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Biotransformations of 2,3-epoxy-3-arylpropanenitriles by *Debaryomyces hansenii* and *Mortierella isabellina* cells

Malgorzata Zagozda, Jan Plenkiewicz *

Faculty of Chemistry, Warsaw University of Technology, Noakowskiego 3, 00-664 Warsaw, Poland

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ABSTRACT

Biotransformations of five substituted *cis*- and *trans*-oxiranecarbonitriles with *Mortierella isabellina* DSM 1414, a microbial whole-cell catalyst producing epoxide hydrolases, were investigated. The reactions were stopped when the conversion of the substrates reached 50%. They yielded the appropriate optically active dihydroxycarbonitriles and oxiranecarbonitriles in low enantiomeric purity. Kinetic resolution of *rac-syn*-2,3-dihydroxy-3-arylpropanenitriles by lipase catalyzed acetylation yielded almost enantiomerically pure (−)-dihydroxynitriles and mixtures of regioisomers of monoacetylated diols. Another microorganism, *Debaryomyces hansenii* DSM 3428, was used as a source of nitrile hydratases in the kinetic resolution of oxiranecarbonitriles. Only two *trans*-configured compounds were transformed into the corresponding oxiranecarboxamides.

1. Introduction

The enzyme-catalyzed enantioselective ring opening or functional group transformation of electrophilic oxiranes is still an important and attractive reaction. In the case of phenylglycidonitrile derivatives, hydrolysis of the cyano group may give, depending on the enzymes used to carry out the reaction, the corresponding optically active oxiranecarboxamides or oxiranecarboxylic acids. On the other hand, the oxirane ring may be hydrolyzed by epoxide hydrolases to yield the appropriate diols. Optically active products of such biotransformations are important substrates in the preparation of several biologically active compounds. For example, oxiranecarboxamides and oxiranecarboxylic acid esters prepared from the corresponding oxiranenitriles are known as useful building blocks in the synthesis of (+)-clausenamide,[1] (+)-neoclausenamide,[2] (−)-dehydroclausenamide,[3] that is, compounds used in the synthesis of Taxol®.[4] The products of these biotransformations are also important and versatile intermediates in the synthesis of several pharmaceuticals such as the selective leukotriene antagonist SK&F 104353[5] or diltiazem,[4b,6] a potent coronary vasodilating agent. It is worth mentioning that some biologically active N-monosubstituted and N,N-disubstituted phenylglycidamide derivatives have been isolated[7] from the *Clausena indica* and *Clausena lansium* plants whose leaves and fruits are used in traditional medicine to treat coughs, asthma, viral hepatitis as well as some dermatological and gastrointestinal disorders.

A highly efficient and enantioselective hydrolysis of the cyano group in 3-aryloxiranecarbonitrile and its 2-methyl- and 2,3-dimethyl-derivatives have recently been described by Wang et al.[8,9] As the enzyme source, they employed *Rhodococcus sp*. AJ 270 cells producing nitrile hydratases and amidases. The biotransformations of racemic *trans*-2,3-epoxy-3-arylpropanenitriles afforded (2*R*,3*S*)-2-arylglycidamides in excellent yield and enantiomeric purity as well as the unstable (2*S*,3*R*)-2-arylglycidacides which were not isolated. In contrast to the racemic *trans*-2,3-epoxy-3-arylpropanenitriles, the biotransformations of the racemic *cis*-counterparts proceeded with great difficulty. This indicates that the steric factors control the reaction of nitrile hydratase with nitrile substrates. Since the biotransformations of the racemic *cis*-2,3-epoxy-3-arylpropanenitriles with the same microorganism stopped at the stage of the corresponding racemic *cis*-amides, the amidases produced by *Rhodococcus sp*. AJ270 were inactive against the investigated *cis*-epoxyamides.

The cited authors[8,9] confirmed the earlier results[10] by concluding that the overall high enantioselectivity of the biotransformations arises from the combined effects of the highly (2*S*)-enantioselective amidase (predominant effect) and the poorly (2*S*) enantioselective nitrile hydratase involved in the reaction.

2. Results and discussion

Herein, we report the enzymatic hydrolysis of the oxiranecarbonitrile derivatives catalyzed by *Debaryomyces hansenii* DSM 3428 and *Mortierella isabellina* DSM 1414 cells. Assuming that the fungi produce nitrilases and/or nitrile hydratases we expected them to be able to transform 2,3-epoxy-3-arylpropanecarbonitrile into

* Corresponding author. Tel.: +48 22 234 75 70; fax: +48 22 628 27 41.
E-mail address: plenki@ch.pw.edu.pl (J. Plenkiewicz).

