Tetrahedron: *Asymmetry* 27 (2016) 930–935

Contents lists available at ScienceDirect

Tetrahedron: *Asymmetry*

journal homepage: www.elsevier.com/locate/tetasy

Azidolysis of epoxides catalysed by the halohydrin dehalogenase from *Arthrobacter* sp. AD2 and a mutant with enhanced enantioselectivity: an (*S*)-selective HHDH

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ARTICLE INFO

Article history:
Received 1 June 2016
Accepted 2 August 2016
Available online 28 August 2016

ABSTRACT

Halohydrin dehalogenase from *Arthrobacter* sp. AD2 catalysed azidolysis of epoxides with high regioselectivity and low to moderate (*S*)-enantioselectivity ($E = 1–16$). Mutation of the asparagine 178 to alanine (N178A) showed increased enantioselectivity towards styrene oxide derivatives and glycidyl ethers. Conversion of aromatic epoxides was catalysed by HheA-N178A with complete enantioselectivity, however the regioselectivity was reduced. As a result of the enzyme-catalysed reaction, enantiomerically pure (*S*)-β-azido alcohols and (*R*)-α-azido alcohols (ee ⩾ 99%) were obtained.

1. Introduction

Halohydrin dehalogenases (HHDHs) are attractive enzymes due to their catalytic promiscuity in catalysis of a number of non-natural reactions.[1] Their natural role is metabolism of vicinal halohydrines to form epoxides. In the reverse reaction, epoxide ring-opening, several non-natural nucleophiles are accepted.[2] HHDH-catalysed conversions have been studied since the late 1960s, however the number and variety of known enzymes is still limited.[3] To date, only bacterial HHDHs have been isolated and characterised. Among them, the best studied is the enzyme from *Agrobacterium radiobacter* AD1 (HheC).[4] It is a homotetrameric protein with 28 kDa subunits and displays high (*R*)-enantioselectivity towards 2,2-disubstituted and aromatic epoxides.[5] HHDH produced by *Arthrobacter* sp. AD2 (HheA) has a 33% amino acid sequence similarity and a similar tertiary and quaternary structure but a much more open active site compared to HheC.[6] The low sequence identities and different active site conformations suggest that enzymes HheA and HheC are considerably different. Although isolated and characterized in 1991,[7] the biocatalytic potential of HheA has not been broadly studied, mostly due to its apparently low enantioselectivity. A low *E*-value was found for the conversion of *para*-nitro-2-bromo-1-phenylethanol to epoxide ($E < 3$), with the slight preference towards (*S*)-enantiomer.[4b] Cyanolysis of a series of structurally different aliphatic epoxides was catalysed by low to moderate (*S*)-enantioselectivity and high regioselectivity.[8] HheA was found to catalyse highly regioselective azidolysis of spiroepoxides again with low enantioselectivity.[9] Based on the aforementioned research, HheA can be considered as a regioselective enzyme, displaying a broad substrate range and low enantioselectivity, slightly preferring (*S*)-enantiomer. Since the only known enantioselective wild-type (WT) HHDH is the HheC of (*R*)-stereopreference, an (*S*)-selective enzyme would be of great interest for application in asymmetric synthesis. Recently, several HheA mutants have been successfully evolved by semirational design.[10] Among them, the N178A variant displayed an outstanding improvement in the kinetic resolution of *rac*-2-chloro-1-phenylethanol ($E > 200$) compared to HheA-WT ($E = 1.7$).

In order to gain more insight into the enantioselectivity of HheA-WT and the mutant N178A in the azidolysis reaction, a variety of epoxides was investigated. Molecular docking analyses were performed to understand the structural basis of the enantioselectivity and regioselectivity of mutant N178A.

