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Enantioselective Ring Opening of Epoxides with Cyanide Catalysed by Halohydrin Dehalogenases: A New Approach to Non-Racemic β-Hydroxy Nitriles

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Abstract: Halohydrin dehalogenases (HheA, HheB and HheC) were found to efficiently catalyse a carbon-carbon bond forming reaction between terminal aliphatic epoxides and cyanide, yielding β-hydroxy nitriles. With all three enzymes nucleophilic ring opening of epoxides proceeds with high regioselectivity to the β-carbon atom. Activity, enantioselectivity and enantiopreference depend on the type of enzyme and the substrate structure. HheC was found to be the most selective among the tested enzymes. The enantioselectivity toward monosubstituted epoxides varies from moderate to high ($E = 5-106$), while resolution of 2,2-disubstituted epoxides proceeds with very high enantioselectivity ($E = 141$ and 200). The results show that halohydrin dehalogenases may become attractive catalysts for the facile preparation of enantiopure β-hydroxy nitriles from racemic epoxides.

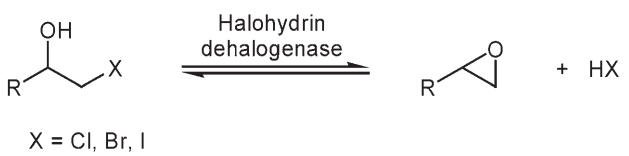
Keywords: cyanide; epoxides; halohydrin dehalogenase; kinetic resolution

Introduction

Halohydrin dehalogenases are enzymes involved in the biodegradation of some halogenated organic compounds. They catalyse the reversible ring closure of vicinal halo alcohols, yielding epoxides and halide ions (Scheme 1).[1]

Several microorganisms are known to possess halohydrin dehalogenases.[2] Although not yet commercially available, these enzymes are highly promising biocatalytic tools for the synthesis of optically pure epoxides, halohydrins and other β-substituted alcohols due to their enantioselectivity[1,3] and capability to accept non-natural nucleophiles.[4] It was found that besides the halide, other small negatively charged ions like azide, cyanide and nitrite are also accepted in the ring opening reaction (Figure 1).[4] In this way some β-azido alcohols,[5] β-nitro alcohols[6] and β-hydroxy nitriles[7,8] were prepared.

Among these nucleophiles cyanide is particularly interesting because of its synthetic versatility. Since a nitrile group can be transformed into an amino, amide, carboxy, or carbonyl group, enantiopure β-hydroxy nitriles can serve as versatile building blocks for the synthesis of biologically active compounds. There are several biocatalytic approaches for their preparation, such as stereoselective microbial reduction of β-keto nitriles,[9,10] lipase-catalysed resolution of β-hydroxy nitrile acetates or alcohols[11] and resolution of β-hydroxy nitriles by ni-



Scheme 1.

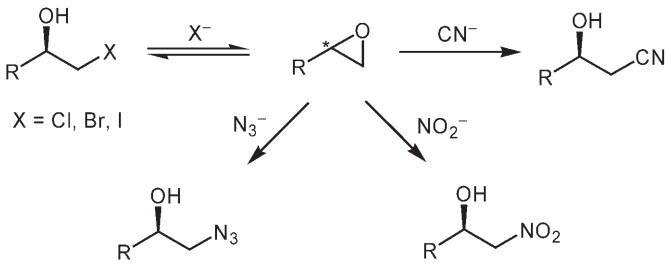
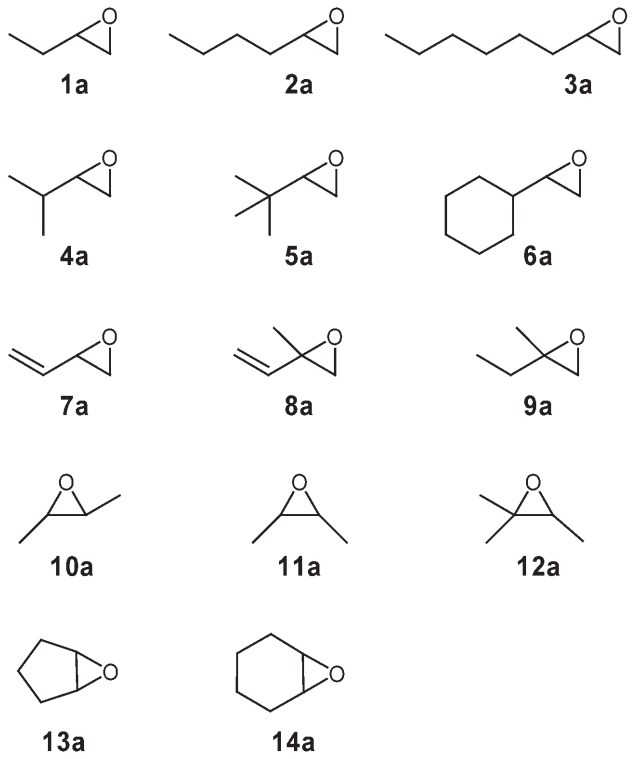