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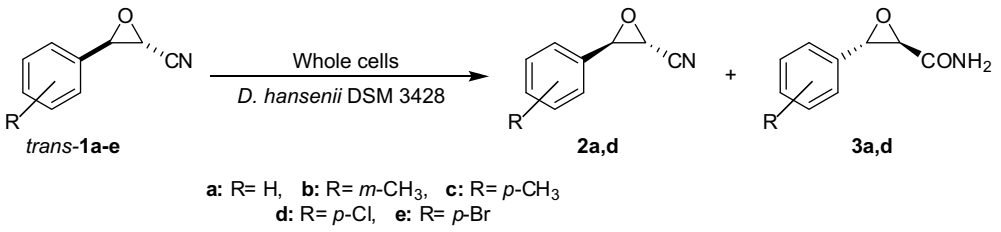


Scheme 1.

the corresponding optically active 3-arylglycidamides or the appropriate acids. Moreover, if the investigated microorganisms proved to be able to produce epoxide hydrolases, it would also be possible to convert the oxiranecarbonitrile substrates into the appropriate optically active 2,3-dihydroxy-3-arylpropanenitriles or 2,3-dihydroxy-3-arylpropaneamides.

The reactions were carried out with whole cells of the microorganisms harvested on the third day of cultivation. To the wet cells suspended in a 2% glucose solution in distilled water the oxiranecarbonitrile substrate was added as a solution in a small volume of ethanol. The mixtures were incubated at 30 °C until the substrate conversion was approximately 50% as estimated by TLC monitoring in a hexane/ethyl acetate mixture.

In the experiments with *D. hansenii* DSM 3428 cells, only the *trans*-isomers of two of the five investigated 2,3-epoxy-3-arylpropanecarbonitriles, namely, **1a** and **1d**, were accepted by the nitrile hydratases produced by this microorganism (see Scheme 1).

As the products of kinetic resolution of racemates of *trans*-**1a** and **1d**, we obtained (2*R*,3*S*)-3-phenylglycidamide **3a**, or (2*R*,3*S*) 3-(*p*-chlorophenyl)glycidamide **3d** and the unreacted (2*R*,3*R*) 2,3-epoxypropanecarbonitriles **2a** and **2d** in good yields, but of low enantiomeric purity. The results of the biotransformation are presented in Table 1.

Attempts at biotransformation of the *cis*-isomers of **1a**–**e** by *D. hansenii* DSM 3428 cells failed and the substrates were isolated unchanged, even after extending the reaction time up to seven days.

Using the same substrates **1a**–**e** and the standard biotransformation procedure, we investigated *M. isabellina* DSM 1414 cells as the catalyst. Unlike *D. hansenii* DSM 3428, that strain was found to produce epoxide hydrolases able to open the oxirane ring with no effect on the cyano substituent. With racemic *trans*-2,3-epoxy-3-arylpropanecarbonitriles, a 50% conversion was achieved in the smoothly proceeding reactions. The products contained the unchanged (2*R*,3*R*)-oxiranecarbonitrile and the corresponding *syn*-2,3-dihydroxy-3-arylpropanenitrile formed by hydrolysis of the oxirane ring (see Scheme 2).

Unfortunately, only the reactions with three of the five investigated substrates **1c**–**e** proceeded, yielding optically active products although with poor stereoselectivity. The phenyl and *m*-methylphenyl derivatives **1a** and **1b** yielded racemic 2,3-dihydroxy-3-arylpropanenitriles **4a** and **4b** and racemic oxiranecarbonitriles **2a** and **2b**. These results are similar to those reported by Wang[9] for the *Rhodococcus sp.* AJ270-catalyzed hydrolysis of the cyano group in *cis*-2-methyl-3-phenyloxiranecarbonitrile. In this reaction, carried out under the kinetic resolution conditions, both the product and substrate were racemates. The results obtained are summarized in Table 2.

Since it was not known which position of the oxirane ring was the target of the attack, we could not forecast the absolute

Table 1
Results of 2,3-epoxy-3-arylpropanenitrile hydrolysis by *Debaryomyces hansenii* DSM 3428

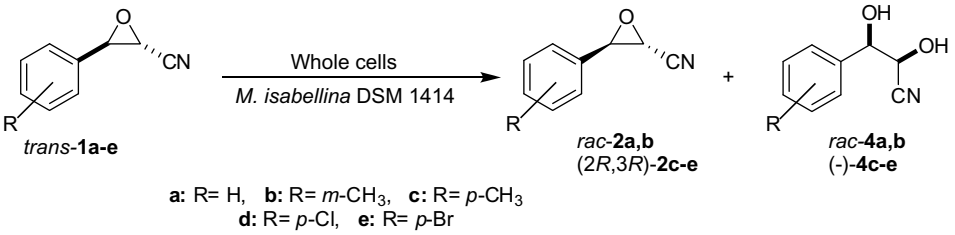
Substrate **1**	Time (h)	C[a] (%)	*trans*-**2**			**3**				*E*
			Y[b] (%)	ee[c] (%)	$[\alpha]_D^{25}$	Y[b] (%)	ee[c] (%)	Mp (°C)	$[\alpha]_D^{25}$	
a	3	45	43	29	+24.3	40	36	149–151[d]	−33.2[d]	2.8
d	6	43	46	19	+16.0	36	25	187–188[e]	−11.8[e]	2.0

[a] Conversions were calculated from the enantiomeric excesses of substrates *trans*-**2** (ee_s) and products **3** using the formula: $c = ee_s/(ee_s + ee_p)$.
[b] Yield after purification on a chromatography column.
[c] Enantiomeric excess determined by HPLC.
[d] Lit.[8] mp = 157–158 °C, $[\alpha]_D^{25} = -160.0$.
[e] Lit.[8] mp = 190–191 °C.

