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Published in:
ChemInform

DOI: 10.1002/adsc.200700146

Enzyme-Catalyzed Nucleophilic Ring Opening of Epoxides for the Preparation of Enantiopure Tertiary Alcohols

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Received: March 21, 2007; Revised: July 11, 2007

Abstract: The halohydrin dehalogenase from *Agrobacterium radiobacter* AD1 (HheC) catalyzes nucleophilic ring opening of epoxides with cyanide and azide. In the case of 2,2-disubstituted epoxides, this reaction proceeds with excellent enantioselectivity (E values up to >200), which gives, by kinetic resolution, access to various enantiopure epoxides and β-substituted tertiary alcohols (*ee* up to 99%). Since the enzyme has a broad substrate range and because these tertiary alcohols are difficult to prepare in other ways, HheC is an attractive biocatalyst for the production of β-cyano and β-azido tertiary alcohols.

Keywords: azides; cyanides; epoxides; halohydrin dehalogenase; kinetic resolution; tertiary alcohols.

Introduction

The asymmetric ring opening of epoxides catalyzed by chiral metal complexes is an important synthetic strategy for the preparation of valuable chiral building blocks, and a variety of nucleophiles have been employed in these reactions.[1] For the enzymatic ring opening of epoxides, halohydrin dehalogenases have recently emerged as a biocatalyst that may complement the use of organometallic catalysts.[2] In the forward direction, halohydrin dehalogenases catalyze the conversion of vicinal halohydrins to the corresponding epoxides, a reaction that is important for the biodegradation of some halogenated xenobiotic compounds.[3] In the reverse of this reaction, the enzymes can accept a variety of alternative anionic nucleophiles such as N_3^-, NO_2^-, and CN^-, to open the epoxide ring.[4,5] Depending on the nature of the epoxide, these reactions proceed with good rate and high enantioselectivity, providing a route to enantioenriched products.[6,7] The structure of the halohydrin dehalogenase from *Agrobacterium radiobacter* strain AD1 (HheC) has been solved.[8] A recent study indicates that the performance of this enzyme for the synthesis of the important statin side chain building block (*R*)-methyl 4-cyano-3-hydroxybutyrate can be improved by directed evolution.[9] Mutants with enhanced and reduced enantioselectivity have been described as well.[10]

Our previous work on ring opening of epoxides indicated that the halohydrin dehalogenase from this organism could be a suitable catalyst for the resolution of epoxides with azide[6] or cyanide[7] as a nucleophile. Since, in the case of 2,2-disubstituted epoxides, the products of such conversions are β-cyano tertiary alcohols, HheC was considered as a biocatalyst for the preparation of these alcohols in enantiopure form. Chiral tertiary alcohols are important building blocks for various optically active products, but methods for their preparation are limited compared to procedures that yield the more easily available primary and secondary alcohols.[11] A frequently used biocatalytic method for the resolution of racemic alcohols employs the enantioselectivity of lipases.[12] However, most lipases do not accept tertiary alcohols, and consequently there are only a few examples of their application for resolving enantiomers of these compounds.[13] Recently, esterase mutants were reported to catalyze the hydrolysis of several acetates of tertiary alcohols with excellent enantioselectivites.[14] Some enantiospecific epoxide hydrolases are also able to hydrolyze 2,2-disubstituted epoxides, yielding diols containing a *tert*-alcohol moiety.[15]

Here we report the use of halohydrin dehalogenase in a new biocatalytic approach to optically pure terti-

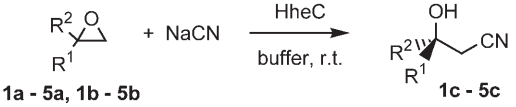
ary alcohols bearing synthetically useful groups at the β-position.