2. Results and discussion

2.1. Enantioselectivity of HheA-WT and N178A mutant

Initially, a set of structurally different epoxides **1a–1k** was chosen to test the enantioselectivity of HheA in the azide mediated

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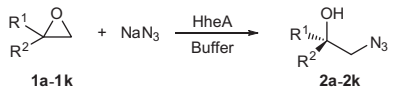
http://dx.doi.org/10.1016/j.tetasy.2016.08.003
0957-4166/

ring-opening reaction (Table 1). The kinetic resolution experiments were performed by using crude extract containing recombinant enzyme prepared from *Escherichia coli* cells. Enzymatic reactions were performed in Tris–SO_4 buffer (pH 7.0) containing 0.5–2% DMSO, NaN_3 (5 mM) and epoxide (5 mM), except **1k** was used in 2 mM concentration due to low aqueous solubility. Reactions were monitored by periodically taking samples from the reaction mixture, followed by extraction and GC or HPLC analysis. HheA showed a broad substrate range by converting all tested epoxides into azido alcohols. The nucleophile attacks at the less hindered carbon of the epoxide moiety resulting in regiospecific reaction. All reactions occurred with low to moderate enantioselectivity (E = 1–16) and a slight (*S*)-preference for the majority of the substrates (Table 1). These results confirmed previous observations of HheA as a biocatalyst displaying poor enantioselectivity and high regioselectivity.[4b,8,9]

Table 1
Kinetic resolution of epoxides **1a**–**1k** catalysed by HheA



1a-1k **2a-2k**

Substrate	R^1	R^2	E-Value[a]	Config.[b]
1a	Et	H	1.5	(*S*)
1b	*n*-Bu	H	9	(*S*)
1c	*i*-Pr	H	7	(*S*)
1d	*t*-Bu	H	10	(*S*)
1e	*c*–Hex	H	2	(*S*)
1f	*n*-Pen	Me	2	(*R*)
1g	CH_2CO_2Me	H	1	(*R*)
1h	$CH_2OCH_2CH{=}CH_2$	H	1	(*R*)[c]
1i	CH_2OPh	H	2	(*S*)[c]
1j	CH_2Ph	H	16	(*S*)
1k	Ph	H	5	(*S*)

[a] E-Values were calculated from ee_p and ee_s.
[b] Absolute configuration of the faster reacting enantiomer.
[c] Reversed absolute configuration due to the Cahn–Ingold–Prelog rule.

Table 2
Enantioselectivity of HheA-WT and HheA-N178A variant in the ring-opening reaction with azide

Substrate	HheA-WT E-Value[a,b]	HheA-N178A E-Value[a,b]
1b	10 (*S*)	10 (*S*)
1g	1 (*R*)	1 (*R*)
1h	1 (*R*)	48 (*R*)[c]
1i	1.5 (*S*)[c]	20 (*R*)[c]
1j	16 (*S*)[c]	6 (*S*)
1k	5 (*S*)[d]	108 (*S*)[d]

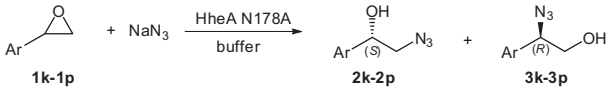
[a] E-Values were calculated from ee_p and ee_s.
[b] Absolute configuration of the faster reacting epoxide is indicated between brackets.
[c] Reversed absolute configuration due to the CPI rule.
[d] Data from Ref. 10.

Recently, a saturation mutagenesis library was constructed based on the knowledge of the X-ray structure of HheA and rational considerations.[10] Residues L141, V136 and N178 located in the two active-site loops were targeted, and resulted in several active mutants with improved properties. The N178A variant, which displayed a remarkable improvement in the kinetic resolution of *rac*-2-chloro-1-phenylethanol (E > 200) with (*S*)-stereopreference, was selected for a more detailed study. For this, a small set of substrates **1b** and **1g**–**1k** that represent different classes of epoxide was chosen to investigate the enantioselectivity of HheA-N178A in the ring-opening reaction with azide (Table 2).

Reaction of aliphatic **1b** and carboxymethyl **1g** substituted epoxides occurred with no difference in enantioselectivity. A significant increase was observed with glycidyl ethers **1h** and **1i**, as well as for previously reported styrene oxide **1k**.[10] For **1h** the E-value increased from 1 to 48, for **1i** from 1.5 to 20 and for **1k** from 5 to 108. A drop of enantioselectivity was observed only with **1j**. Styrene oxide derivatives and glycidyl ethers were azidolysed with up to 48-fold higher enantioselectivity, which exposed this class of epoxides as favourable substrates for N178A mutant. To further explore this enzyme, a series of aromatic epoxides was tested and results are represented in Table 3. Racemic substrates used for this screening were commercially available or synthesised according to literature procedure (see Experimental section).

