RSC Advances

PAPER

View Article Online
View Journal | View Issue

Cite this: *RSC Adv.*, 2019, **9**, 16418

Highly α-position regioselective ring-opening of epoxides catalyzed by halohydrin dehalogenase from *Ilumatobacter coccineus*: a biocatalytic approach to 2-azido-2-aryl-1-ols†

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Received 20th May 2019
Accepted 20th May 2019

DOI: 10.1039/c9ra03774h

rsc.li/rsc-advances

Halohydrin dehalogenases are usually recognized as strict β-position regioselective enzymes in the nucleophile-mediated ring-opening of epoxides. Here we found the HheG from *Ilumatobacter coccineus* exhibited excellent α-position regioselectivity in the azide-mediated ring-opening of styrene oxide derivatives **1a–1k**, producing the corresponding 2-azido-2-aryl-1-ols **2a–2k** with the yields up to 96%.

Introduction

1,2-Azido alcohols are versatile precursors in organic synthesis for a wide variety of target compounds such as triazoles,[1] triazole-fused dihydrooxazinones,[2] 2-oxazolidinones,[3] 1,4-oxazepines and 1,3-oxazines,[4] oxazaborolidines,[5] and 1,3-oxazolidines.[6] More importantly, they have been widely employed for the regioselective preparation of 1,2-amino alcohols and highly oxygenated compounds such as carbohydrates and nucleosides.[7]

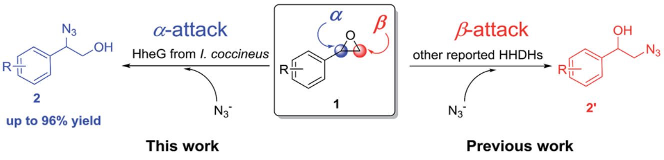
The epoxy group is a highly reactive moiety, which makes it easily react with a broad range of nucleophiles and provides a convenient access to diverse highly functionalized molecules.[8] Thus among the transformations used for the preparation of 1,2-azido alcohols, the epoxide ring-opening is straightforward and effective method which has been widely studied. Though trimethylsilyl azide and other metal azides have been used to introduce the azide group,[9] the sodium azide is generally employed under various reaction conditions.[10]

Halohydrin dehalogenases (HHDHs) are valuable enzymes from short dehydrogenases/reductases superfamily, which exhibit promiscuous catalytic functions in the synthesis and conversion of epoxides.[11] HHDHs are not only able to catalyze the dehalogenation of vicinal haloalcohols to produce epoxides, but also can accept several anionic nucleophiles in epoxide ring-opening reactions with the formation of a broad range of β-functionalized alcohols, which are useful biocatalytic routes to prepare some important pharmaceuticals and biologically active compounds.[12] Azide can be processed by HHDHs as one of the nucleophiles to produce β-azido alcohols. However, all the reported HHDHs have been described to transform the azide group to the epoxides at β-position (Scheme 1, red), generating the 2-azido-1-phenylethan-1-ols (**2′**).[12a,12h,12j] In this study, we explored the HheG from *I. coccineus* for highly α-position regioselective ring-opening of styrene oxide derivatives **1** with azide (Scheme 1, blue), which gave the 2-azido-2-aryl-1-ols (**2**) with the yields up to 96%.

Results and discussion

Recently Schallmey and coworkers have reported that the HHDH from *I. coccineus*, namely HheG, exhibited significant activity in the azidolysis of cyclohexene oxide and limonene oxide.[13] Analysis of the HheG's structure revealed a large open cleft harboring the active site to accept these cyclic epoxide substrates, which was in sharp contrast to other known HHDH structures. Attracted by these results, we inferred the HheG might possess a special regioselectivity in the ring-opening process.

With this conjecture in mind, we first investigated the HheG-catalyzed ring-opening of styrene oxide **1a** with azide on an



Scheme 1 HHDH-catalyzed α-position and β-position regioselective ring-opening of epoxides with azide.