Results and Discussion

Production of Tertiary β-Cyanohydrins with HheC

To explore the applicability of HheC for the production of optically active β-cyano tertiary alcohols, we studied the conversion of several 2,2-disubstituted epoxides (**1b–5b**) and compared it with the conversion of monosubstituted epoxides (**1a–5a**) (Table 1). Each substrate was tested for enantioselective ring opening using HheC as the catalyst and cyanide as the nucleophile. The resolution of monosubstituted epoxides (**1a**–**5a**) proceeded with varying degrees of enantioselectivity ($E = 2$–106). In all cases, the (*R*)-enantiomer was preferentially converted, except for **4b**, in which case there is a change in priority due to the ester substituent. The conversion of all substrates with an additional 2-methyl substituent (**1b**–**5b**) occurred with a much higher enantioselectivity ($E > 200$). In the monosubstituted series, enantioselectivity was dependent on the R^1 substituent of the epoxide, and the highest *E* value was obtained with **2a**. In contrast, when the additional 2-methyl substituent was present (R^2), HheC displayed a high *E* value (>200) independent of the nature of the R^1 substituent, which could be an alkyl, aryl, or ester group.

Table 1. Kinetic resolution of 2,2-disubstituted (**1b**–**5b**) and corresponding monosubstituted epoxides (**1a**–**5a**) catalyzed by halohydrin dehalogenase.[a]



1a - 5a, 1b - 5b 1c - 5c

Substrate	R^1	R^2	E[b]	Configuration[c]
1a[d]	Et	H	29	*R*
1b[d]	Et	Me	>200	*R*
2a[d]	*c*-Hex	H	106	*R*
2b	*c*-Hex	Me	>200	*R*
3a	CH_2Ph	H	2	*R*
3b	CH_2Ph	Me	>200	*R*
4a	CO_2Me	H	n.d.[f]	-
4b	CO_2Me	Me	>200	*S*[g]
5a[e]	CH_2CO_2Me	H	15	*R*
5b	CH_2CO_2Me	Me	>200	*R*

[a] *General conditions:* 5 mM epoxide in 10 mL Tris-SO_4, pH 7.5, 15 mM NaCN, 0.9 or 3.5 μM HheC.
[b] E values were calculated from ee_p and ee_s.
[c] Absolute configuration of the faster reacting enantiomer.
[d] Data from Majerić et al.[7]
[e] Data from Majerić et al.[16]
[f] Not determined due to very low activity.
[g] Change in configuration due to the CIP rule.

Modelling

The remarkable effect of the methyl substituent on enantioselectivity is most striking for epoxide **1b**, which has a methyl and an ethyl group on the chiral centre. This is a rare example of highly efficient chiral discrimination by an enzyme for a substrate that bears substituents with similar electronic properties and with only such as small difference in size. To find a structural explanation for the positive effect of the additional 2-methyl substituent on halohydrin dehalogenase enantioselectivity, substrate **1b** was modelled in the active site of the X-ray structure of HheC. Since no structure of the apoenzyme is known, the HheC model was derived from a structure of the enzyme complexed with (*R*)-*para*-nitrostyrene oxide. In order to mimic the productive complex, the epoxide oxygen and terminal carbon atom of the substrates were positioned in such a way that attack of a nucleophile bound in the halide binding site would be possible. Using these constraints, and avoiding steric conflicts, the (*R*)-enantiomer of molecule **1b** fits rather tightly into the active site pocket with the methyl group pointing into a small bulge bordered by the amino acids Trp139, Asn176 and Thr134 (Figure 1). A bulkier group at this position would lead to steric conflict with those amino acids and thus the (*S*)-form of **1b** cannot bind in a productive manner. The less sterically demanding monosubstituted (*S*)-**1a** can be positioned in a way that is close to the productive conformation of (*R*)-**1b**, which can explain the observation that it is still a weak substrate and that the enantioselectivity with **1a** is low.