Table 2
Results of *trans*-**1a**-**e** hydrolysis by *Mortierella isabellina* DSM 1414 cells

Substrate *trans*-**1**	Time (h)	*C* (%)	**2**			**4**				*E*
			Y[c] (%)	ee[d] (%)	$[\alpha]_D^{24}$	Y[c] (%)	ee[d] (%)	Mp (°C)	$[\alpha]_D^{24}$	
a	5.5	~50[a]	49	*rac*	—	32	*rac*	Oil	—	—
b	4	~50[a]	34	*rac*	—	41	*rac*	Oil	—	—
c	5	44[b]	38	13	+15.8	43	17	Oil	−4.6	1.6
d	24	45[b]	46	44	+25.4	34	55	99–101	−11.4	5.3
e	96	35[b]	25	18	+14.5	19	34	92–94	−9.1	2.4

[a] Approximate conversion on the basis of spot area on TLC plates.
[b] Conversions were calculated from the enantiomeric excesses of substrates *trans*-**2** (ee_s) and products **4** using the formula: $c = ee_s/(ee_s + ee_p)$.
[c] Yield after purification on chromatography column.
[d] Enantiomeric excess determined by HPLC.

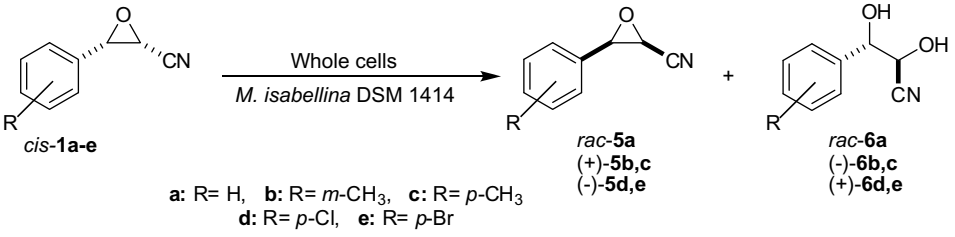


Scheme 2.

Table 3
Results of hydrolysis of *cis*-**1a-e** by *Mortierella isabellina* DSM 1414 cells

Substrate *cis*-**1**	Time (h)	*C* (%)	**5**			**6**				*E*
			Y[c] (%)	ee[d] (%)	$[\alpha]_D^{25}$	*Y*[c] (%)	ee[d] (%)	Mp (°C)	$[\alpha]_D^{25}$	
a	24	~50[a]	43	*rac*	–	39	*rac*	Oil	–	–
b	24	48[b]	39	23	+23.8	32	25	Oil	−3.1	2.1
c	4	39[b]	29	23	+22.4	24	37	91–92	−15.2	2.7
d	24	59[b]	47	36	−19.5	29	25	98–99	+8.9	2.3
e	72	51[b]	47	20	−15.1	26	19	96–97	+3.2	1.8

[a] Approximate conversion on the basis of spot area on TLC plates.
[b] Conversions were calculated from the enantiomeric excesses of substrates **5** (ee_s) and products **6** using the formula $c = ee_s/(ee_s + ee_p)$.
[c] Yield after purification on a chromatography column.
[d] Enantiomeric excess determined by HPLC.



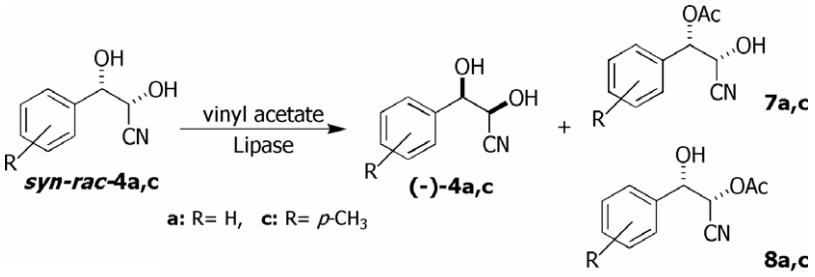
Scheme 3.

configuration of the optically active 2,3-dihydroxy-3-arylpropanenitriles obtained.

Unlike with the *D. hansenii* DSM 3428 cells, the cultures of *Mortierella isabellina* DSM 1414 produced epoxide hydrolases active against *cis*-2,3-epoxy-3-aryloxiranecarbonitriles.

The reactions, which were carried out under the same conditions, yielded optically active products of the kinetic resolution. Only the unsubstituted compound **1a** gave racemic mixtures. The hydrolytic opening of the oxirane ring of *cis*-2,3-epoxy-3-arylpropanecarbonitriles **1a–e** proceeded much slower than that of the *trans*-isomers, while the enantiomeric purities of the *anti*-2,3-dihydroxy-3-arylpropanenitriles **6a–e** as well as of the unchanged *cis*-carbonitriles **5a–e** were even lower than in the products of the *trans*-oxiranecarbonitrile isomers (see Table 3, see Scheme 3).

