselective biohydrolysis of nitriles because nitrile-hydrolyzing enzymes exhibit stereoselectivity and therefore the biotransformation of nitriles would provide a unique route to optically active carboxylic acids and their derivatives.[5] A few examples of enantioselective biotransformations of nitriles have been reported by Ohta,[6] Turner,[7] Wang[8] and others[9] using different microbial whole cells.



Scheme 1. Enzyme-catalyzed hydrolysis reactions of nitriles.

Surprisingly, however, the enantioselective biotransformations of dinitriles remains largely unexplored. This is particularly surprising when compared with the extensive investigations into the enzymatic desymmetrization of diesters and diols.[10] Ohta[11] and Turner[12] and their co-workers independently investigated the hydrolysis of *O*-substituted 3-hydroxyglutaronitriles using different *Rhodococcus* whole-cell catalysts and obtained the corresponding monocyanocarboxylic acids in good enantiomeric excess. When 3-benzylglutaronitrile was used as substrate, the stereoselectivity of the reaction, however, was extremely low.[11] It is also noteworthy that biotransformation of a disubstituted malononitrile catalyzed by *Rhodococcus rhodochrous* IFO15564 yielded an amido-acid with excellent enantioselectivity.[13]

Rhodococcus sp. AJ270 is a powerful and robust nitrile hydratase/amidase-containing microorganism. It can efficiently hydrolyze a variety of nitriles[3] and dinitriles[14] with excellent chemo- and regioselectivity. Recently we have shown that *Rhodococcus* sp. AJ270 also exhibits high enantioselectivity against different types of racemic nitriles.[8] To further explore its potential in asymmetric synthesis we undertook the current study of

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desymmetrization of 3-alkyl- and 3-aryl-substituted glutaronitriles,[15] envisaging a simple and convenient synthesis of optically active 4-cyanobutanoic acid derivatives which are important and versatile chiral building blocks in organic synthesis.

2. Results

2.1. Biotransformation of 3-phenylglutaronitrile

To begin our study we first examined the hydrolysis of 3-phenylglutaronitrile **1a**[16] in the presence of *Rhodococcus* sp. AJ270 cells in aqueous phosphate buffer at 30°C. The reaction proceeded efficiently to afford optically active 4-cyano-3-phenylbutanoic acid **2a** as the sole product in addition to recovered starting nitrile **1a**. The absolute configuration of the preferred enantiomer **2a** was assigned as *S*-based on comparison of the optical rotation of its chemically modified products with that of authentic samples (vide infra). As shown in Table 1, longer incubation time led to higher conversion of dinitrile **1a** while the enantioselectivity of the reaction decreased rapidly with increasing conversion. For example, the enantiomeric excess (e.e.) of **2a** dropped from 61 to 39% when the conversion progressed from 56 to 88%. To improve the solubility of substrate **1a** in aqueous buffer and therefore facilitate its conversion, small amounts of acetone (3 mL) were added to the reaction mixture (50 mL). In contrast to our expectation, no improvement in the conversion was observed. The reaction was in fact somewhat retarded. Very surprisingly, however, the enantioselectivity of the biotransformation of **1a** was significantly enhanced, with the e.e. of **2a** increasing from 39 to 88% within the same time period. A variety of chemicals were then screened as an additive or co-solvent and the results were summarized in Table 1. β-Cyclodextrin (β-CD) was found to greatly improve the enantioselectivity and also increased the chemical conversion slightly. A biphasic system of hexane and aqueous phosphate buffer (25 mL/25 mL) was found to be similarly effective in improving the enantiocontrol of the reaction, other water immiscible solvents such as dichloromethane and ethyl acetate completely inhibited the biotransformation of **1a** (Scheme 2).

Table 1. Biohydrolysis of 3-phenylglutaronitrile **1a**

Entry	Time (h)	Additive[a]	Yield[d] (%)	E.e.[e] (%)
1	12	–	56	61
2	24	–	88	39
3	24	Acetone	67	88
4.	24	Toluene	35	56
5	24	CH_3CO_2Et	–	–
6	24	CH_2Cl_2	–	–
7	24	Cyclohexanone	–	–
8	8	Hexane[b]	48	84
9	12	β-CD[c]	61	80

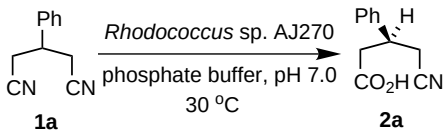
[a] Organic solvent (3 mL) was used as an additive.
[b] The reaction was performed in an aqueous buffer-hexane (25 mL/25 mL) biphasic system.
[c] β-CD (0.3 g) was used.
[d] Isolated yield.
[e] Determined by chiral HPLC analysis.