In order to verify this interpretation, all (*R*) and (*S*) enantiomers of the epoxides in Table 1 were docked into the binding pocket of HheC using the program GLIDE.[17] The docking solutions were analyzed according the binding of the epoxide ring (Table 2). A solution was considered as productive when the epoxide ring was placed in the orientation described above. Other docking solutions were considered non-productive. In some cases a conformation near the productive conformation was observed. No docking position with the epoxide ring in the epoxide binding site was found for the (*R*) form of **4b**. This epoxide carries an ester group and it was found to become docked in a reverse manner with the ester carbonyl oxygens interacting with Ser132. Although no protein flexibility was taken into account, the results in Table 2 are in agreement with the proposed geometric explanation for the preference of HheC for (*R*)-epoxides.[18]

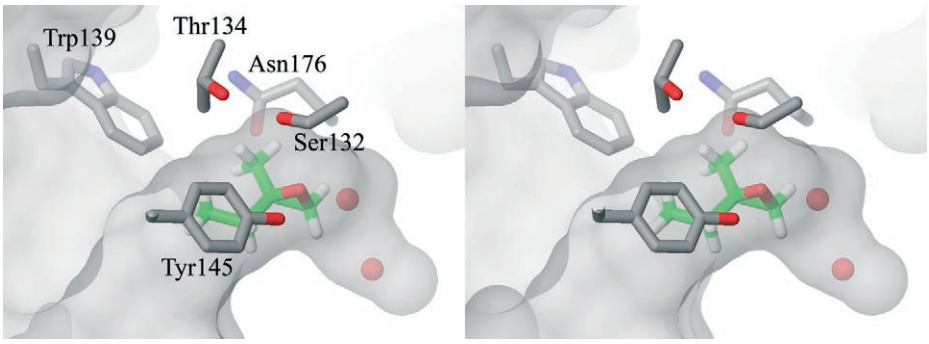


Figure 1. Stereo image of the modelling of (*R*)-**1b** in the active site of halohydrin dehalogenase. The side chains of the two catalytic residues Tyr145 and Ser 132 and the side chains of the amino acids restricting the space for the second substituent are shown. The substrate is shown in green. The red dots show the two water molecules in the proposed binding site of the nucleophile.

Table 2. Docking poses obtained with the docking program GLIDE for the molecules in Table 1.[a]

Substrate	**a** (R^2=H)		**b** (R^2=Me)	
	(*R*)	(*S*)	(*R*)	(*S*)
1	P	~	P	~
2	P	NP	~	NP
3	~	NP	~	NP
4	NP	P	no pose	~
5	P	NP	~	NP

[a] P, productive conformation; NP, non-productive conformation; ~, conformation close to productive.

Azide and Cyanide as Nucleophiles

To further explore the potential of halohydrin dehalogenase for producing enantiopure β-substituted *tert*-alcohols, we tested a series of 2,2-disubstituted epoxides with azide and cyanide. As illustrated in Table 3, HheC catalyzed the formation of a wide range of *tert*-alcohols both with cyanide and with azide ions.

All these substrates were hardly sensitive towards the non-enzyme-catalyzed ring opening reaction with cyanide under the conditions used. The same was observed in our previous work.[7] The insignificance of the chemical conversion of the 2,2-disubstituted epoxides to β-cyano alcohols was reflected in the very high product *ee*s ($\geq$ 99%). Although azide is a stronger nucleophile than cyanide and non-enzyme-catalyzed azidolysis was suspected to be faster than with cyanide, spontaneous azidolysis was also much slower than the enzyme-catalyzed reaction and again had no effect on optical purity of the product. The only exception was the transformation of **4b** where due to the low enzyme activity the spontaneous ring opening reaction played a significant role, reflected in the lower *ee* (90%) of product **4d**.

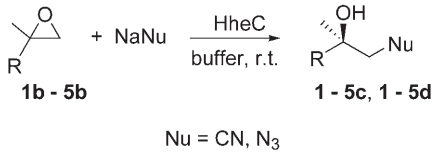
All HheC-catalyzed reactions of 2,2-disubstituted epoxides proceeded with very high enantioselectivity and regioselectivity, yielding products with *ee* $\geq$99%, regardless of the nature of the larger 2-substituent (R^1). The conversions proceeded with retention of the absolute configuration at the chiral oxirane carbon. Variations in the electronic or steric nature of the R^1 substituent thus had little influence on the enantioselectivity and no effect on the stereopreference of the reactions. However, the reaction rate strongly depended on the nucleophile and with all epoxides the highest rate was found with azide. Enzyme activity was also dependent on the substrate and low activity in some cases may be a disadvantage for the synthetic utility of the biotransformation. This problem may be possibly be overcome by developing mutants with increased activity.[9,10]