Poor enantiomeric purities of *syn*- and *anti*-2,3-dihydroxy-3-arylpropanenitriles prompted us to attempt their purification by kinetic resolution using the lipase-catalyzed esterification. The solutions of racemic *syn*-2,3-dihydroxy-3-phenyl- and 2,3-dihydroxy-3-(*p*-methylphenyl)propanenitrile **4a** and **4c** in a non-polar organic solvent were treated with vinyl acetate in the presence of *Novozym* 435 *(Candida Antarctica)* or *Amano AK* (*Pseudomonas fluorescens*) lipase and the reactions were carried on until the conversion was approximately 50%. The regioselectivity of the reaction was rather low and two isomeric monoacetylated diols **7a** and **7c** and **8a** and **8c** were formed (see Scheme 4).



Scheme 4.

Table 4
Results of lipase catalyzed acetylation of *syn-rac*-**4a**,**c**

4	Lipase	Molar excess of vinyl acetate	Temp. (°C)	Time (days)	Solvent	*syn*-(−)-**4a**,**c**			Monoester ratio **7**/**8**[c]
						Y[a] (%)	ee[b] (%)	$[\alpha]_D^{24}$ (EtOH)	
a	*Novozym 435*	10	35	18	Toluene	41	34	−11.0 (*c* 0.71)	2/1
a	*Amano AK*	10	35	9	Toluene	44	68	−22.0 (*c* 0.76)	3/1
c	*Novozym 435*	10	35	21	Toluene	34	77	−23.8 (*c* 0.80)	7/1
c	*Amano AK*	10	35	6	Toluene	38	99	−37.9 (*c* 0.66)	9/1
a	*Novozym 435*	5	22	18	TBME	39	59	−13.6 (*c* 0.81)	4/1
a	*Amano AK*	5	22	11	TBME	43	89	−28.7 (*c* 0.84)	5/1
c	*Novozym 435*	5	22	30	TBME	35	94	−36.1 (*c* 0.69)	7/1
c	*Amano AK*	5	22	21	TBME	38	92	−31.7 (*c* 0.76)	2/1

[a] Yield after purification on a chromatography column.
[b] Enantiomeric excess determined by HPLC.
[c] Monoester ratio determined on the basis of ^{1}H NMR spectra.

The reaction conditions were optimized by changing the solvents and other parameters as shown in Table 4.

Separation of the monoacetylated diols failed, therefore the isomer ratio was established by analysis of the ^{1}H NMR spectra. The particular signals were attributed to the isomers on the basis of the literature[11] data. As it can be seen the 2-acetoxy compound is predominant. The enantiomeric purities ee of the separated *levorotatory* diols were above 90%. A similar purification procedure was applied to the racemic mixture of *anti*-2,3-dihydroxy-3-(*p*-bromophenyl)propanenitrile **6e**. In the resulting acetylation catalyzed by the *Amano AK* lipase we obtained a 3:1 mixture of 2- and 3-monoacetoxy derivatives and the *dextrarotatory* diol of 95% enantiomeric purity and in 35% yield.

3. Conclusion

Our results indicate that whole cells of *D. hansenii* DSM 3428 can selectively catalyze the hydrolysis of the cyano group in some *trans*-2,3-epoxy-3-aryl-propanenitriles to give the corresponding amides in high yields but with low enantioselectivity. On the other hand, *M. isabellina* DSM 1414 cells catalyze the oxirane ring opening of both *cis*- and *trans*-2,3-epoxy-3-aryl-propanenitriles yielding the corresponding optically active 2,3-hydroxy-3-arylpropanenitriles.

4. Experimental

4.1. General

^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded with a Varian Mercury 400 MHz spectrometer in $CDCl_3$ solution, and chemical shifts (δ) are reported in parts per million. Optical rotations were measured in EtOH using a PolAAr 32 polarimeter. Elemental analyses were performed with a CHNS/O Perkin–Elmer type 2400 instrument. Enantiomeric excesses (ee%) were determined by HPLC analysis on a Thermo-Separation Products P-100 instrument with Chiracel OD-H column in *n*-hexane/*iso*-propanol as the eluent in comparison with racemates. The biotransformation reactions were monitored by TLC on Silica Gel 60 F_{254} plates. *Amano AK* was kindly granted by Amano and *Novozym* SP 435 by Novozymes.

4.2. Synthesis of 2,3-epoxy-3-arylpropanenitriles 1a–e

2,3-Epoxy-3-arylpropanenitriles were prepared from the appropriate aldehyde and chloroacetonitrile in a Darzens reaction according to the described[12] procedure. The *cis*-/*trans*-isomers separation was accomplished by column chromatography.

4.2.1. 2,3-Epoxy-3-phenylpropanenitrile 1a

Yield 73%; colorless oil; bp = 123–125 °C/15 Torr (lit.[13] bp = 119–121 °C/6 Torr). Mixture of diastereoisomers.