2.2. Enzymatic desymmetrization of other 3-substituted glutaronitrile substrates

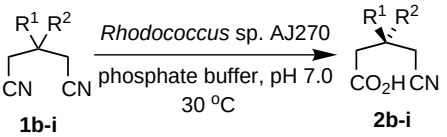
To test the generality of *Rhodococcus* sp. AJ270-catalyzed desymmetrization and also to understand the influence of the structure of substrates on both the biotransformation efficiency and enantioselectivity, a series of 3-aryl- and alkyl-substituted glutaronitrile substrates **1b–i** were prepared[16] and subjected to reaction (Scheme 3). The effect of adding acetone on the enantioselectivity was also examined.

For those 3-arylglutaronitriles listed in Table 2, the introduction of a bulky substituent including chloro **1c**, methyl **1d** and methoxy **1e** at the *para* position of the phenyl ring resulted in lower conversion of the dinitriles **1** into their monocyanoacid products **2** (entries 3, 5 and 9). High conversion was obtained from the hydrolysis of 3-(4-fluorophenyl)glutaronitrile **1b** and 3-cyclohexylglutaronitrile **1g** (entries 1 and 11). 3-Benzylglutaronitrile **1h** was the best substrate, yielding almost complete conversion in 20 h (entry 13). Attempts were made to transform 3-methyl-3-phenylglutaronitrile **1i**, a quaternary carbon-containing prochiral dinitrile, into homochiral product **2i**. Unfortunately, however, no reaction took place and only the starting material was recovered after incubation with *Rhodococcus* sp. AJ270 cells for 6 days (entry 15).

In the absence of any additive, only low to moderate enantioselectivity (e.e. 25–64%) was obtained for all substrates, as summarized in Table 2. This is comparable to the result from the hydrolysis of the parent 3-phenylglutaronitrile (**1a**) (entries 1 and 2 in Table 1). The presence of a methyl group at the *ortho* **1e** rather than the *para* position (**1d**) on the phenyl ring caused a drastic decrease in the enantiocontrol (entries 5 and 7 in Table 2). Both cyclohexyl and benzyl substituted glutaronitrile analogues afforded monocyano acids **2g** and **2h** in low enantiomeric purity (entries 11 and 13 in Table 2). When acetone was used as an additive, a significant improvement in the enantioselectivity of the



Scheme 2. Biohydrolysis of 3-phenylglutaronitrile **1a**.



Scheme 3. Desymmetrization of 3-substituted glutaronitrile substrates.

Table 2. Enzymatic desymmetrization of 3-substituted glutaronitrile substrates **1b**–**1i**

Entry	R^1	R^2	Additive[a]	Time (h)	Product (%)[b]	E.e. (%)[c]
1	4-F-C_6H_4	H	–	24	**2b** (81)	25
2	4-F-C_6H_4	H	Acetone	24	**2b** (16)	76
3	4-F-C_6H_4	H	Acetone	84	**2b** (55)	32
4	4-Cl-C_6H_4	H	–	72	**2c** (37)	26
5	4-Cl-C_6H_4	H	Acetone	72	**2c** (25)	63
6	4-CH_3-C_6H_4	H	–	24	**2d** (42)	64
7	4-CH_3-C_6H_4	H	Acetone	24	**2d** (25)	95
8	4-CH_3-C_6H_4	H	Acetone	84	**2d** (67)	80
9	2-CH_3-C_6H_4	H	–	20	**2e** (51)	30
10	2-CH_3-C_6H_4	H	Acetone	20	**2e** (40)	35
11	4-CH_3O-C_6H_4	H	–	48	**2f** (36)	50
12	4-CH_3O-C_6H_4	H	Acetone	48	**2f** (17)	79
13	4-CH_3O-C_6H_4	H	Acetone	84	**2f** (53)	60
14	Cyclohexyl	H	–	20	**2g** (63)	31
15	Cyclohexyl	H	Acetone	20	**2g** (60)	83
16	$C_6H_5CH_2$	H	–	20	**2h** (90)	29
17	$C_6H_5CH_2$	H	Acetone	20	**2h** (62)	32
18	C_6H_6	CH_3	–	144	**2i** (0)	–

[a] 3 mL of acetone was used.
[b] Isolated yield.
[c] Determined by chiral HPLC analysis.

reaction was seen in most cases. For example, a more than two-fold enhancement of enantioselectivity was achieved for 3-cyclohexylglutaronitrile **1g** while the enantiomeric excess of 4-cyano-3-(4-methylphenyl)-butanoic acid **2d** increased from 64% to as high as 95% after acetone was added to the reaction mixture. Acetone did not seem to have this great enhancement effect on all substrates; as exemplified by the observation of only a marginal increase in enantioselectivity in the hydrolysis of **1e** and **1h**. It should also be noted that in many cases acetone inhibited the nitrile hydratase, leading to lower catalytic efficiency.