Figure 1. Ring opening reactions catalysed by halohydrin dehalogenase.

trilases.[12] A valuable alternative to these methods would be the use of halohydrin dehalogenases in a kinetic resolution of epoxides with the formation of a C–C bond at the same time. Although halohydrin dehalogenases have been known for several years, their potential for the synthesis of β-hydroxy nitriles has not been well explored. Until now, there are only two reports describing this conversion.[7,8] A halohydrin dehalogenase isolated from a *Corynebacterium* sp. strain N-1074, called H-lyase A (closely related to HheA, see below), catalysed the ring opening of 1,2-epoxybutane resulting in racemic 3-hydroxypentanenitrile.[7] H-Lyase B (closely related to HheB), isolated from the same microorganism, showed enantioselectivity towards epichlorohydrin, yielding enantiomerically enriched (*R*)-γ-chloro-β-hydroxybutyronitrile upon reaction with cyanide.[8] In order to explore the catalytic scope of the halohydrin dehalogenase-catalysed synthesis of β-hydroxy nitriles, more thorough studies are needed.

Here we describe the substrate specificity and enantioselectivity in cyanide-mediated epoxide ring opening of three different halohydrin dehalogenases. The enzymes are obtained from *Arthrobacter* sp. AD2 (HheA), *Mycobacterium* sp. GP1 (HheB) and *Agrobacterium radiobacter* AD1 (HheC). Based on sequence analysis and comparison with other genes, they can be divided into three distinct groups that share 24 to 32% sequence identity, which are referred as groups A, B and C.[1] The best studied among these enzymes is HheC. It is a homotetrameric protein with 28 kDa subunits. The crystal structure and catalytic mechanism of HheC have been determined recently.[13]



Scheme 2.

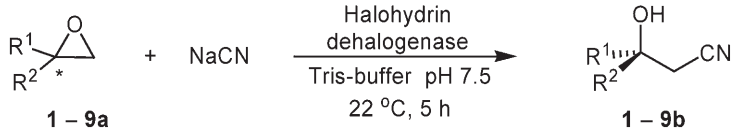
Results and Discussion

Since the three halohydrin dehalogenases isolated in our laboratory have not been studied as biocatalysts in the epoxide ring opening reaction by cyanide, we decided to start a systematic evaluation of this reaction. Our investigations are focused on the enantioselectivity of the wild-type dehalogenases (HheA, HheB and HheC) and the dependence of the activity and selectivity on the substrate structure. Several structurally different aliphatic epoxides (**1**–**14a**) were used as substrates to cover variations in the number and size of substituents on the oxirane ring (Scheme 2).

All enzymatic reactions were carried out in Tris-SO_4 buffer (pH 7.5) containing 0.5% DMSO to increase the solubility of the substrate, NaCN (15 mM) and epoxide (5 mM), except with **3a** and **14a** for which, because of low aqueous solubility, a 2 mM solution of epoxide was used. Reactions were performed with purified enzyme at room temperature and monitored by periodically taking samples, followed by a GC analysis.

All three enzymes showed a broad substrate range with the terminal epoxides **1**–**9a** (Table 1). All mono- and disubstituted terminal epoxides tested were converted and in each case GC analysis indicated formation of only one product. The structures of the products were confirmed using chemically synthesized racemic standards **1**–**9b** that were co-injected on GC columns. With non-terminal epoxides **10**–**14a**, no product formation was observed within 24 h under the described experimental conditions, indicating that the enzymes have no significant activity with these compounds. The enzymatic reactions with terminal epoxides proceeded with preferential attack of CN^- at the less substituted carbon atom, in all cases with high regioselectivity. Only epoxide **8a** was found to be unstable in water, due to the presence of a vinyl group at the α-carbon atom of the oxirane ring. This substrate undergoes spontaneous hydrolysis (*ca.* 5% per h) and chemical formation of the diol was competitive with enzymatic conversion. Replacing the vinyl group on the 2,2-disubstituted oxirane ring with an ethyl group resulted in substrate **9a**, which was resistant to hydrolysis. For each substrate, the experiments were repeated in the absence of enzyme to determine the rate of the chemical ring opening reaction. The non-enzyme-catalysed formation of β-hydroxy nitriles was found to be absent or very slow. Chemical conversion was negligible at epoxide and cyanide concentrations of 5 and 15 mM, respectively, and only became significant at higher concentrations and with prolonged reaction time. With 50 mM **1a** and 100 mM NaCN 4% of racemic product **1b** was observed after 2 h at room temperature.