4.2.2. 2,3-Epoxy-3-(*m*-methylphenyl)propanenitrile 1b

Yield 62%; colorless oil; bp = 74–75 °C/0.1 Torr. Anal. Calcd for $C_{10}H_9NO$ (159.18): C, 75.45; H, 5.70; N, 8.80. Found: C, 75.28; H, 5.65; N, 8.73. *trans*-**1b**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.37 (s, 3H, CH_3-Ar); 3.41 (d, J = 2.0 Hz, 1H, Ar-CH–); 4.25 (d, J = 2.0 Hz, 1H, –CH–CN); 7.26–7.41 (m, 4H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.28; 44.50; 58.48; 116.02; 122.76; 126.08; 128.81; 130.54; 132.61; 138.83. *cis*-**1b**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.40 (s, 3H, CH_3-Ar); 3.76 (d, J = 3.6 Hz, 1H, Ar-CH–); 4.21 (d, J = 3.6 Hz, 1H, –CH–CN); 7.21–7.35 (m, 4H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.35; 44.98; 57.66; 115.01; 123.29; 126.77; 128.51; 130.43; 131.23; 138.42.

4.2.3. 2,3-Epoxy-3-(*p*-methylphenyl)propanenitrile 1c

Yield 80%; colorless oil; bp = 87–88 °C/0.4 Torr (lit.[14] bp = 140–145 °C/17 Torr). Mixture of diastereoisomers.

4.2.4. 2,3-Epoxy-3-(*p*-chlorophenyl)propanenitrile 1d

Yield 73%; colorless crystals; *trans*-1d: mp = 66–67 °C (lit.[14] mp = 68–69 °C), *cis*-1d: mp = 78–80 °C (lit.[14] mp = 79–81 °C).

4.2.5. 2,3-Epoxy-3-(*p*-bromophenyl)propanenitrile 1e

Yield 78%; colorless crystals. Anal. Calcd for C_9H_6BrNO (224.05): C, 48.25; H, 2.70; N, 6.25; Br, 35.66. Found: C, 48.29; H, 2.89; N, 6.22; Br, 35.61. *trans*-**1e**: mp = 90–92 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.39 (d, J = 2.0 Hz, 1H, Ar-CH–); 4.26 (d, J = 2.0 Hz, 1H, –CH–CN); 7.13–7.17 (m, 2H, Ar-H); 7.51–7.54 (m, 2H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 44.54; 57.88; 115.65; 123.93; 127.20; 131.73; 132.15. *cis*-**1e**: mp = 96–97 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.79 (d, J = 3.6 Hz, 1H, Ar-CH–); 4.22 (d, J = 3.6 Hz, 1H, –CH–CN); 7.27–7.31 (m, 2H, Ar-H); 7.56–7.59 (m, 2H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 44.97; 57.14; 114.70; 123.93; 127.89; 130.40; 131.90.

4.3. Microorganisms

The DSM 1414 strain of *M. isabellina* and DSM 3428 strain of *D. hansenii* were purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ).

4.4. Cultivation of microorganisms

The microorganism was grown on a plate with an agar medium for 3 days at 27 °C until well sporulated. Spores from the surface

culture were used to inoculate flasks containing sterile liquid medium (sterilized at 121 °C for 15 min), next incubated at 30 °C for 3 days on a rotary shaker at 140 rpm.

Growing medium for both microorganisms investigated contains malt extract (30 g), soya peptone (3 g), dissolved in 1.0 L of distilled water, and adjusted pH to 5.6.

The biomass of fungal cells of *M. isabellina* was separated by filtration under reduced pressure, while *D. hansenii* was separated by centrifugation of the medium.

4.5. Hydrolysis of *trans*-2,3-epoxy-3-phenylpropanenitrile 1a and *trans*-2,3-epoxy-3-(*p*-chlorophenyl)-propanenitrile 1d by D. hansenii DSM 3428

Wet cells of the microorganism (58 g) were suspended in 116 mL of 2% glucose solution in sterile water, and a solution of 0.24 g (1.5 mmol) of **1a** in 1.5 mL of ethanol or 0.3 g (1.5 mmol) of **1d** in 1.5 mL of DMF was added. The reaction was carried at 30 °C using an orbital shaker (140 rpm). The reaction monitored by TLC was quenched after about 50% of the substrate reacted. Ethyl acetate (90 mL) was added to the reaction flask, shaken for 20 min, and 25 g of Celite® was added next. After filtration, the supernatant was extracted three times with ethyl acetate. The extract was dried with sodium sulfate and evaporated to dryness. The residue was separated on silica gel column with hexane–ethyl acetate (gradient of concentration) yielding unreacted nitriles (2*R*,3*R*)-**2a** or **2d** and the amides (2*R*,3*S*) **3a** or **3d**. The enantiomeric excesses were determined by HPLC analysis using a Chiracel OD-H column (in *n*-hexane–*iso*-propanol, 7/3 v/v, $f = 0.6$ mL/min). The ^{1}H NMR spectra of the amides were consistent with those presented in the literature.[8]