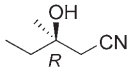
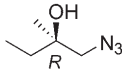
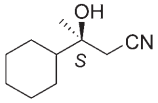
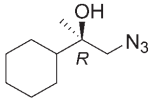
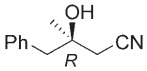
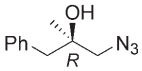
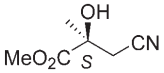
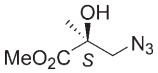
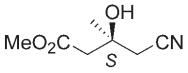
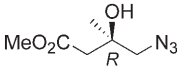
To demonstrate the synthetic usefulness of the described biocatalytic transformations, a preparative scale conversions were carried out (Table 4). In a typical experiment, 300 mg of **2b** was processed with 0.5 equivs. of NaN_3 and 9 mg of purified halohydrin dehalogenase in 40 mL Tris-SO_4 buffer at room temperature. After 2.5 h product (*R*)-**2d** was isolated from the incubation mixture in 41% yield and very high *ee*. Similarly, a reaction with NaCN was performed. More enzyme and a longer reaction time were required to achieve comparable conversion and to extract and purify enantiopure (*R*)-**2c** in 40% yield (Table 4). Both with NaCN and NaN_3 addition of 0.5 equivs. was enough to obtain *ca.* 45% conversion of epoxide, with no reduction of product *ee* due to chemical reaction of epoxide with nucleophile. Also tertiary alcohol **5c** could be isolated in highly enantioenriched form and good yield after extraction and chromatography.

Conclusions

Halohydrin dehalogenase was applied for the kinetic resolution of racemic 2,2-disubstituted epoxides. By

Table 3. Kinetic resolution of 2,2-disubstituted epoxides (**1b–5b**) catalyzed by HheC on an analytical scale.[a]



Substrate	Nu	Product		t [h]	Conversion [%][c]	ee_s [%][c]	ee_p [%][c]
1b	CN		**1c**	5	34	51	99
1b	N_3		**1d**	0.5	50	>99	>99
2b	CN		**2c**	5	47	88	>99
2b	N_3		**2d**	1.5	45	82	>99
3b	CN		**3c**	5	30[b]	42	99
3b	N_3		**3d**	2	47	89	>99
4b	CN		**4c**	5	21[b]	26	>99
4b	N_3		**4d**	5	29[b]	40	90
5b	CN		**5c**	3	47	87	>99
5b	N_3		**5d**	0.5	47	89	>99

[a] *General conditions:* 5 mM epoxide in 10 mL Tris-SO_4, pH 7.5, 15 mM NaCN or 7.5 mM NaN_3, 0.9 μM HheC.
[b] 3.5 μM of HheC.
[c] Determined by GC or HPLC analysis.

nucleophilic ring opening, optically pure tertiary alcohols were obtained in good yield at low nucleophile concentration, in aqueous medium and at room temperature. These products contain a synthetically useful cyano or azido group at the β-position and can give access to a variety of building blocks in enantiopure form. The results obtained open a new biocatalytic way for the preparation of enantiomerically pure tertiary alcohols.

Experimental Section

General Remarks

^1H and ^{13}C NMR spectra were recorded on a Varian 400 (100 MHz) spectrometer in $CDCl_3$. Chemical shift values are denoted in δ values (ppm) relative to residual solvent peaks ($CHCl_3$, ^1H $\delta = 7.26$, ^{13}C $\delta = 77.16$). Enzymatic reactions were monitored by GC or HPLC using commercially available chiral columns. GC analysis was performed on a Hewlett–Packard 6890 series gas chromatograph equipped with an FID detector (set at 225 °C), a split injector (set at 225 °C), using N_2 as a carrier gas and an autosampler. HPLC analysis was performed on a Merck L-6200 A instrument with UV detection at 254 nm. Column chromatography was done using silica gel, Merck type 9385 (230–400 mesh). TLC was performed on 0.25 mm silica gel 60-F plates, Merck.