Table 1. Kinetic resolution of *rac* **1**–**9a**.



1 – 9a **1 – 9b**

	Enzyme	Substrate	Initial activity[a] [μmol min^{-1} mg^{-1}]	Conversion[b] [%]	Epoxide		β-Hydroxy nitrile		*E* value[c]
					ee [%]	Config.[d, f]	ee [%]	Config.[d]	
1	HheA	**1a**	1.4	48	43	(*R*)	46	(*S*)	4
2	HheA	**2a**	1.8	46	22	(*R*)	26	(*S*)	2
3	HheA	**3a**	0.8	54	nd	(*R*)	27	(*S*)	2
4	HheA	**4a**	0.6	33	35	(*R*)	71	(*R*)[e]	8
5	HheA	**5a**	0.3	34	51	(*R*)	97	(*R*)[e]	109
6	HheA	**6a**	0.6	27	28	(*S*)	75	(*S*)[e]	9
7	HheA	**7a**	0.7	40	44	(*R*)	66	(*R*)[e]	7
8	HheA	**8a**	0.6	30	18	(*S*)	42	(*S*)[e]	3
9	HheA	**9a**	1.2	54	15	(*S*)	13	(*R*)	1
10	HheB	**1a**	0.4	43	6	(*R*)	8	(*S*)	1
11	HheB	**2a**	1.2	36	40	(*R*)	71	(*S*)	9
12	HheB	**3a**	0.2	31	nd	(*R*)	75	(*S*)	10
13	HheB	**4a**	0.2	24	20	(*R*)	62	(*R*)[e]	5
14	HheB	**5a**	0.1	18	18	(*R*)	80	(*R*)[e]	10
15	HheB	**6a**	0.3	21	10	(*R*)	37	(*R*)[e]	2
16	HheB	**7a**	0.2	29	19	(*S*)	48	(*S*)[e]	3
17	HheB	**8a**	0.8	42	46	(*S*)	63	(*S*)[e]	7
18	HheB	**9a**	2.2	85	69	(*R*)	71	(*R*)	2
19	HheC	**1a**	1.0	58	99	(*S*)	71	(*R*)	29
20	HheC	**2a**	0.2	32	32	(*S*)	67	(*R*)	7
21	HheC	**3a**	0.1	41	nd	(*S*)	54	(*R*)	5
22	HheC	**4a**	0.8	57	71	(*S*)	54	(*S*)[e]	7
23	HheC	**5a**	0.1	20	22	(*R*)	86	(*R*)[e]	16
24	HheC	**6a**	1.1	49	92	(*S*)	94	(*S*)[e]	106
25	HheC	**7a**	0.7	51	82	(*S*)	79	(*S*)[e]	21
26	HheC	**8a**	2.1	53	100	(*S*)	90	(*S*)[e]	141
27	HheC	**9a**	0.3	34	51	(*S*)	99	(*R*)	>200

[a] Expressed in μmol of product formed per min per mg enzyme.
[b] Data given for conversion are calculated according to formula $c = ee_s/(ee_s + ee_p)$, and are in good correlation with conversions determined experimentally using GC. Large deviations were observed only with substrate **8a** where, due to the chemical instability of the epoxide, a significant amount of diol was formed as well.
[c] *E* values were calculated from ee_p and ee_s according to formula $E = \ln[(1 - ee_s)/(1 + ee_s/ee_p)]/\ln[(1 + ee_s)/(1 + ee_s/ee_p)]$, except for **3a**, where *E* was calculated from *c* and ee_p.[14] *E* values above 100 should be considered as approximate values.
[d] Absolute configurations were determined by GC analysis on a chiral stationary phase by comparison of retention times with reference materials.
[e] Apparent inversion of configuration of epoxides (*S*)-**4**–**8a** to alcohols (*R*)-**4**–**8b** is due to a different substituent priority according to the Cahn–Ingold–Prelog convention.
[f] Configuration of the remaining enantiomer.