4.6. Hydrolysis of *cis*- or *trans*-2,3-epoxy-3-aryl-propanenitriles by *M. isabellina* DSM 1414

To an Erlenmeyer flask (300 mL) covered by cotton wool, 110 mL of 2% glucose solution in sterilized water and *M. isabellina* DSM 1414 cells (55 g wet weight) were added. The appropriate isomer of the substrate (1.1 mmol) in 2 mL of ethanol was added in one portion to the flask, and the mixture incubated at 30 °C using an orbital shaker (140 rpm). The reaction monitored by TLC was quenched after a specified period of time (see Tables 2 and 3) by the addition of ethyl acetate and shaking for an additional 20 min. The biomass was filtered off, and washed with a small volume of ethyl acetate. The organic phase of the filtrate was separated, and the water phase extracted with ethyl acetate. The combined extracts after drying with sodium sulfate and solvent evaporation were chromatographed on silica gel column with gradient of hexane–ethyl acetate mixture, yielding the appropriate unreacted 2,3-epoxy-3-arylpropanenitrile and 2,3-dihydroxy-3-arylpropanenitrile as the product of kinetic resolution. The enantiomeric excesses were determined by HPLC analysis using a Chiracel OD-H column (in *n*-hexane–*i*-propanol, 7/3 v/v for unreacted nitriles and 9/1 v/v for cyanohydrins, $f = 0.6$ mL/min). ^{1}H, ^{13}C NMR spectra and elemental analyses of the isolated products are reported below.

4.6.1. *rac-anti*-2,3-Dihydroxy-3-phenylpropanenitrile 6a

Yield 39%; colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.09 (s, 1H, –OH); 3.46 (d, $J = 6.0$ Hz, 1H, –OH); 4.50 (dd, $J = 6.4$ Hz, $J = 6.0$ Hz, 1H, –CH); 4.90 (d, $J = 6.4$ Hz, 1H, –CH); 7.38–7.45 (m, 5H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 66.42; 74.94; 118.27; 127.06; 129.07; 129.52; 137.11. Anal. Calcd for $C_9H_9NO_2$ (163.17): C, 66.25; H, 5.56; N, 8.58. Found: C, 66.18; H, 5.53; N, 8.55.

4.6.2. *rac-syn*-2,3-Dihydroxy-3-phenylpropanenitrile 4a

Yield 32%; colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.83 (d, $J = 3.2$ Hz, 1H, –OH); 3.32 (d, $J = 8.8$ Hz, 1H, –OH); 4.55 (dd, $J = 8.8$ Hz, $J = 4.0$ Hz, 1H, –CH); 5.00 (dd, $J = 4.0$ Hz, $J = 3.2$ Hz, 1H, –CH); 7.37–7.47 (m, 5H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 67.27; 74.73; 117.15; 126.22; 128.92; 129.24; 136.74. Anal. Calcd for $C_9H_9NO_2$ (163.17): C, 66.25; H, 5.56; N, 8.58. Found: C, 66.32; H, 5.59; N, 8.60.

4.6.3. *anti*-(−)-2,3-Dihydroxy-3-(*m*-methylphenyl)-propanenitrile 6b

Yield 32%; colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.38 (s, 3H, CH_3-Ar); 2.88 (br s, 1H, –OH); 3.21 (br s, 1H, –OH); 4.51 (d, $J = 6.4$ Hz, 1H, –CH); 4.88 (d, $J = 6.4$ Hz, 1H, –CH); 7.19–7.32 (m, 4H, Ar-H).^{13}C NMR (100 MHz, $CDCl_3$): δ 21.38; 66.16; 74.72; 117.96; 123.82; 127.35; 128.71; 130.05; 136.77; 138.64. Anal. Calcd for $C_{10}H_{11}NO_2$ (177.20): C, 67.78; H, 6.26; N, 7.90. Found: C, 67.81; H, 6.28; N, 7.93.

4.6.4. *rac-syn*-2,3-Dihydroxy-3-(*m*-methylphenyl)-propanenitrile 4b

Yield 41%; colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.38 (s, 3H, CH_3-Ar); 2.82 (br s, 1H, –OH); 3.35 (br s, 1H, –OH); 4.54 (d, $J = 4.0$ Hz, 1H, –CH); 4.96 (d, $J = 4.0$ Hz, 1H, –CH); 7.18–7.33 (m, 4H, Ar-H).^{13}C NMR (100 MHz, $CDCl_3$): δ 21.41; 67.31; 74.68; 117.48; 123.24; 126.82; 128.75; 129.86; 136.77; 138.65. Anal. Calcd for $C_{10}H_{11}NO_2$ (177.20): C, 67.78; H, 6.26; N, 7.90. Found: C, 67.89; H, 6.29; N, 7.95.

4.6.5. *anti*-(−)-2,3-Dihydroxy-3-(*p*-methylphenyl)-propanenitrile 6c

Yield 24%; colorless crystals. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.37 (s, 3H, CH_3-Ar); 2.70 (br s, 1H, –OH); 2.97 (br s, 1H, –OH); 4.51 (d, $J = 6.4$ Hz, 1H, –CH); 4.90 (d, $J = 6.4$ Hz, 1H, –CH); 7.22–7.24 (m, 2H, Ar-H); 7.32–7.34 (m, 2H, Ar-H).^{13}C NMR (100 MHz, $CDCl_3$): δ 21.21; 66.33; 74.67; 117.66; 126.62; 129.58; 133.87; 139.38. Anal. Calcd for $C_{10}H_{11}NO_2$ (177.20): C, 67.78; H, 6.26; N, 7.90. Found: C, 67.69; H, 6.21; N, 7.94.