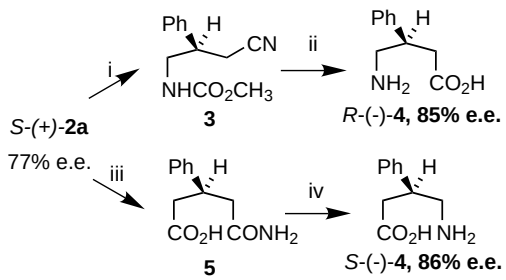
2.3. Synthesis of both enantiomers of 4-amino-3-phenylbutanoic acids (β-phenyl-γ-aminobutanoic acids)

To determine the absolute configuration of homochiral product **2** and to demonstrate its synthetic potential, we attempted chemical transformations to prepare both enantiomers of 4-amino-3-phenylbutanoic acids (β-phenyl GABA).

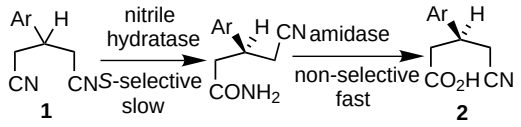
The biotransformed product **2a** underwent a straightforward Curtius rearrangement followed by acidic hydrolysis to give (*R*)-(−)-4-amino-3-phenylbutanoic acid (*R*)-(−)-**4** with e.e. of 85%. Chemical hydration of the cyano group of **2a** followed by Hoffmann rearrangement afforded (*S*)-(+)-4-amino-3-phenylbutanoic acid (*S*)-(+)-**4** (Scheme 4). The configurations of both enantiomers of 4-amino-3-phenylbutanoic acids **4** were determined by comparing the sign of the specific rotations with those of authentic samples reported in the literature.[17] Since the stereogenic center remained intact during the course of chemical manipulation, the absolute configuration of the desymmetrization product **2a** was assigned as *S*.

3. Discussion

As a nitrile hydratase/amidase-containing microorganism, *Rhodococcus* sp. AJ270 was able to hydrolyze 3-alkyl- and 3-arylglutaronitriles in a selective manner. Isolation of the monocyano acid **2** as the sole product from the reaction indicated that the nitrile hydratase involved in this microbial cell acts as a regiospecific hydrating enzyme against dinitrile **1**. In other words, the nitrile hydratase only acted on one cyano group and left the other intact. The amidase, on the other hand, was highly efficient, converting all monocyano amide intermediate formed from hydration step into the acid rapidly and completely, as demonstrated by the fact that in all cases no monocyano amide was obtained. It is apparent that the enantioselectivity of the overall hydrolysis was derived from the action of nitrile hydratase (Scheme 5). This is in sharp contrast to the hydrolysis of racemic nitriles, in which the amidase acted as a kinetic resolving enzyme.[6–9]



Scheme 4. Synthesis of (*R*)- and (*S*)-4-amino-3-phenylbutanoic acids. (i) a. $ClCO_2Et$, Et_3N, −5°C; b. NaN_3; c. MeOH, reflux 3 h, 43%. (ii) HCl/H_2O, reflux 48 h, 78%. (iii) $H_2O_2/NaOH$, 79%. (iv) $Br_2/NaOH$, 71%.



Scheme 5. Suggested pathway for desymmetrization of glutaronitriles.

As evidenced by the correlation between (+)-4-cyano-3-phenylbutanoic acid **2a** and both (*R*)- and (*S*)-4-amino-3-phenylbutanoic acids through different chemical transformations (Scheme 4), the nitrile hydratase involved in *Rhodococcus* sp. AJ270 is (*S*)-enantioselective. Assuming that the introduction of a substituent into the phenyl ring of **2** did not affect the direction of the optical rotation, the resulting (+)-3-aryl-4-cyanobutanoic acids **2b**–**2f** were assigned as (*S*)-enantiomers. The stereochemistry of the products (−)-**2g** and (−)-**2h** was not unambiguously determined, though they were tentatively assigned as (*S*)-isomers.