Table 4. Preparative-scale kinetic resolutions of epoxides with cyanide or azide as the nucleophile.

Substrate	t [h]	Conv. [%][a]	Epoxide Yield [%][b]	Epoxide ee_s [%]	*tert*-Alcohol	*tert*-Alcohol Yield [%][b]	*tert*-Alcohol ee_p [%]
2b	24	42	52	71 (*S*)	**2c**	40	>99 (*S*)
2b	2.5	43	41	76 (*S*)	**2d**	41	>99 (*R*)
5b	2	45	37	81 (*S*)	**5c**	35	>99 (*S*)

[a] Determined by GC analysis.
[b] Isolated yield.

Bulb-to-bulb distillation was performed using a glass oven B-585 instrument from Büchi.

The commercial grade reagents and solvents were used without further purification. Racemic 2-methyl-1,2-epoxybutane (**1b**) was purchased from Acros Organics. The compounds 1,2-epoxybutane (**1a**), 2,3-epoxypropylbenzene (**3a**), methyl 2-methylglycidate (**4b**) and (*R,R*)-*N,N'*-bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexanediaminochromium(III) chloride were supplied by Aldrich. The epoxides 2-cyclohexyl-2-methyloxirane (**2b**) and 2-benzyl-2-methyloxirane (**3b**) were prepared from corresponding ketone using trimethylsulfonium methyl sulfate (Aldrich). Epoxide **4a** was prepared by oxidation of the methyl acrylate with NaOCl. Epoxide **5a** was prepared by reduction of methyl 4-chloroacetoacetate with $NaBH_4$, followed by ring closure to methyl 3,4-epoxybutyrate as described.[19] Methyl 2-methyloxirane acetate (**5b**) was prepared starting from 3-methyl-3-butenol. In the first step the alcohol was oxidized by Jones reagent, followed by esterification and finally epoxidation of methyl 3-methyl-3-butenoate by *m*-CPBA. Racemic alcohols **1c–5c** and **1d–5d** were prepared by ring opening reactions of the corresponding epoxides with sodium cyanide or sodium azide in water.

Halohydrin dehalogenase from *Agrobacterium radiobacter* AD1 (HheC, wild-type enzyme, acc. no. AAK92099) was produced in recombinant form in *E. coli* cultivated in LB medium. The enzyme was purified from sonicated cells by column chromatography as described before.[20]

General Procedure for Enzymatic Reactions on Analytical Scale

Enzymatic reactions were performed at ambient temperature (22 °C). Substrate (0.054 mmol, final concentration 5 mM) was added from a stock solution in DMSO (50 μL, 0.5%) to Tris-SO_4 buffer (10 mL, 0.2 M, pH 7.5), followed by addition of a stock solution in water of NaCN (0.75 mL, 162 mmol, final concentration 15 mM) or NaN_3 (0.75 mL, 80 mmol, final concentration 7.5 mM). Reactions were initiated by addition of enzyme (0.25 or 1.0 mg) to a final concentration of 0.9 or 3.5 μM. The progress of the reaction was followed by periodically taking samples (1 mL) from reaction mixture. These were extracted with diethyl ether (1 mL) containing an internal standard (1-chlorohexane or mesitylene), dried over Na_2SO_4 and analyzed by GC or HPLC.

Preparative-Scale Enzymatic Reactions

Pure epoxides **2b** or **5b** were dissolved in Tris-SO_4 buffer, followed by addition of NaCN or NaN_3, and purified enzyme. The mixture was stirred at room temperature and monitored by gas chromatography (GC). The reaction was stopped at conversion <45% and the mixture was extracted with ethyl acetate. The organic phase was dried over Na_2SO_4 and evaporated. The crude product was chromatographed on a silica gel using pentane/CH_2Cl_2 (4:6) as eluent yielding pure alcohols. The NMR data were identical with synthesized racemic reference compounds.