The three enzymes converted the terminal epoxides **1**–**9a** with different rates and enantioselectivities. In general, HheA and HheB catalysed their reactions with lower enantioselectivity, but for both enzymes it was observed that enantioselectivity rises with increasing bulkiness of the substituent, although the activity drops (Table 1, entries 1, 4, 5 and 10, 13, 14). The influence of bulkiness is more evident for HheA, where an increase of enantioselectivity was observed from $E = 4$ for **1a**, to $E = 8$–9 for **4a** and **6a**, reaching $E = 109$ for substrate **5a**, which has the bulkiest substituent. Most of the tested substrates were converted with low enantioselectivity by HheB. The highest *E* values ($E = 10$) were obtained with substrates having a longer chain (entries 11 and 12), or a bulky substituent (entry 14).

The results show that HheC is the most selective among the tested enzymes. Previously this enzyme showed a high selectivity in azide- and nitrite-mediated ring opening reactions of aliphatic and aromatic epoxides.[4–6] Whereas its selectivity towards monosubstitut-

ed aliphatic epoxides varied from moderate to high (entries 19–25), the enzyme showed a enhanced enantioselectivity with 2,2-disubstituted epoxides (entries 26 and 27). For monosubstituted epoxides its initial activity significantly dropped with increasing length of alkyl substituent (initial activity for R = Et > *n*-Bu > *n*-Hex, entries 19–21), as well as with branching (initial activity for R = Et > *i*-Pr > *t*-Bu, entries 19, 22, 23). Branching of the alkyl substituent did not increase enantioselectivity, except for substrate **6a**, which bears a cyclohexyl substituent. The enantioselectivity of HheC towards **6a** is the highest among the monosubstituted epoxides (E = 106), with no loss of activity (entry 24).

Introduction of a methyl group as a second substituent at the chiral centre led to a huge increase in enantioselectivity with E values above 100 for **8a** (compare entries 25 and 26) and **9a** (compare entries 19 and 27). A similar enhanced selectivity with disubstituted epoxides was reported for some bacterial epoxide hydrolases.[15] The 2,2-disubstituted epoxides turned out to be the best substrates for HheC with regard to enantioselectivity and these conversions yielded highly optically enriched product (ee 99%). The high enantioselectivity towards 2,2-disubstituted oxiranes (entries 26 and 27) and the lower activity of HheC toward substrates with larger substituents (entries 20, 21, and 23) disclose HheC as a biocatalyst suitable for the resolution of epoxides bearing a fully substituted chiral centre with small substituents. In this way, enantiomerically pure tertiary alcohols containing a synthetically useful nitrile group can be obtained.

Absolute configurations of the nitriles produced by halohydrin dehalogenases were determined using chemically prepared standard compounds of known configuration. We found that the stereochemical course of the enzymatic reactions mostly depends on the biocatalyst and less on the substrate structure. The (*S*)-enantiomers were transformed more rapidly in reactions catalysed by HheA and HheB. However, for HheB a vinyl substituent (**7a**, **8a**) turns the enantiopreference in the opposite direction (entries 16 and 17), while for HheA there was an inversion of selectivity for 2,2-disubstituted epoxides (**8a**, **9a**) as well as for **6a** (entries 6, 8, and 9). The stereopreference of HheC was found to be opposite to that of the A- and B-type enzymes. For all tested substrates, except **5a**, the (*R*)-enantiomer was converted first. This (*S*)-enantiopreference with **5a** is the only example of reversed enantioselectivity for HheC. With all other substrates tested in this study and in all our previous work with other nucleophiles[4–6] HheC behaved as an (*R*)-selective enzyme. The steric properties of substrate **5a** are probably responsible for this inversion of stereopreference.

Halohydrin dehalogenases are cofactor-independent enzymes that are easy to prepare from recombinant *E. coli* cells and they show high stability under the incubation conditions used when oxidation of sulfhydryl groups is prevented, which is important because the catalytic activity is not very high (k_{cat} up to about 1 s^{-1}). High substrate concentrations are tolerated (e.g., up to 300 mM for epoxybutane), allowing preparative scale applications of these novel biocatalysts.

Conclusion

Wild-type halohydrin dehalogenases were able to catalyse the cyanide-mediated ring opening reaction of epoxides yielding enantiomerically enriched β-hydroxy nitriles. HheA, HheB and HheC showed different catalytic properties concerning activity, enantioselectivity and enantiopreference. Although HheB had a low enantioselectivity towards aliphatic epoxides, this can be considered as an advantage when only regioselectivity is an issue. Chemical methods[16] for epoxide ring opening usually proceed with low regioselectivity, require a Lewis acid catalyst, high temperatures, a prolonged reaction time, and the use of organic solvents. In contrast, the biocatalytic ring opening with cyanide in water proceeds under mild conditions with high regioselectivity. Therefore, when racemic 3-substituted 3-hydroxy nitriles are the desired products, HheB is the catalyst of choice because of its high regioselectivity, low enantioselectivity, and broad substrate range. HheA shows potential as a biocatalyst for the resolution of epoxides bearing bulky substituents. In spite of its low enantioselectivity towards the majority of the tested substrates, it showed promising results with **5a**, which is an indication that more suitable substrates can be found.