The enantiomeric excesses obtained from the reaction varied from 25 to 64% or from 32 to 95% in the presence of acetone, depending on the nature of the 3-substituent on the glutaronitrile **1**. A phenyl group or an aryl group bearing an electron-donating substituent in the *para* position led to higher enantioselectivity. This reflects clearly that the nitrile hydratase involved in *Rhodococcus* sp. AJ270 is very sensitive to both electronic and steric effects in the substrates even when the substituent is relatively remote from the cyano function. The intriguing additive or co-solvent effect on the enhancement of the enantioselectivity of the desymmetrization reaction is not yet fully understood. It has been shown[8] that in a kinetic resolution process, the solubility of the racemic substrate influences the e.e. of the products. In contrast to kinetic resolution, however, the solubility of prochiral substrate **1** does not make any difference on two enantiotopic cyano groups although acetone or β-cyclodextrin did increase the solubility of the glutaronitrile substrates **1** in aqueous buffer. The enhancement in enantioselectivity therefore probably results from the interaction of the additive or organic solvent with the biocatalyst. This additive–catalyst interaction is also in agreement with the observed inhibitory effect of adding an organic co-solvent and it is clear that *Rhodococcus* sp. AJ270 could not tolerate organic solvents such as dichloromethane and ethyl acetate.

One of the advantages of desymmetrization of a prochiral dinitrile over a diester is the formation of a nitrogen-containing chiral compound. Optically active 4-cyano-3-substituted butanoic acids **2** are therefore very important and versatile chiral intermediates and their chemical manipulation would yield a wide variety of organic compounds.[15] A simple and straightforward synthesis of both enantiomers of 4-amino-3-phenylbutanoic acids has also been demonstrated. (*R*)-(−)-4-Amino-3-phenylbutanoic acid (*R*)-β-phenyl-γ-aminobutanoic acid (β-phenyl-GABA) are clinically used as a mood elevator and tranquilizer.[17,18]

4. Conclusions

Hydrolysis of 3-aryl- and 3-alkyl-substituted glutaronitriles catalyzed by *Rhodococcus* sp. AJ270 cells gave the corresponding optically active (4*S*)-(+)-3-aryl- and (−)-3-alkyl-4-cyanobutanoic acids with low to moderate enantiomeric excess. The enantioselectivity of this desymmetrization reaction was determined by the nature of the 3-substituent. The presence of an additive such as acetone enhanced the enantioselectivity dramatically, giving as high as 95% enantiomeric excess in some cases. Our study has also demonstrated the novel and convenient chemoenzymatic process for the preparation of both enantiomers of 4-amino-3-phenylbutanoic acids.

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 a Bruker AM 300 spectrometer. 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 in 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 (e.e.) values of all products were obtained by means of chiral HPLC using racemic samples as references. The enantiomeric excesses of all compounds were obtained with a Shimadzu LC-10AVP HPLC system using a Chiracel OD column. A mixture of 2-propanol:hexane [9:1] as the mobile phase at a flow rate of 0.8 mL/min was used for compounds **2a**–**d** while for **2e** and **2f** a mixture of 2-propanol:hexane [18:1] as the mobile phase at a flow rate of 0.2 mL/min was employed. Products **2g** and **2h** were converted into their benzyl esters with benzyl alcohol and their enantiomeric excesses were obtained using 2-propanol:hexane [18:1] as the mobile phase at a flow rate of 0.8 mL/min. The enantiomeric excess values of *R*- and *S*-4-amino-3-phenylbutanoic acids **4** were obtained using a $CR^{(+)}$ column with $HClO_4$ aqueous solution (pH 1.5) as the mobile phase at a flow rate of 1 mL/min.

5.2. Preparation of 3-aryl- and 3-alkyl-substituted glutaronitriles 1[16]

The starting 3-substituted glutaronitriles **1a**–**f** were prepared from the reaction between aromatic aldehydes and cyano acetic acid according to the literature,[16a] while glutaronitriles **1g** and **1h** were prepared from cyanolation of the corresponding diols.[16b] Dinitrile **1i** was obtained according to a literature method[1b,c] by

reacting acetonitrile with 3-methylcinnamonitrile. All unknown starting dinitriles were fully characterized by their spectra data.