4.6.6. *syn*-(−)-2,3-Dihydroxy-3-(*p*-methylphenyl)-propanenitrile 4c

Yield 43%; colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.34 (s, 3H, CH_3-Ar); 3.42 (br s, 1H, –OH); 4.03 (d, $J = 8.0$ Hz, 1H, –OH); 4.49 (dd, $J = 8.0$ Hz, $J = 3.6$ Hz, 1H, –CH); 4.92 (d, $J = 3.6$ Hz, 1H, –CH); 7.18–7.20 (m, 2H, Ar-H); 7.28–7.30 (m, 2H, Ar-H).^{13}C NMR (100 MHz, $CDCl_3$): δ 21.15; 67.29; 74.50; 117.57; 126.13; 129.49; 133.79; 138. 96. Anal. Calcd for $C_{10}H_{11}NO_2$ (177.20): C, 67.78; H, 6.26; N, 7.90. Found: C, 67.62; H, 6.29; N, 7.71.

4.6.7. *anti*-(+)-2,3-Dihydroxy-3-(*p*-chlorophenyl)-propanenitrile 6d

Yield 29%; colorless crystals. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.78 (d, $J = 3.2$ Hz, 1H, –OH); 2.94 (d, $J = 6.8$ Hz, 1H, –OH); 4.49 (dd, $J = 6.8$ Hz, $J = 6.4$ Hz, 1H, –CH); 4.93 (dd, $J = 6.4$ Hz, $J = 3.2$ Hz, 1H, –CH); 7.21–7.37 (m, 4H, Ar-H).^{13}C NMR (100 MHz, $CDCl_3$): δ 67.23; 73.88; 117.23; 127.56; 129.08; 135.08; 135.14. Anal. Calcd for $C_9H_8ClNO_2$ (197.62): C, 54.70; H, 4.08; Cl, 17.94; N, 7.09. Found: C, 54.74; H, 4.10; Cl, 17.99; N, 7.14.

4.6.8. *syn*-(−)-2,3-Dihydroxy-3-(*p*-chlorophenyl)-propanenitrile 4d

Yield 34%; colorless crystals. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.41 (br s, 1H, –OH), 3.95 (br s, 1H, –OH), 4.52 (d, $J = 4.0$ Hz, 1H, –CH); 4.96 (d, $J = 4.0$ Hz, 1H, –CH); 7.28–7.39 (m, 4H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 67.10; 73.99; 117.19; 127.65; 129.06;

135.04; 135.26. Anal. Calcd for $C_9H_8ClNO_2$ (197.62): C, 54.70; H, 4.08; Cl, 17.94; N, 7.09. Found: C, 54.86; H, 4.12; Cl, 17.98; N, 7.16.

4.6.9. *anti*-(+)-2,3-Dihydroxy-3-(*p*-bromophenyl)-propanenitrile 6e

Yield 26%; colorless crystals. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.71 (br s, 1H, –OH); 3.01 (br s, 1H, –OH); 4.79 (d, J = 5.6 Hz, 1H, –CH); 5.01 (d, J = 5.6 Hz, 1H, –CH); 7.28–7.39 (m, 2H, Ar-H); 7.56–7.59 (m, 2H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 66.32; 74.61; 116.87; 123.59; 127.89; 131.93; 135.42. Anal. Calcd for $C_9H_8BrNO_2$ (242.07): C, 44.66; H, 3.33; Br, 33.01; N, 5.79. Found: C, 44.70; H, 3.40; Br, 33.12; N, 5.77.

4.6.10. *syn*-(−)-2,3-Dihydroxy-3-(*p*-bromophenyl)-propanenitrile 4e

Yield 19%; colorless crystals. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.52 (br s, 1H, –OH); 4.07 (br s, 1H, –OH); 4.51 (d, J = 4.0 Hz, 1H, –CH); 4.92 (d, J = 4.0 Hz, 1H, –CH); 7.27–7.31 (m, 2H, Ar-H); 7.50–7.54 (m, 2H, Ar-H).^{13}C NMR (100 MHz, $CDCl_3$): δ 67.01; 73.99; 117.25; 123.18; 127.94; 131.99; 135.76. Anal. Calcd for $C_9H_8BrNO_2$ (242.07): C, 44.66; H, 3.33; Br, 33.01; N, 5.79. Found: C, 44.85; H, 3.51; Br, 33.14; N, 5.61.