The catalytic properties of HheC strongly differ from those of the other two halohydrin dehalogenases. The enantiopreference of HheC is opposite to that of HheA and HheB. It is an (*R*)-selective enzyme and it shows the highest enantioselectivity among the tested enzymes. The large enhancement of enantioselectivity that is obtained when a second substituent is present on the chiral centre of the epoxide ring is remarkable. The high enantioselectivity toward 2,2-disubstituted epoxides makes HheC a promising biocatalyst for the synthesis of enantiomerically pure tertiary alcohols.

The halohydrin dehalogenase-catalysed production of β-hydroxy nitriles is a C–C bond forming reaction. In general C–C bond forming enzymes are useful tools for the synthesis of important groups of compounds in a chemo-, regio- and enantioselective manner. In this way, enantioselective syntheses of cyanohydrins, β-hydroxy ketones, and α-hydroxy carbonyl compounds were achieved.[17] The range of C–C bond forming enzymes is now expanded with three halohydrin dehalogenases. These halohydrin dehalogenases also complement the biocatalytic scope of hydroxynitrile lyases, since both catalyse formation of products that contain a hydroxy and a nitrile group and that are, therefore, of the same bifunctional nature, but halohydrin dehalo-

genases yield β-cyanohydrins whereas hydroxynitrile lyases yield α-cyanohydrins.[18]

Experimental Section

General

^{1}H and ^{13}C NMR spectra were recorded on Varian 300 (75.48 MHz) or Varian 400 (100.00 MHz) spectrometer in $CDCl_3$. Chemical shift values are denoted in δ values (ppm) relative to residual solvent peak ($CHCl_3$, ^{1}H δ = 7.26, ^{13}C δ = 77.16). Optical rotations were measured on a Polartronic MH8 (Schmidt + Haensch) polarimeter. Gas chromatography (GC) was performed on Shimadzu GC-17A and Hewlett-Packard 6890 gas chromatographs equipped with FID detectors using He as a carrier gas. Enzymatic reactions were monitored using a HP-1 column (60 m × 0.25 mm × 0.25 μm, Agilent). Optical purities were determined using a Chiraldex G-TA column (30 m × 0.25 mm × 0.25 μm, Astec). Concentrations of epoxides and hydroxynitriles were derived from calibration curves.

The commercially available substrates 1,2-epoxybutane (**1a**), 1,2-epoxyoctane (**3a**), cyclopentene oxide (**13a**), cyclohexene oxide (**14a**) were supplied by Aldrich Chemie, as were the enantiomerically pure (*R*)-**1a**, (*R*)-**2a** and (*R*)-**3a**. 1,2-Epoxyhexane (**2a**), 2-vinyloxirane (**7a**), (*R*)-**7a** and *trans*-2,3-epoxybutane (**10a**) were acquired from Fluka. 1,2-Epoxy-3-methylbutane (**4a**), 3,3-dimethyl-1,2-epoxybutane (**5a**) were purchased from Lancaster and 1,2-epoxy-2-methylbutane (**9a**), *cis*-2,3-epoxybutane (**11a**), 2,3-epoxy-2-methylbutane (**12a**) from Acros Organics.

Enzymes were prepared from recombinant *E. coli* cells expressing the *hheA*, *hheB*, or *heeC* genes according to a previously described protocol.[1]

Synthesis of Substrate 6a

To a solution of cyclohexanecarboxaldehyde (2.24 g, 20 mmol) in CH_2Cl_2 (20 mL) trimethylsulphonium methyl sulphate was added (4.4 g, 23.3 mmol). Aqueous NaOH (50%, 10 mL) was added and the reaction mixture was stirred overnight. Water was added and the organic phase was separated. The water phase was extracted twice with CH_2Cl_2. The combined organic phase was washed twice with water, shaken for 20 min with a saturated solution of sodium metabisulphite to remove unreacted aldehyde, and washed twice with water. The organic phase was dried and evaporated. Bulb-to-bulb distillation under reduced pressure (10 mm Hg, 60–70 °C) afforded **6a** as a colourless liquid; yield: 1.3 g (43%). ^{1}H NMR ($CDCl_3$): δ = 1.05–1.29 (6H, m), 1.65–1.74 (4H, m), 1.85–1.88 (1H, m), 2.51 (1H, dt, J_1 = 4.5 Hz, J_2 = 1.0 Hz), 2.70 (2H, m); ^{13}C NMR ($CDCl_3$): δ = 25.8, 25.9, 26.6, 44.3, 29.0, 40.6, 46.3, 57.0 ppm.