5.2.1. 3-(4-Chlorophenyl)glutaronitrile 1c.[16a] Yield 52%. Bp: 179–180°C (1 mmHg); ν_{max} (KBr)/cm^{-1} 2248 (CN); δ_H ($CDCl_3$) 7.42 (d, *J* 8.4, 2H), 7.27 (d, *J* 8.4, 2H), 3.43 (quin., *J* 6.9, 1H), 2.86 (d, *J* 7.0, 4H); δ_C 136.4, 134.7, 129.7, 128.2, 116.8, 38.0, 23.5; *m*/*z* (EI) 206 (6), 204 (M^+, 18%), 166 (33), 164 (100). Found: C, 64.35; H, 4.34; N, 13.28. $C_{11}H_9ClN_2$ requires: C, 64.59; H, 4.43; N, 13.69%.

5.2.2. 3-(4-Methylphenyl)glutaronitrile 1d.[16a] Yield 45%. Bp: 156–158°C (1 mmHg); ν_{max} (KBr)/cm^{-1} 2244 (CN); δ_H ($CDCl_3$) 7.23 (d, *J* 8.1, 2H), 7.16 (d, *J* 8.2, 2H), 3.35 (quin., *J* 6,9, 1H), 2.79 (d, *J* 6.9, 4H), 2.35 (s, 3H); δ_C ($CDCl_3$) 138.4, 134.9, 129.9, 126.5, 117.0, 38.1, 23.4, 20.9; *m*/*z* (EI) 184 (M^+, 21%), 144 (100). Found: C, 78.61; H, 6.43; N, 15.27. $C_{12}H_{12}N_2$ requires: C, 78.23; H, 6.56; N, 15.20.

5.2.3. 3-(2-Methylphenyl)glutaronitrile 1e.[16a] Yield 42%. Bp: 134–136°C (0.5 mmHg); ν_{max} (KBr)/cm^{-1} 2249 (CN); δ_H ($CDCl_3$) 7.20–7.29 (m, 4H), 3.68 (quin., *J* 6.9, 1H), 2.76 (d, *J* 6.9, 4H), 2.39 (S, 3H); δ_C ($CDCl_3$) 136.3, 135.8, 131.4, 128.5, 127.2, 124.9, 117.3, 33.7, 23.0, 21.2; *m*/*z* (EI) 184 (M^+, 13%), 144 (100). Found: C, 77.97; H, 6.64; N, 15.29. $C_{12}H_{12}N_2$ requires: C, 78.23; H, 6.56; N, 15.20%.

5.2.4. 3-(4-Methoxyphenyl)glutaronitrile 1f.[16a] Yield 48%. Mp: 71–72°C; ν_{max} (KBr)/cm^{-1} 2250.7 (CN); δ_H ($CDCl_3$) 7.23 (d, *J* 8.6, 2H), 6.96 (d, *J* 8,6, 2H), 3.84 (s, 3H), 3.40 (quin., *J* 6.9, 1H), 2.84 (d, *J* 6.9, 4H); δ_C ($CDCl_3$) 159.5, 129.7, 127.6, 114.5, 116.9, 55.1, 37.7, 23.5; *m*/*z* (EI) 200 (M^+, 17%), 160 (100). Found: C, 72.23; H, 5.93; N, 13.82. $C_{12}H_{12}N_2O$ requires: C, 71.98; H, 6.04; N, 13.99%.

5.3. General procedure for the biotransformations of 3-substituted glutaronitriles

To an Erlenmeyer flask (150 mL) with a screw cap was added *Rhodococcus* sp. AJ270 cells[3] (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. The 3-substituted glutaronitrile as a fine powder, or dissolved in organic solvents (3 mL) or in hexane (25 mL) solution, or as a gelation with β-CD (300 mg) and buffer (Table 1) was added in one portion to the flask and the mixture was incubated at 30°C using an orbital shaker (200 rpm). The reaction, monitored by TLC, was quenched after the specified period of time (see Tables 1 and 2) by removing the biomass via filtration through a Celite pad. The resulting aqueous solution was basified to pH 12 with aqueous NaOH (2 M). Extraction with ethyl acetate gave, after drying ($MgSO_4$) and removing solvent, the unconverted nitrile. The aqueous solution was then acidified using aqueous HCl (2 M) to pH 2 and extracted with ethyl acetate. Pure 3-substituted 4-cyanobutanoic acid **2** was obtained after removal of the solvent.