The mutant enzyme showed high enantioselectivity towards substituted styrene oxides **1k**–**1o** and the same enantiopreference for (*S*)-epoxides. Conversion proceeded rapidly to 50%, but after that it drastically slowed down, resulting in efficient kinetic resolution and formation of enantiomerically pure secondary alcohols **2** (ee ⩾ 99%). The only exception was pyridine derivative **1p** that was converted with moderate enantioselectivity and lower rate compared to phenyl-substituted epoxides (Table 3, entry 8). These results clearly confirm that replacement of the active site residue Asn178 by alanine is crucial in controlling the enantioselectivity of HheA. Besides the fact that this mutation beneficially influences the enantioselectivity and activity,[10] it has however a detrimental effect on the regioselectivity. While HheA-WT shows high preferential attack at the terminal position, mutant N178A catalyses

Table 3
Conversion of epoxides **1k**–**1p** catalysed by HheA-N187A[a]



1k-1p **2k-2p** **3k-3p**

Entry	Substrate	Ar	t (h)	Conv. (%)	ee **1** (%)	ee **2** (%)	ee **3** (%)	Ratio (**2**:**3**)
1	*rac*-**1k**	Ph	2	45	72 (*R*)	99 (*S*)	91 (*R*)	57:43
2	*rac*-**1l**	4-Cl-Ph	0.75	50	>99 (*R*)	>99 (*S*)	>99 (*R*)	69:31
3	*rac*-**1m**	4-Br-Ph	0.75	50	>99 (*R*)	>99 (*S*)	>99 (*R*)	70:30
4	(*S*)-**1m**	4-Br-Ph	0.75	100	/	100 (*S*)	100 (*R*)	70:30
5	(*R*)-**1m**	4-Br-Ph	1	0	/	/	/	/
6	*rac*-**1n**	4-CN-Ph	2	51	85 (*R*)	>99 (*S*)	n.d.[b]	81:19
7	*rac*-**1o**	4-NO_2-Ph	1.5	52	98 (*R*)	>99 (*S*)	n.d.[b]	94:6
8	*rac*-**1p**	2-Py	20	35	43 (*R*)	63 (*R*)[c]	/	100:0

[a] General conditions: 2 mM epoxide in Tris–SO_4, 2 mM NaN_3, cell-free extract.
[b] Not determined.
[c] Apparent inversion of configuration of epoxides (*S*)-**1p** to alcohols (*R*)-**2p** is due to a different substituent priority according to the CIP rule.

formation of both regioisomers **2** and **3**. The less hindered carbon atom is predominantly attacked, however, the α/β ratio **2**:**3** depends on the *para*-substituent. The highest regioisomeric ratio was observed with epoxides bearing electron-withdrawing substituents **1n**–**1p**, while the lowest was for styrene oxide **1k** (57:43). Nevertheless, the formation of primary alcohols **3** obviously is enzyme catalysed, since they were isolated in highly enantioenriched form (up to >99% ee). Not surprisingly, the configuration on the stereogenic centre was found to be (*R*). In order to confirm the stereochemical course of the nucleophilic α-attack and formation of (*R*)-**3**, enzymatic reactions were performed with pure enantiomers. For these experiments epoxide **1m** was chosen as substrate. Reaction of racemic **1m** showed formation of the mixture of secondary (*S*)-**2m** and primary (*R*)-**3m** alcohols in the ratio of 70:30 (Table 3, entry 3). Enantiomerically pure (*R*)-**1m** and (*S*)-**1m** were obtained from racemic **1m** by preparative HPLC and subjected to enzymatic reaction. (*S*)-**1m** was completely converted with identical stereochemical pattern as racemic compound (*RS*)-**1m** and both products were formed in the ratio **2m**:**3m** = 70:30, equal to reaction with racemic substrate (Table 3, entry 4). From the other hand, no reaction occurred with (*R*)-enantiomer (Table 3, entry 5). These results confirm that the enzyme-catalysed reaction is highly enantioselective, and nucleophilic β-attack occurs at the sterically less hindered C3 atom of (*S*)-enantiomer with the retention of the absolute configuration, while the α-attack (C2 atom) is accompanied by inversion of configuration. As a result, (*R*)-configured azido alcohol **3** is formed from the (*S*)-epoxide.