4.7. Transesterification procedure of *syn*-4a

syn-2,3-Dihydroxy-3-phenylpropanenitrile **4a** 0.1 g (0.63 mmol) was dissolved in 12 mL of TBME (*tert*-butyl methyl ether), and vinyl acetate 0.29 mL (3.2 mmol) as well as 0.13 g of *Amano AK* lipase was added. The mixture was stirred at room temperature and the conversion was monitored by TLC. After the appropriate time, the reaction was stopped by filtering off the enzyme and the solvent was evaporated under reduced pressure. The crude mixture was separated by column chromatography on silica gel with a hexane–ethyl acetate (3:1 v/v) mixture as the eluent, yielding the appropriate unreacted 2,3-dihydroxy-3-phenylpropanenitrile **4a** and the mixture of two isomeric monoacetylated diols **7a** and **8a** as the products of kinetic resolution (see Table 4). ^{1}H and ^{13}C NMR spectra of enantiomerically enriched cyanohydrin (−)-**4a** were identical with those obtained by hydrolysis of *trans*-**1a**. The spectra and elemental analyses of the obtained monoacetylated diols are reported below.

Mixture of 3-acetoxy-2-hydroxy-3-phenylpropanenitrile **7a** and 2-acetoxy-3-hydroxy-3-phenylpropanenitrile **8a** in a ratio of 5/1. Colorless liquid. Anal. Calcd for $C_{11}H_{11}NO_3$ (205.21): C, 64.38; H, 5.40; N, 6.83. Found: C, 64.42; H, 5.46; N, 6.89.

4.7.1. 3-Acetoxy-2-hydroxy-3-phenylpropanenitrile 7a

^{1}H NMR (400 MHz, $CDCl_3$): δ 2.20 (s, 3H, $-CH_3$); 3.77 (br s, 1H, –OH); 4.65 (d, J = 4.4 Hz, 1H, –CH); 5.96 (d, J = 4.4 Hz, 1H, –CH); 7.38–7.44 (m, 5H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.89; 66.24; 75.79; 116.81; 126.75; 128.92; 129.45; 136.42; 170.40.

4.7.2. 2-Acetoxy-3-hydroxy-3-phenylpropanenitrile 8a

^{1}H NMR (400 MHz, $CDCl_3$): δ 2.12 (s, 0.6H, $-CH_3$); 3.10 (br s, 0.2H, –OH); 5.04 (d, J = 4.4 Hz, 0.2H, –CH); 5.45 (d, J = 4.4 Hz, 0.2H, –CH); 7.38–7.44 (m, 1H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.27; 65.53; 72.91; 114.72; 126.38; 128.81; 129.29; 133.82; 168.81.

4.8. Transesterification procedure of *syn*-4c

syn-2,3-Dihydroxy-3-(*p*-methylphenyl)propanenitrile **4c** 0.08 g (0.48 mmol) was dissolved in 8 mL of toluene and vinyl acetate 0.44 mL (4.8 mmol) as well as 0.08 g of *Amano AK* lipase was added. The mixture was stirred at 35 °C and the conversion was monitored by TLC. After the appropriate time, the reaction was stopped by filtering off the enzyme and the solvent was evaporated under reduced pressure. The crude mixture was separated by column chromatography on silica gel with a hexane–ethyl acetate (3:1 v/v) mixture as the eluent, yielding the appropriate unreacted 2,3-dihydroxy-3-(*p*-methylphenyl)propanenitrile **4c** and the mixture of two isomeric monoacetylated diols **7c** and **8c** as the products of kinetic resolution (see Table 4).

The ^{1}H and ^{13}C NMR spectra spectra of enantiomerically enriched cyanohydrin (−)-**4c** were identical with those obtained by hydrolysis of *trans*-**1c**. The spectra and elemental analyses of the obtained monoacetylated diols are reported below.

Mixture of 3-acetoxy-2-hydroxy-3-(*p*-methylphenyl)propanenitrile **7c** and 2-acetoxy-3-hydroxy-3-(*p*-methylphenyl)propanenitrile **8c** in ratio 9/1. Colorless liquid. Anal. Calcd for $C_{12}H_{13}NO_3$ (219.24): C, 65.74; H, 5.98; N, 6.39. Found: C, 65.55; H, 5.88; N, 6.29.

4.8.1. 3-Acetoxy-2-hydroxy-3-(*p*-methylphenyl)-propanenitrile 7c

^{1}H NMR (400 MHz, $CDCl_3$): δ 2.20 (s, 3H, $-CH_3$); 2.35 (s, 3H, $-CH_3$); 3.66 (br s, 1H, –OH); 4.63 (d, J = 4.8 Hz, 1H, –CH); 5.93 (d, J = 4.8 Hz, 1H, –CH); 7.20–7.34 (m, 4H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.91; 21.26; 66.45; 75.63; 116.39; 126.67; 129.61; 130.86; 139.63; 170.51.

4.8.2. 2-Acetoxy-3-hydroxy-3-(*p*-methylphenyl)-propanenitrile 8c

^{1}H NMR (400 MHz, $CDCl_3$): δ 2.13 (s, 0.3H, $-CH_3$); 2.36 (s, 0.3H, $-CH_3$); 2.98 (br s, 0.1H, –OH); 5.01 (d, J = 4.8 Hz, 0.1H, –CH); 5.43 (d, J = 4.8 Hz, 0.1H, –CH); 7.20–7.34 (m, 0.4H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.29; 21.19; 65.58; 74.16; 115.08; 126.58; 129.54; 130.77; 139.50; 169.43.

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