Synthesis of Racemic β-Hydroxy Nitriles 1–9b; General Procedure[11]

To a solution of (*i*-Pr)$_2$NH (2.01 mL, 15 mmol) in dry THF (15 mL), a 1.6 M solution of *n*-BuLi in hexanes (8.75 mL, 14 mmol) was added under an inert atmosphere of N_2 at 0 °C. The mixture was stirred for 15 min, cooled to −78 °C, and then a solution of CH_3CN (0.78 mL, 15 mmol) in THF (7.5 mL) was added. After 1 h stirring at −78 °C, a solution of propionaldehyde (0.9 mL, 12.5 mmol) in THF (7.5 mL) was added. After 3 h of stirring at −78 °C, the reaction was quenched with a saturated aqueous solution of NH_4Cl and treated with aqueous 1 M HCl, and the pH was adjusted to neutral. The reaction mixture was extracted with Et_2O. The organic extracts were dried over Na_2SO_4, filtered, and solvent was evaporated. Crude product was purified by column chromatography on silica gel using hexane/ethyl acetate (7:3) as eluent.

Using the same procedure, β-hydroxy nitriles **2**–**9b** were also prepared from the corresponding carbonyl compounds.

3-Hydroxypentanenitrile (1b): ^{1}H NMR ($CDCl_3$): δ = 1.00 (3H, t, J = 7.0 Hz), 1.78 (2H, dq, J_1 = 8.0 Hz, J_2 = 8.0 Hz), 1.94 (1H, bs), 2.48 (1H, dd, J_1 = 17.0 Hz, J_2 = 6.0 Hz), 2.56 (1H, dd, J_1 = 17.0 Hz, J_2 = 5.0 Hz), 3.85–3.90 (1H, m); ^{13}C NMR ($CDCl_3$): δ = 9.9, 25.9, 29.7, 69.8, 118.25.

3-Hydroxyheptanenitrile (2b): ^{1}H NMR ($CDCl_3$): δ = 0.92 (3H, t, J = 7.0 Hz), 1.34–1.48 (4H, m), 1.56–1.63 (2H, m), 2.05 (1H, bs), 2.48 (1H, dd, J_1 = 17.0 Hz, J_2 = 6.0 Hz), 2.56 (1H, dd, J_1 = 17.0 Hz, J_2 = 5.0 Hz), 3.92–3.96 (1H, m); ^{13}C NMR ($CDCl_3$): δ = 14.2, 22.7, 26.3, 26.9, 66.0, 118.1.

3-Hydroxynonanenitrile (3b): ^{1}H NMR ($CDCl_3$): δ = 0.88 (3H, t, J = 7.0 Hz), 1.29–1.46 (8H, m), 1.56–1.61 (2H, m), 1.92 (1H, bs), 2.48 (1H, dd, J_1 = 17.0 Hz, J_2 = 6.0 Hz), 2.56 (1H, dd, J_1 = 17.0 Hz, J_2 = 5.0 Hz), 3.90–3.98 (1H, m); ^{13}C NMR ($CDCl_3$): δ = 14.3, 22.8, 24.6, 26.3, 29.3, 31.9, 36.8, 68.9, 118.2.

3-Hydroxy-4-methylpentanenitrile (4a): ^{1}H NMR ($CDCl_3$): δ = 0.96 (3H, d, J = 7.0 Hz), 0.98 (1H, d, J = 7.0 Hz), 1.81 (1H, dsept, J_1 = 6.5 Hz, J_2 = 6.5 Hz), 2.06 (1H, bs), 2.50 (1H, dd, J_1 = 17.0 Hz, J_2 = 7.0 Hz), 2.55 (1H, dd, J_1 = 17.0 Hz, J_2 = 5.0 Hz), 3.67–3.73 (1H, m); ^{13}C NMR ($CDCl_3$): δ = 17.6, 18.7, 23.9, 33.5, 72.8, 118.6.

3-Hydroxy-4,4-dimethylpentanenitrile (5a): ^{1}H NMR ($CDCl_3$): δ = 1.65 (9H, s), 2.27 (1H, d, J = 5.0 Hz), 2.41 (1H, dd, J_1 = 16.5 Hz, J_2 = 9.5 Hz), 2.54 (1H, dd, J_1 = 6.5 Hz, J_2 = 3.0 Hz), 3.65 (1H, ddd, J_1 = 9.5 Hz, J_2 = 5.0 Hz, J_3 = 3.0 Hz); ^{13}C NMR ($CDCl_3$): δ = 18.7, 21.8, 25.5, 35.2, 75.7, 120.0.