2.2. Docking studies and quantum chemical calculations

To gain insight into how the single mutation at the residue 178 results in such a drastic change of enantioselectivity and regioselectivity for the azidolysis of *para*-substituted styrene oxides, in silico mutagenesis of N178 to A and docking of **1k** and **1m** enantiomers to the mutant HheA-N178A were performed. To investigate the electronic effects of the important interactions within the active site, a hybrid approach combining quantum chemical (B3LYP/6-311G(d)) and semiempirical (PM6) calculations within the ONIOM scheme were carried out for the docked structures with the lowest energy. A model of the enzyme active site was built by selecting the most important amino acids within 10 Å from the catalytic triad (positions of their atoms fixed). The position of amino acids and the azide ion were frozen during the optimizations. Optimized geometries obtained for enantiomers of **1k** and **1m** are presented at the Figures 1 and 2, respectively.

The epoxide oxygen atom of (*R*)-**1k** is positioned in such a way that can form two strong hydrogen bonds (distances below 3.0 Å) with Tyr147 and Ser134 whereas (*S*)-enantiomer forms weaker hydrogen bonds (Fig. 1).

Although the epoxide carbon atoms are in the vicinity of the nucleophile, those positions are geometrically unfavourable for the nucleophilic attack to occur. On the other hand, for (*S*)-**1k**, additional complex was found (Fig. 1, thinner grey model). The aromatic ring is positioned in a similar location as the previous one, but the epoxide ring is rotated and its oxygen atom pointed towards Ser135, making two strong H-bonds with Ser134 and Ser135. This binding is geometrically in a correct position for the nucleophilic attack to occur since azide ion is located ca. 3.3 Å from Cβ. Cβ hydrogen atom is positioned the vicinity of the Cα hydrogen atom of Ala178 (H–H 1.7 Å). However, this is a non-productive complex, suggesting that a conformational change at the active site of HheA might occur.

Furthermore, to evaluate the impact of a *para*-substituent on the aromatic ring, docking and subsequent ONIOM calculations of **1m** enantiomers to the mutant HheA-N178A were performed. Overlay of the obtained optimized geometries for (*R*)- and (*S*)-enantiomers of **1m** in the active site are presented in Figure 2.

Similar binding patterns to that of **1k** can be observed. Again, the most productive binding (Fig. 2, thinner grey model) for the reaction to occur is that in which (*S*)-epoxide ring is rotated making two stronger H-bonds with Ser134 and Ser135. Azide ion is close and geometrically in the best position for the nucleophilic attack. The distance from nucleophile to the epoxide Cα atom is ca. 0.6 Å longer than that to Cβ resulting in a higher regioselectivity of the attack. Due to the bigger size of the aromatic substituent (*para*-bromine), a slight shift in the positions of the aromatic group and epoxide ring compared to binding of equivalent **1k**-enantiomers is observed. Exchange of Asn178 to Ala increased the conformational flexibility of Ser135 hydroxyl group. The strong

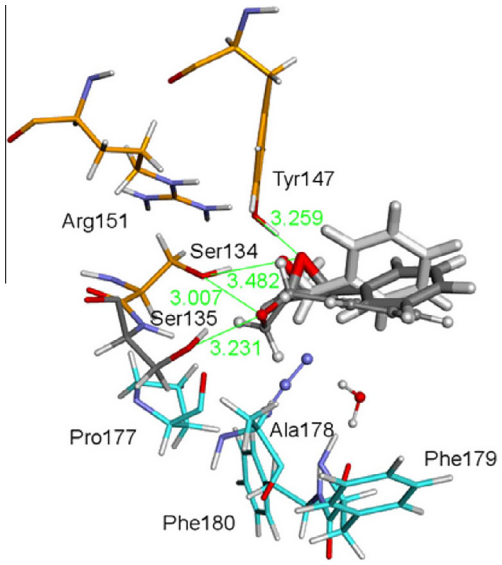


Figure 1. Overlay of **1k** enantiomers ((*R*)-white, (*S*)-grey thicker and thinner model) and the azide ion in the HheA-N178A active site model represented by some structurally important amino acids obtained by ONIOM calculations. Hydrogen bonds of (*S*)-enantiomers are indicated as green lines, distances given in Å.