(*S*)-3-Cyclohexyl-3-hydroxybutyronitrile (2c): The reaction was carried out in 25 mL Tris-SO_4 buffer (200 mM, pH 7.5), for 24 h following the general procedure (42% conversion) using 200 mg (1.42 mmol) of racemic **2b**, 35 mg (0.71 mmol) of NaCN and 9 mg of halohydrin dehalogenase (HheC in 1.2 mL buffer). After extraction and purification by chromatography pure (*S*)-**2b** (105 mg, 52%, *ee* 71%) and (*S*)-**2c** (95 mg, 40%, *ee* >99%) were isolated. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.98–1.33 (m, 5H), 1.30 (s, 3H), 1.50 (m, 1H), 1.60–1.83 (m, 6H), 2.52 (dd, J_1 = 16.5 Hz, J_2 = 1.0 Hz, 1H), 2.55 (d, J = 16.5 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 23.9, 26.1, 26.2, 26.3, 26.8, 27.5, 29.6, 47.1, 73.1, 117.9.

(*R*)-1-Azido-2-cyclohexylpropan-2-ol (2d): The reaction was carried out for 2.5 h in 40 mL Tris-SO_4 buffer (200 mM, pH 7.5), following the general procedure (43% conversion) using 300 mg (2.14 mmol) of racemic **2b**, 69 mg (1.06 mmol) NaN_3 and 9 mg of halohydrin dehalogenase (HheC in 280 μL buffer). After extraction and purification by chromatography pure (*S*)-**2b** was isolated (155 mg, 51%, *ee* 76%) and (*R*)-**2d** (163 mg, 41%, *ee* >99%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.92–1.29 (m, 5H), 1.11 (s, 3H), 1.46 (tt, J_1 = 12.0 Hz, J_2 = 3.0 Hz, 1H), 1.65–1.86 (m, 6H), 3.25 (d, J = 12.0 Hz, 1H), 3.36 (d, J = 12.1 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 21.8, 26.7, 26.8, 26.9, 26.9, 28.1, 45.8, 59.9, 75.1.

(*S*)-Methyl 4-cyano-3-hydroxy-3-methylbutanoate (5c): The reaction was carried out in 30 mL Tris-SO_4 buffer (200 mM, pH 7.5), following the general procedure for 2 h (45% conversion) using 300 mg (2.14 mmol) of racemic **5b** (200 mg, 1.53 mmol), 38 mg (0.77 mmol) of NaCN and 15 mg of halohydrin dehalogenase (HheC in 280 μL buffer). After extraction and purification by chromatography pure (*S*)-**5b** (75 mg, 37%, *ee* 81%) and (*S*)-**5c** (84 mg, 35%, *ee* >99%) were obtained. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.43 (s, 3H), 2.63 (d, J = 16.5 Hz, 1H), 2.65 (s, 2H), 2.71 (d, J = 16.5 Hz, 1H), 3.75 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 27.3, 30.9, 43.6, 52.4, 69.5, 117.3, 172.6.

Determination of Enantiomeric Purity

Enantiomeric excesses (*ee*) were determined by chiral GC analysis on the Chiraldex G-TA column (30 m × 0.25 mm × 0.25 μm) (Column I), or HPLC analysis on Chiralpak AS-H column (25 × 0.46 cm) (Column II) (Table 5) or as described earlier.[7] Compounds are defined in Table 1, except 3-hydroxy-4-phenylbutyronitrile (**3a′**) and methyl 4-cyano-3-hydroxybutanoate (**5a′**).

Table 5. Chiral analysis of epoxides, cyano alcohols and β-azido alcohols.