3-Cyclohexyl-3-hydroxypropionitrile (6a): ^{1}H NMR ($CDCl_3$): δ = 0.98–1.31 (5H, m), 1.43–1.54 (1H, m), 1.61–1.90 (5H, m), 2.05 (1H, d, J = 5.0 Hz), 2.50 (1H, dd, J_1 = 16.5 Hz, J_2 = 6.5 Hz), 2.58 (1H, dd, J_1 = 16.5 Hz, J_2 = 5.0 Hz), 3.64–3.73 (1H, m); ^{13}C NMR ($CDCl_3$): δ = 23.8, 25.9, 26.1, 26.4, 28.1, 29.2, 43.3, 72.2, 118.5.

3-Hydroxy-4-pentenenitrile (7a): ^{1}H NMR ($CDCl_3$): δ = 2.20 (1H, d, J = 4.5 Hz), 2.58 (1H, dd, J_1 = 17.0 Hz, J_2 = 6.0 Hz), 2.64 (1H, dd, J_1 = 17.0 Hz, J_2 = 5.0 Hz), 4.45–4.48 (1H, m), 5.31 (1H, d, J = 10.0 Hz), 5.41 (1H, d, J = 17 Hz), 5.93 (1H, ddd, J_1 = 17.0 Hz, J_2 = 10.0 Hz, J_3 = 6.0 Hz); ^{13}C NMR ($CDCl_3$): δ = 26.3, 68.8, 117.5, 117.9, 135.6.

3-Hydroxy-3-methyl-4-pentenenitrile (8a): ^{1}H NMR ($CDCl_3$): δ = 0.97 (3H, t, J = 7.5 Hz), 1.35 (3H, s), 1.63–1.68 (3H, m), 2.50 (1H, s), 2.51 (1H, s); ^{13}C NMR ($CDCl_3$): δ = 8.3, 26.3, 31.1, 34.4, 71.6, 118.0.

3-Hydroxy-3-methylpentanenitrile (9a): ^{1}H NMR ($CDCl_3$): δ = 0.97 (3H, t, J = 7.5 Hz), 1.35 (3H, s), 1.63–1.68 (2H, m), 2.51 (2H, d, J = 1.0 Hz); ^{13}C NMR ($CDCl_3$): δ = 8.3, 26.3, 31.0, 34.4, 71.5, 118.0.

Table 2. Chiral GC analysis of epoxides and β-hydroxy nitriles.

Compound	Conditions	Retention time [min]	
		Epoxide (**a**)	β-Hydroxy nitrile (**b**)
1	40 °C 4 min, 10 °C/min to 120 °C, 15 min at 120 °C	3.9 (*R*)/4.1 (*S*)	22.0 (*R*)/23.8 (*S*)
2	60 °C 7 min, 15 °C/min to 150 °C, 5 min at 150 °C	4.1 (*S*)/4.3 (*R*)	15.1 (*R*)/15.4 (*S*)
3	40 °C 50 min, 15 °C/min to 150 °C, 5 min at 150 °C	52.7 (S)/52.8 (*R*)	66.7 (*R*)/67.1 (*S*)
4	40 °C 4 min, 10 °C/min to 150 °C, 5 min at 150 °C	4.7 (*R*)/4.9 (*S*)	16.8 (*S*)/17.1 (*R*)
5	40 °C 4 min, 10 °C/min to 120 °C, 15 min at 120 °C	6.2 (*R*)/6.5 (*S*)	26.0 (*S*)/26.5 (*R*)
6	60 °C 7 min, 15 °C/min to 170 °C, 10 min at 170 °C	11.8 (*R*)/11.9 (*S*)	21.4 (*S*)/21.7 (*R*)
7	40 °C 5 min, 10 °C/min to 150 °C, 3 min at 150 °C	3.4 (*S*)/3.5 (*R*)	16.3 (*S*)/16.7 (*R*)
8	40 °C 5 min, 15 °C/min to 135 °C, 5 min at 135 °C	4.2 (*R*)/4.6 (*S*)	15.2 (*S*)/15.6 (*R*)
9	40 °C 5 min, 15 °C/min to 135 °C, 5 min at 135 °C	4.3 (*R*)/4.6 (*S*)	15.1 (*R*)/15.3 (*S*)