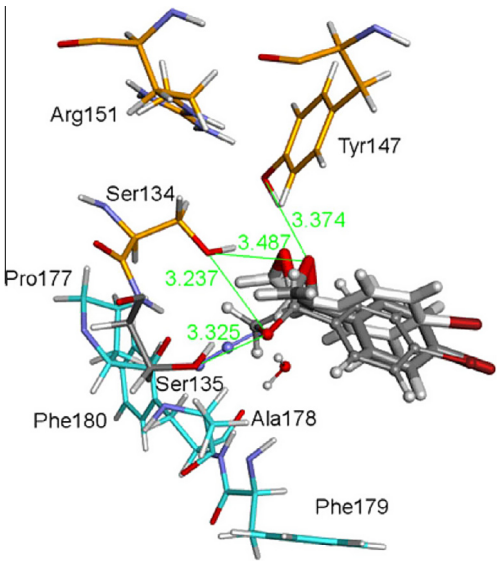


Figure 2. Overlay of **1m** enantiomers ((*R*)-white, (*S*)-grey, thicker and thinner model, bromine atom represented with dark red colour) and the azide ion in the HheA-N178A active site model represented by some structurally important amino acids obtained by ONIOM calculations. Hydrogen bonds are indicated as green lines, distances given in Å.

H-bond (ca. 3 Å) between Asn amide nitrogen atom and Ser OH group, present in the HheA-WT, vanished in the mutant. Loss of that H-bond at the same time increased potency of Ser OH group to act as an H-bond donor towards the (*S*)-enantiomer of the substrate. Since the mutation dramatically changed the reaction outcome, it can be assumed that the observed enantio- and regioselectivity of reactions are also affected by the possible greater conformational adjustment of the protein not included in present quantum chemical study.[10]

3. Conclusions

In conclusion, it was shown that halohydrin dehalogenase HheA catalyzed ring-opening reaction of various structurally diverse epoxides with azide as the nucleophile. This reaction proceeded with high regioselectivity and low to moderate enantioselectivity. HheA-N178A mutant showed enhanced enantioselectivity and reduced regioselectivity towards the azidolysis of styrene oxide derivatives. All *para*-substituted styrene oxides could be resolved in excellent enantioselectivities and enantiomerically pure (*S*)-β-azido alcohols and (*R*)-α-azido alcohols could be obtained. It is remarkable that a single mutation results in such a dramatic change in the enantioselectivity. It is clear that residue 178 in the halide binding pocket plays a critical role in determining the enantioselectivity of these epoxide azidolysis. HheA-N178A represents a first highly (*S*)-enantioselective HHDH. With the molecular modelling studies, binding modes of enantiomers and the important interactions within the enzyme active site were determined.

4. Experimental

4.1. General

The commercial grade reagents and solvents were used without further purification. The commercially available racemic substrates 1,2-epoxybutane **1a**, 1,2-epoxyhexane **1b**, allyl glycidyl ether **1h**, 2,3-epoxypropyl-benzene **1j** and styrene oxide **1k**, as well as azidotrimethylsilane, (*R*,*R*)-*N*,*N*′-bis(3,5-di-tertbutylsalicylidene)-1,2-cyclohexanediaminochromium(III) chloride, trimethylsulfoxonium iodide, absolute DMSO, L-arabinose, ampicillin sodium salt, β-mercaptoethanol, sorbitol, and EDTA were purchased from Sigma–Aldrich. Racemic 1,2-epoxy-3-methylbutane **1c**, 3,3-dimethyl-1,2-epoxybutane **1d**, 1,2-epoxy-3-phenoxypropane **1i** and 4-chlorostyrene oxide **1l** were purchased from Alfa Aesar. Bacto-tryptone, yeast extract and bacto-agar were purchased from Difco. Complete Protease Inhibitor Cocktail Tablets were supplied by Roche, while glycerol was obtained from Kemika. *Rac*-2-cyclohexyl-oxirane **1e**[8] and *rac*-methyl-3,4-epoxybutyrate **1g**[11] were prepared as previously described. Enantiomerically pure (*R*)-**1m** and (*S*)-**1m** were obtained from racemic **1m** by preparative HPLC using a semipreparative Chiralpak AS column (250 × 46 mm, Daicel) with 0.5% 2-PrOH/Hexane as eluent (4 mL/min). Racemic azido alcohols were prepared by ring-opening reactions of the corresponding epoxide with sodium azide in water.[12] Enzymes, HheA-WT and mutant HheA-N178A were prepared by overexpression in *E. coli* strain MC1061 according to a previously described protocol and used as cell-free extract.[9,10]