5.3.1. Enzymatic hydrolysis of 3-phenylglutaronitrile 1a. (3*S*)-4-Cyano-3-phenylbutanoic acid 2a: reaction time 24 h (with acetone) (67%) $[\alpha]_D^{20}$ +22 (*c* 1, $CHCl_3$), e.e. 88%. Mp: 123–124°C; ν_{max} (KBr)/cm^{-1} 2553–3215 (COOH), 2247 (CN), 1697 (C=O); δ_H ($CDCl_3$) 8.97 (br, 1H), 7.26–7.41 (m, 5H), 3.51 (quin., *J* 7.0, 1H), 2.94 (dd, *J* 7.7, 16.7, 1H), 2.85 (dd, *J* 7.2, 16.8, 1H), 2.76 (d, *J* 6.6, 2H); δ_C ($CDCl_3$) 176.5, 139.9, 129.1, 128.0, 126.9, 117.7, 38.5, 37.7, 24.4; *m*/*z* (EI) 189 (M^+, 15%), 171 (10), 162 (36), 143 (38), 134 (57), 107 (100). Found: C, 69.79; H, 5.89; N, 7.44. $C_{11}H_{11}NO_2$ requires C, 69.83; H, 5.86; N, 7.40%.

5.3.2. Enzymatic hydrolysis of 3-(4-fluorophenyl) glutaronitrile 1b. (3*S*)-4-Cyano-3-(4-fluorophenyl)butanoic acid 2b: reaction time 24 h (with acetone) (16%) $[\alpha]_D^{20}$ +46.7 (*c* 0.9, $CHCl_3$) e.e. 76%. Mp: 100.5–101.5°C; ν_{max} (KBr)/cm^{-1} 2632–3072 (COOH), 2255 (CN), 1712 (C=O); δ_H ($CDCl_3$) 8.37 (br, 1H), 7.05–7.28 (m, 4H), 3.51 (quin., *J* 7.0, 1H), 2.90 (dd, *J* 7.6, 16.7, 1H), 2.84 (dd, *J* 7.3, 16.7, 1H), 2.74 (d, *J* 6.6, 2H); δ_C ($CDCl_3$) 176.3, 163.9, 160.6, 135.7, 135.6, 128.7, 128.6, 117.5, 116.2, 115.9, 38.6, 37.1, 24.6; *m*/*z* (EI) 207 (M^+, 10%), 180 (31), 161 (21), 152 (32), 148 (20), 125 (100). Found: C, 63.48; H, 4.98; N, 6.89. $C_{11}H_{10}FNO_2$ requires: C, 63.76; H, 4.86; N, 6.76%.

5.3.3. Enzymatic hydrolysis of 3-(4-chlorophenyl) glutaronitrile 1c. (3*S*)-3-(4-Chlorophenyl)-4-cyanobutanoic acid 2f: reaction time 72 h (with acetone) (25%) $[\alpha]_D^{25}$ +14.3 (*c* 1.4, $CHCl_3$) e.e. 63%. Mp: 119–120°C; ν_{max} (KBr)/cm^{-1} 2538–3208 (COOH), 2254 (CN), 1707 (C=O); δ_H ($CDCl_3$) 9.01 (br, 1H), 7.34 (d, *J* 8.4, 2H), 7.21 (d, *J* 8.5, 2H), 3.48 (quin., *J* 7.0, 1H), 2.88 (dd, *J* 7.5, 16.7, 1H), 2.82 (dd, *J* 7.4, 16.8, 1H), 2.73 (d, *J* 6.6, 2H); δ_C ($CDCl_3$) 176.4, 138.4, 133.9, 129.3, 128.4, 117.4, 38.5, 37.2, 24.4; *m*/*z* (EI) 225 (7), 223 (M^+, 17%), 198 (16), 196 (46), 179 (8), 177 (23), 170 (12), 168 (35), 166 (7), 164 (21), 143 (32), 141 (100). Found: C, 58.75; H, 4.69; N, 6.28. $C_{11}H_{10}ClNO_2$ requires: C, 59.07; H, 4.51; N, 6.26%.

5.3.4. Enzymic hydrolysis of 3-(4-methylphenyl) glutaronitrile 1d. (3*S*)-4-Cyano-3-(4-methylphenyl)butanoic acid 2d: reaction time 24 h (42%) (e.e. 64%); 24 h (25%) (with acetone) $[\alpha]_D^{20}$ +24 (*c* 1, $CHCl_3$) e.e. 95%. Mp: 114–115°C; ν_{max} (KBr)/cm^{-1} 2589–3208 (COOH), 2247 (CN), 1700 (C=O); δ_H ($CDCl_3$) 9.26 (br, 1H), 7.20 (s, 4H), 3.50 (quin., *J* 7.0, 1H), 2.93 (dd, *J* 7.7, 16.6, 1H), 2.85 (dd, *J* 7.3, 16.7, 1H), 2.76 (d, *J* 6.6, 2H), 2.38 (s, 3H); δ_C ($CDCl_3$) 176.8, 137.7, 137.0, 129.7, 126.8, 117.8, 38.7, 37.4, 24.5, 21.1; *m*/*z* (EI) 203 (M^+, 19%), 176 (39), 157 (17), 148 (27), 121 (100). Found: C, 71.02; H, 6.71; N, 6.81. $C_{11}H_{11}NO_2$ requires: C, 70.92; H, 6.45; N, 6.89%.