Kinetic Resolution of Epoxides

To 20 mL of Tris-SO_4 buffer (0.2 M, pH 7.5) containing epoxide (0.1 mmol, 5 mM) and DMSO (100 μL, 0.5%) purified enzyme was added (0.5 mg, final concentration *ca.* 0.8 μM). Because of the low solubility of **3a** and **14a**, they were used as 2 mM solutions. Enzymatic reactions were performed at ambient temperature (22 °C). The progress of the reaction was followed by periodically taking samples (0.5 mL) from the reaction mixture. Samples were extracted with diethyl ether (1 mL) containing an internal standard (1-chlorohexane or mesitylene), dried over Na_2SO_4 and analysed by GC. The conversion of epoxides was monitored on an achiral column. Non-enzymatic ring opening was determined by monitoring β-hydroxy nitrile formation in the absence of enzyme. The enantiomeric excess (ee) of the remaining epoxides was determined by chiral GC under conditions described in Table 2. All separations were carried out on a Chiraldex G-TA column.

Absolute Configurations

Absolute configurations were assigned by chiral GC analysis using reference compounds. Commercially available were optically pure (*R*)-**1a**, (*R*)-**2a**, (*R*)-**3a** and (*R*)-**7a**. Enantiomerically enriched epoxides (*S*)-**5a**, (*S*)-**8a** and (*S*)-**9a** were prepared by Sharpless asymmetric dihydroxylation[19] of 3,3-dimethyl-1-butene, isoprene, or 2-methyl-1-butene followed by ring closure (TsCl, NaH, THF).[20] Their absolute configurations (*S*) were assigned by comparison of the optical rotations of the formed diols with literature data.[21,22] Enantiomerically enriched β-hydroxy nitriles (*S*)-**4b** and (*S*)-**6b** were obtained by lipase-catalysed resolution of racemic alcohols using *Pseudomonas cepacia* lipase (Amano). Their (*S*) absolute configuration was assigned by comparison of the optical rotations of the formed acetates with literature data.[11]

Synthesis of (*S*)-5a, (*S*)-8a and (*S*)-9a; General Procedure

According to Sharpless et al.[19] enantiomerically enriched diols were prepared using AD-mix-α, starting from 3,3-dimethyl-1-butene, isoprene, or 2-methyl-1-butene (Fluka). Their absolute configurations were assigned on the basis of optical rotations. Specific optical rotation values are: (*S*)-3,3-dimethylbutane-1,2-diol $[\alpha]_D$: +13.9 (*c* 1.25 $CHCl_3$), Lit.[21] (*R*) $[\alpha]_D$: −23.4 (*c* 0.76 $CHCl_3$); (*S*)-2-methyl-butane-1,2-diol $[\alpha]_D$: −3.9 (*c* 2.89 $CHCl_3$), Lit.[22] (*R*) $[\alpha]_D$: +8.7 (*c* 1.35 $CHCl_3$). From isoprene, a mixture of two diols was obtained, they were not separated but used as a mixture. The absolute configuration of (*S*)-2-methylbut-3-ene-1,2-diol was assigned by analogy to the stereochemistry of dihydroxylation of 3,3-dimethyl-1-butene and 2-methyl-1-butene. Diols were cyclised to (*S*)-**5a** (ee 63%), (*S*)-**8a** (ee 8%) and (*S*)-**9a** (ee 50%) according to a literature procedure.[20] Since these epoxides are highly volatile, they were not isolated but used as ethereal solutions.

Synthesis of (*S*)-4b and (*S*)-6b; General Procedure

Racemic alcohols **4b** or **6b** (200 mg) were dissolved in vinyl acetate (20 mL) and hexane (100 mL). The reaction was initiated by the addition of *Pseudomonas cepacia* lipase (200 mg). The progress of the reaction was followed by GC and reactions were terminated when *ca.* 50% conversion to acetate was reached. Enzyme was filtered off, solvent was evaporated, and crude product was purified on silica gel using hexane/ethyl acetate (9:1) as eluent. The enantiomerically enriched (*R*)-acetates and (*S*)-alcohols (*S*)-**4b** (ee 61%) and (*S*)-**6b** (ee 90%) were isolated. Specific optical rotation values are: (*R*)-1-(cyanomethyl)-2-methylpropyl acetate $[\alpha]_D$: −60 (*c* 0.75 $CHCl_3$), Lit.[11] (*R*) $[\alpha]_D$: −52.2 (*c* 0.76 $CHCl_3$); (*R*)-2-cyano-1-cyclohexylethyl acetate $[\alpha]_D$: −32 (*c* 0.85 $CHCl_3$), Lit.[11] (*R*) $[\alpha]_D$: −31.4 (*c* 0.52 $CHCl_3$).

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