4.2. Synthesis of racemic substrates

4.2.1. 1,2-Epoxy-2-methylheptane 1f

Epoxide **1f** was prepared according to a literature procedure.[13] DMSO (20 mL) was added dropwise to a mixture of trimethylsulfoxonium iodide (5.1 g, 23.1 mmol) and sodium hydride (a 60% disp. in mineral oil; 0.94 g, 23.1 mmol) cooled to 0 °C, under argon. After stirring for 20 min at room temperature, a solution of 2-heptanone (2.0 g, 17.8 mmol) in DMSO (10 mL) was added dropwise. Reaction mixture was stirred for 22 h. Water was added (20 mL) and the mixture extracted with diethyl ether (3 × 30 mL). The combined organic layers were washed with water (2 × 30 mL) and brine (20 mL), dried over Na_2SO_4, filtered and evaporated under reduced pressure. Column chromatography (SiO_2; hexane–ethyl acetate, 9.5:0.5) furnished the pure epoxide **1f** (1.1 g, 48%) as a colourless liquid. d_H (300 MHz, $CDCl_3$) 0.87–0.91 (3H, m), 1.27–1.34 (11H, m), 2.57 (1H, d, *J* 5.0 Hz), 2.60 (1H, d, *J* 5.0 Hz); d_C (150 MHz, $CDCl_3$) 14.0, 20.9, 22.6, 24.9, 31.8, 36.7, 53.9, 57.1.

4.2.2. 4-Bromostyrene oxide 1m

Epoxide **1m** was prepared according to a literature procedure.[13] 4-Bromobenzaldehyde (1.0 g, 5.4 mmol) and trimethylsulfonium iodide (1.76 g, 8.6 mmol) were dissolved in DMSO (8 mL). Potassium *tert*-butoxide (0.92 g, 8.2 mmol) was dissolved in 8 mL of DMSO and added to the reaction mixture. Reaction mixture was stirred over night at room temperature under argon. Reaction was quenched by addition of water (16 mL). Reaction mixture was extracted with dichloromethane (3 × 30 mL), combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure. Column chromatography (SiO_2; hexane–ethyl acetate, 9:1) furnished the pure epoxide **1m** (0.79 g, 74%) as a colourless liquid. d_H (300 MHz, $CDCl_3$) 2.74 (1H, dd, J_1 5.5 Hz, J_2 2.5 Hz), 3.13 (1H, dd, J_1 5.5 Hz, J_2 4.0 Hz), 3.82 (1H, dd, J_1 4.0 Hz, J_2 2.5 Hz), 7.14 (2H, d, *J* 8.5 Hz), 7.46 (2H, d, *J* 8.5 Hz); d_C (75 MHz, $CDCl_3$) 51.2, 51.8, 122.0, 127.2, 131.7, 136.8.