5.3.5. Enzymatic hydrolysis of 3-(2-methylphenyl) glutaronitrile 1e. (3*S*)-4-Cyano-3-(4-methylphenyl)butanoic acid 2e: reaction time 20 h (with acetone) (40%) $[\alpha]_D^{25}$ +3.16 (*c* 1.25, $CHCl_3$) e.e. 35%. Oil: ν_{max} (KBr)/cm^{-1} 2958–3250 (COOH), 2248 (CN), 1711 (C=O); δ_H ($CDCl_3$) 7.20–7.26 (m, 4H), 3.81 (quin., *J* 7.0, 1H), 2.91 (dd, *J* 7.7, 16.7, 1H), 2.83 (dd, *J* 7.0, 16.8, 1H), 2.70 (d,

J 6.8, 2H), 2.40 (s, 3H); δ_C ($CDCl_3$) 175.4, 137.7, 135.2, 130.0, 127.3, 126.6, 124.2, 116.9, 37.5, 32.2, 22.6, 18.5; *m*/*z* (EI) 203 (M^+, 26%), 163 (31), 144 (33), 121 (100). Found 202.0874. $C_{12}H_{13}NO_2$ requires 202.0868.

5.3.6. Enzymatic hydrolysis of 3-(4-methoxyphenyl) glutaronitrile 1f. (3*S*)-4-Cyano-3-(4-methoxyphenyl)-butanoic acid 2f: reaction time 48 h (with acetone) (17%) $[\alpha]_D^{25}$ +22.4 (*c* 1.25, $CHCl_3$) e.e. 79%. Mp: 101–102°C; ν_{max} (KBr)/cm^{-1} 2848–3050 (COOH), 2248 (CN), 1708 (C=O); δ_H ($CDCl_3$) 8.68 (br, 1H), 7.17 (d, *J* 8.6, 2H), 6.89 (d, *J* 8.6, 2H), 3.80 (s, 3H), 3.45 (quin., *J* 7.0, 1H), 2.87 (dd, *J* 7.6, 16.6, 1H), 2.80 (dd, *J* 7.3, 16.6, 1H), 2.71 (d, *J* 6.6, 2H); δ_C ($CDCl_3$) 176.6, 159.2, 132.0, 128.0, 114.4, 117.8, 55.3, 38.8, 37.1, 24.7; *m*/*z* (EI) 219 (M^+ 24%), 192 (16), 179 (47), 160 (18), 137 (100). Found: C, 65.49; H, 6.13; N, 6.36. $C_{12}H_{13}NO_3$ requires: C, 65.74; H, 5.98; N, 6.39%.

5.3.7. Enzymatic hydrolysis of 3-cyclohexylglutaronitrile 1g. (−)-4-Cyano-3-cyclohexylbutanoic acid 2g: reaction time 20 h (with acetone) (60%) $[\alpha]_D^{20}$ −3.53 (*c* 1.7, $CHCl_3$) e.e. 83% (determined on the corresponding benzyl ester using chiral HPLC). Mp: 46–48°C; ν_{max} (KBr)/cm^{-1} 2603–3100 (COOH), 2244 (CN), 1705 (CO); δ_H ($CDCl_3$) 8.51 (br, 1H), 2.58 (dd, *J* 4.7, 16.7, 1H), 2.51 (d, *J* 6.1, 2H), 2.40 (dd, *J* 8.7, 16.6, 1H), 2.01–2.12 (m, 1H), 0.9–1.79 (m, 11H); δ_C ($CDCl_3$) 177.1, 117.6, 38.8, 35.9, 34.4, 28.8, 28.6, 25.1, 18.3; *m*/*z* (EI) 194 (M^+−1, 4%), 166 (4), 136 (28), 109 (20), 95 (21), 83 (28), 67 (27), 55 (83), 41 (100). Found: C, 67.28; H, 8.81; N, 7.04. $C_{11}H_{17}NO_2$ requires: C, 67.66; H, 8.78; N, 7.17%.