Compound	Column[a]	Conditions	R_t [min]
1d	I	40 °C 5 min, 10 °C min^{-1} to 100 °C, 7 min at 100 °C	16.1 (*R*)/16.3 (*S*)
2b	I	100 °C 8 min, 15 °C min^{-1} to 170 °C, 6 min at 170 °C	7.0 (*R*)/7.6 (*S*)
2c	I	100 °C 8 min, 15 °C min^{-1} to 170 °C, 6 min at 170 °C	17.4 (*S*)/17.8 (*R*)
2d	I	100 °C 10 min, 10 °C min^{-1} to 150 °C, 5 min at 170 °C	18.5 (*R*)/18.7 (*S*)
3a	I	100 °C 13 min, 15 °C min^{-1} to 170 °C, 10 min at 170 °C	12.5 (*R*)/12.8 (*S*)
3a′	I	100 °C 13 min, 15 °C min^{-1} to 170 °C, 10 min at 170 °C	24.9 (*R*)/25.3 (*S*)
3b	I	100 °C 5 min, 10 °C min^{-1} to 150 °C, 16 min at 150 °C	8.6 (*R*)/8.7 (*S*)
3c	I	100 °C 5 min, 10 °C min^{-1} to 150 °C, 16 min at 150 °C	24.3 (*R*)/24.6 (*S*)
3d	II	2-PrOH/heptane (2:98), 1 mL min^{-1}	11.2 (*S*)/12.1 (*R*)
4a	I	100 °C 8 min, 15 °C min^{-1} to 170 °C, 3 min at 170 °C	5.1/6.6
4b	I	100 °C 8 min, 15 °C min^{-1} to 170 °C, 3 min at 170 °C	4.7 (*S*)/5.3 (*R*)
4c	I	100 °C 8 min, 15 °C min^{-1} to 170 °C, 3 min at 170 °C	13.9 (*S*)/14.6 (*R*)
4d	I	100 °C 8 min, 15 °C min^{-1} to 170 °C	11.5 (*S*)/12.1 (*R*)
5a	I	100 °C 8 min, 15 °C min^{-1} to 170 °C, 10 min at 170 °C	6.9 (*R*)/8.3 (*S*)
5a′	I	100 °C 8 min, 15 °C min^{-1} to 170 °C, 10 min at 170 °C	15.0 (*S*)/15.4 (*R*)
5b	I	100 °C 6 min, 15 °C min^{-1} to 170 °C, 5 min at 170 °C	5.5 (*R*)/5.9 (*S*)
5c	I	100 °C 6 min, 15 °C min^{-1} to 170 °C, 5 min at 170 °C	12.2 (*S*)/12.5 (*R*)
5d	I	100 °C 6 min, 15 °C min^{-1} to 150 °C, 5 min at 150 °C	10.7 (*R*)/11.0 (*S*)

[a] Column I (Chiraldex G-TA); Column II (Chiralpak AS-H).

Determination of Absolute Configuration

Absolute configurations were assigned by chiral GC analysis using reference compounds. In the case of epoxides **3a** and **3b** assignment was based on previously reported data.[21] The enantiomerically enriched epoxides (*R*)-**2b**, (*S*)-**4b**, (*R*)-**5a** and (*R*)-**5b** were prepared by (*R,R*)-(salen)CrCl catalyzed ring opening with $TMSN_3$ according to Label and Jacobsen.[22]

Calculation of Enantioselectivity

E values were calculated from ee_p and ee_s according to formula $E = \ln[(1-ee_s)/(1+ee_s/ee_p)]/\ln[(1+ee_s)/(1+ee_s/ee_p)]$.

Molecular Modelling

For modelling of substrate binding to HheC, we used the enzyme complexed with (*R*)-*para*-nitrostyrene epoxide (PDB code: 1ZMT) into which molecule **1b** was manually positioned using the program MAESTRO 7.5 (Schrödinger, LLC, New York) after removal of the substrate. The oxygen of the epoxide ring was positioned within hydrogen bonding distance of the OH groups of the catalytic residues Tyr145 and Ser132. The terminal carbon atom was placed in the direction of the water molecule that is present in the halide-binding site.

Docking with the (*R*) and (*S*) enantiomers of the molecules listed in Table 1 in the active site of HheC was done with the program GLIDE 4.0.[17] The molecules were generated with the Built panel of MAESTRO 7.5 and prepared with Schrödinger's program LigPrep according to the user manual. The protein structure was optimized with the "pprep" procedure using default values. The docking with GLIDE was performed in "xp" mode.

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