4.2.3. 4-Cyanostyrene oxide 1n

Epoxide **1n** was prepared according to a modified literature procedure.[14] Sodium borohydride (106 mg, 2.7 mmol) was added to a solution of 2-bromo-1-(4-cyanophenyl)-ethanone (568 mg, 2.54 mmol) in methanol (7 mL) and stirred at 0 °C. After 1.5 h, water (10 mL) was added and reaction mixture was stirred for 1 h at room temperature. Reaction mixture was extracted with dichloromethane (3 × 10 mL), combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure. The dry residue was dissolved in a small amount of diethyl ether, following by addition of potassium hydroxide (15 mL, 1 M) and the reaction mixture was stirred over night at room temperature. Reaction mixture was extracted with dichloromethane (4 × 10 mL), combined organic layers were washed with brine, dried over Na_2SO_4, filtered and evaporated under reduced pressure. Column chromatography (SiO_2; hexane–ethyl acetate, 7:3) furnished the pure epoxide **1n** (289 mg, 78%) as a colourless liquid. d_H (300 MHz, $CDCl_3$) 2.74 (1H, dd, J_1 5.5 Hz, J_2 2.5 Hz), 3.18 (1H, dd, J_1 5.5 Hz, J_2 4.0 Hz), 3.88–3.90 (1H, m), 7.37 (2H, d, *J* 8.5 Hz), 8.62 (2H, d, *J* 8.5 Hz); d_C (75 MHz, $CDCl_3$) 51.5, 51.6, 112.0, 118.6, 126.1, 132.3, 143.3.

4.2.4. 4-Nitrostyrene oxide 1o

Epoxide **1o** was prepared according to a literature procedure.[14] Sodium borohydride (85 mg, 2.2 mmol) was added to a solution of 2-bromo-1-(4-nitrophenyl)-ethanone (500 mg, 2 mmol) in methanol/THF (1:1.2, 5 mL) at 0 °C. After that, solution of sodium hydroxide (3 mL, 2 M) was added and reaction mixture was stirred for 1 h at room temperature. The solvent was evaporated, acetic acid was added (to pH 4) and reaction mixture was extracted with dichloromethane (4 × 10 mL), combined organic layers were washed with solution of sodium hydrogen carbonate, water, brine, dried over Na_2SO_4, filtered and evaporated under reduced pressure. The dry residue was dissolved in a small amount of diethyl ether, potassium hydroxide (15 mL, 1 M) was added and reaction mixture was stirred over night at room temperature. Reaction mixture

was extracted with dichloromethane (4 × 10 mL), combined organic layers were washed with brine, dried over Na_2SO_4, filtered and evaporated under reduced pressure. Column chromatography (SiO_2; hexane–ethyl acetate, 7:3) furnished the pure epoxide **1o** (0.30 g, 90%) as a yellow powder. d_H (300 MHz, $CDCl_3$) 2.78 (1H, dd, J_1 5.5 Hz, J_2 2.5 Hz), 3.19 (1H, dd, J_1 5.5 Hz, J_2 4.0 Hz), 3.96 (1H, dd, J_1 4.0 Hz, J_2 2.5 Hz), 7.42 (2H, d, J 8.5 Hz), 8.18 (2H, d, J 8.5 Hz); d_C (150 MHz, $CDCl_3$) 51.4, 51.6, 123.8, 126.2, 145.3, 147.8.

4.2.5. 2-Pyridyl-oxirane 1p

Epoxide **1p** was prepared according to a literature procedure.[15] Vinyl pyridine (1.0 g, 9.5 mmol) was dissolved in tetrahydrofurane (100 mL) at 0 °C in an inert atmosphere. Water (150 mL) was added dropwise until a saturated solution was formed. After that, *N*-bromosuccinimide (1.86 g, 10.5 mmol) was added and reaction mixture was stirred for 2 h at room temperature. Reaction mixture was concentrated at rotary evaporator, THF was removed and aqueous layer was extracted with dichloromethane (3 × 50 mL). Combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure. Column chromatography (SiO_2; dichloromethane–methanol, 98:2) furnished the pure bromoalcohol as a colourless liquid in 64% yield (1.2 g). To a solution of 2-bromo-1-pyridin-2-yl-ethanol (500 mg, 2.5 mmol) in methanol (5 mL) potassium carbonate (400 mg, 2.9 mmol) was added and reaction mixture stirred during 3 h at room temperature. Then water (10 mL) was added and reaction mixture was extracted with dichloromethane (3 × 15 mL), combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure. Column chromatography (SiO_2; dichloromethane–methanol, 98:2) furnished the pure epoxide **1p** (167 mg, 55%) as a colourless liquid. d_H (300 MHz, $CDCl_3$) 2.94 (1H, dd, J_1 6.0 Hz, J_2 2.5 Hz), 3.18 (1H, dd, J_1 6.0 Hz, J_2 4.0 Hz), 4.02 (1H, dd, J_1 4.0 Hz, J_2 2.5 Hz), 7.20–7.29 (2H, m), 7.68 (1H, dt, J_1 7.5 Hz, J_2 1.5 Hz), 8.55–8.56 (1H, m); d_C (75 MHz, $CDCl_3$) 50.4, 52.8, 119.7, 123.1, 136.8, 149.4, 157.2.