5.3.8. Enzymatic hydrolysis of 3-benzylglutaronitrile 1h. (−)-3-Benzyl-4-cyanobutanoic acid 2h: reaction time 20 h (with acetone) (62%) $[\alpha]_D^{20}$ −2.86 (*c* 1.4, $CHCl_3$) e.e. 32% (determined on the corresponding benzyl ester using chiral HPLC). Mp: 63–64°C; ν_{max} (KBr)/cm^{-1} 2524–3064 (COOH), 2243 (CN), 1714 (CO); δ_H ($CDCl_3$) 7.23–7.40 (m, 5H), 2.87 (dd, *J* 5.4, 13.5, 1H), 2.80 (dd, *J* 6.5, 13.7, 1H), 2.46–2.58 (m, 5H); δ_C ($CDCl_3$) 176.7, 136.7, 128.1, 127.9, 126.0, 116.8, 38.3, 36.0, 32.7, 20.1; *m*/*z* (EI) 203 (M^+, 7%), 163 (17), 162 (29), 144 (24), 143 (29), 117 (59), 91 (100). Found: C, 71.22; H, 6.41; N, 6.91. $C_{12}H_{13}NO_2$ requires: C, 70.92; H, 6.45; N, 6.89%.

5.4. Synthesis of (*S*)-(+)-4-amino-3-phenylbutanoic acid 4

To a solution of β-phenyl-γ-cyanobutanoic acid **2a** (189 mg, 1 mmol, e.e. 77%) in 1N aqueous NaOH solution (1.5 mL) were added successively 30% hydrogen peroxide (3.5 mL) and 10% aqueous NaOH solution (1.5 mL). The mixture was stirred overnight and then acidified to pH 1–2 with 18% aqueous hydrochloric acid. Extraction with ethyl acetate (3×15 mL) afforded, after drying and removing solvent, a white solid (164 mg). The resulting solid was dissolved in 1N aqueous NaOH solution (1 mL) and freshly prepared aqueous NaOBr solution (70 μL of Br_2 was added to 1 mL of 5N aqueous NaOH solution) was added. The mixture was stirred for 12 h at room temperature and then acidified to pH 1–2 with 18% hydrochloric acid. After filtration and extraction with ether (3×20 mL), the aqueous solution was purified on an ion-exchange column using 8% ammonia solution as the mobile phase to give (*S*)-**4** as a colorless solid (100 mg, overall yield 56%). Mp: 193–194°C (lit.[17] mp 194–196°C); $[\alpha]_D^{25}$ +4.5 (*c* 2, H_2O, pH 7.0) [lit.[17] $[\alpha]_D^{25}$ +6.3 (*c* 2.9, H_2O, pH 7.0)]; e.e. 87% (Chiral HPLC); ν_{max} (KBr)/cm^{-1} 2648–3020 (COOH, NH_2^+), 1629, 1533, 1391; δ_H (D_2O) 7.19–7.32 (m, 5H), 2.97–3.20 (m, 3H), 2.34–2.51 (m, 2H).

5.5. Synthesis of (*R*)-(−)-4-amino-3-phenylbutanoic acid 4

To an ice/water-bath cooled solution of 4-cyano-3-phenylbutanoic acid **2a** (284 mg, 1.5 mmol, e.e. 77%) in dry acetone (5 mL) was added sequentially triethylamine (0.29 mL, 2.1 mmol) and ethyl chloroformate (0.21 mL, 2.25 mmol). The reaction mixture was stirred at 0°C for 2.5 h, and then a solution of sodium azide (176 mg, 2.7 mmol) in water (0.5 mL) was added. After the mixture was stirred for a further 2 h, water (10 mL) was added and the resulting mixture was extracted with toluene (4×10 mL). The organic layer was dried on $MgSO_4$ overnight. After removal of toluene (about 30 mL) under vacuum, absolute methanol (5 mL) was added and the solution was heated under reflux for 3 h. The solvent was then removed under reduced pressure and the residue was chromatographed (petroleum ether/ethyl acetate 3:1) to afford a colorless liquid (140 mg). It was further hydrolyzed by stirring with 6N hydrochloric acid (15 mL) under reflux for 48 h. Purification on an ion-exchange column using 8% ammonia solution as the mobile phase followed by evaporation of the solvent gave a colorless solid *R*-**4** (90 mg, overall yield 34%). Mp: 180–181°C (lit.[17] mp 193–194°C); $[\alpha]_D^{25}$ −5.3 (*c* 1.85, H_2O, pH 7.0) [lit.[17] $[\alpha]_D^{25}$ −6.0 (*c* 2.5, H_2O, pH 7.0)] e.e. 85% (chiral HPLC); IR and ^1H NMR spectra were identical to those of *S*-(+)-**4**.

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