4.3. Kinetic resolution of epoxides

To Tris–SO_4 buffer (50 mM, pH 7.0) a stock solution of epoxide in DMSO was added at room temperature (final concentration 5 mM for **1a**–**1j** and 2 mM for **1k**–**1p**), followed by addition of a stock solution of NaN_3 in water (0.5 mL, final conc. 5 mM or 2 mM). Reactions were initiated by addition of 1 mL of cell-free extract in TEMG buffer (final volume 10 mL). The progress of the reaction was followed by periodically taking samples (0.5 mL) from reaction mixture. Samples were extracted with MTBE (1.0 mL) containing mesitylene as internal standard and analysed by GC for conversion and regioselectivity ratio. In parallel, chiral GC and/or HPLC analyses were performed to determine the enantiomeric purity of the product and remaining substrate. The non-catalysed reactions of epoxides **1k**–**1p** with NaN_3 were followed by monitoring epoxide consumption in the absence of enzyme.

4.4. Molecular modelling

Quantum mechanical docking calculation were performed by systematic scanning of the HheA-N178A active site (PDB code: 1ZMO,[6] N178 residue changed to alanine) using search algorithm implemented in our program code *qcc*.[19] Three molecular translational degrees of freedom were scanned with the step of 1.0 Å whereas three molecular rotational degrees of freedom were scanned with increments of 45°. Besides these 6 molecular degrees of freedom, rotations around single bonds that correspond to the torsional movement of epoxide ring in investigated compounds were also investigated. In each case the model of the active site was built. Single point calculation for the model of active site and investigated compounds were performed using the PM6 method from Gaussian 09 program package.[20] All energy values from single point calculations were arranged in the 7-way array and search for all local minima was performed by using combinatorial algorithm built in program for multivariate analysis *moonee*.[21]

All these local minima were subjected to geometry optimization procedure using the semiempirical PM6 method and subsequent clustering of geometries was performed and classified on the basis of the energy values. Results were inspected visually and on the basis of the energy values some structure were selected and reoptimized using the QM/QM 2-layer ONIOM approach with semiempirical PM6 method for the outer layer, and density functional theory B3LYP/6-31G(d) method, for the inner layer of system.[22,23]

4.5. Determination of enantiomeric purity and absolute configuration

The enantiomeric excesses (ee's) of the formed azido alcohols and remaining epoxides were determined by chiral GC or HPLC analyses under conditions described in Supplementary information.

Absolute configurations were assigned by chiral GC or HPLC analysis using reference compounds. In the case of epoxides **1a**–**1e**,[8] **1g**,[11] **1j**[5a], **1k**[16] and **1o**[5b] assignment was based on previously reported data. The enantiomerically enriched epoxides (*R*)-**1f**, (*S*)-**1h**, (*S*)-**1i**, (*R*)-**1l**, (*R*)-**1m**, (*R*)-**1n** and (*R*)-**1p** were prepared by (*R,R*)-(salen)CrCl catalysed ring-opening with $TMSiN_3$ according to Lebel and Jacobsen.[17] Absolute configuration of primary azido alcohol **3m** was assigned by comparison of elution order on HPLC column with published data.[18] Absolute configurations of **3k** and **3l** were assigned by analogy.

Acknowledgement

Financial support by the Unity through Knowledge Fund (UKF grant no. 51) is gratefully acknowledged.

Supplementary data

Supplementary data (chiral GC and HPLC analyses and NMR spectra) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetasy.2016.08.003.

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