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† Electronic supplementary information (ESI) available: Experiment procedures and NMR data. See DOI: 10.1039/c9ra03774h

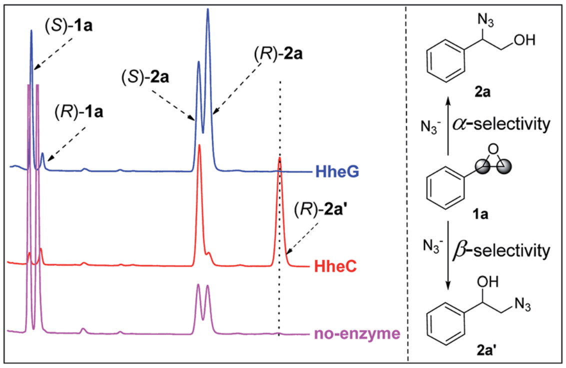


Fig. 1 Ring-opening of **1a** catalyzed by HheG (blue line), HheC (red line), and no enzyme (pink line).

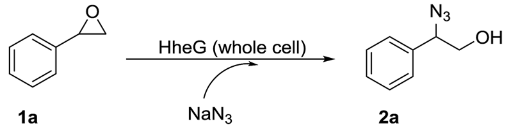
analytical scale. The HheC-catalyzed and non-enzymatic reactions were also carried out as controls. The HPLC analysis results were depicted in Fig. 1. In the absence of enzyme, ring-opening of **1a** preferred α-position regioselectivity (Fig. 1, pink line) and the reaction rate was pH-dependent.[14] Previous study has revealed HheC was a *R*-enantioselective and β-regioselective enzyme for ring-opening of epoxides by several nucleophiles. HheC catalyzed azide ring-opening of **1a** to produced 2-azido-1-phenylethan-1-ol (**2a′**) with 79% β-position regioselectivity and 98% ee (*R*).[12a] Herein, the azide was excessive and we found that the HheC showed about 100% conversion of **1a** with about 50% β-position regioselectivity (Fig. 1, red line). Formation of 2-azido-2-phenylethan-1-ol (**2a**) might be contributed by the chemical reaction *via* α-position ring-opening of **1a**. Interestingly, the HheG showed highly α-position regioselectivity in the ring-opening process (Fig. 1, blue line) without β-position selectivity, which was different from the other reported HHDH regioselectivity.

Subsequently, we tested the conditions of the HheG-catalyzed azide-mediated ring-opening (ARO) reaction using epoxide **1a** as the model substrate. All the reactions were performed on analytical scale, and the relative yields of **2a** at different conditions were determined by HPLC analysis to screen the optimal conditions. First the reaction pH/buffer and temperature were examined and the results were summarized in Table 1. It could be found that the optimal pH/buffer was 7.0/PBS (entry 4). Reaction in 5.0/HAc–NaAc gave a very low yield (entry 1). Reaction in the higher pH/PBS caused a dramatic decrease of the yield (entries 5 and 6 *vs.* entry 4). A good yield was obtained in 8.0/Tris–H_2SO_4, which was dramatic higher than that obtained in 8.0/PBS (entry 6 *vs.* entry 8). Temperature study revealed the optimal reaction temperature was 30 °C (entry 4). An excellent yield was also obtained at 25 °C (entry 10). Increasing reaction temperature to 35–45 °C resulted in a sharp decrease of the yield (entries 11–13 *vs.* entry 4), which corresponded to the previous study of the thermostability of the HheG.[15]

We further investigated the ratio of azide to substrate **1a**. From the results of Table 2 (entries 1–4), we could find that the optimal ratio for the ARO reaction was 1.5 : 1 (entry 2). Increasing the ratio to 2 : 1 and 3 : 1 did not improve the yield (entries 3 and 4 *vs.* entry 2). The recombinant cell density was subsequent investigated at the ratio of 1.5 : 1 (entries 5–7). A higher yield was obtained by increasing cell density (entries 5–7 *vs.* 2). However, the yields generated by 15 and 20 g cdw L^{-1} of cells were almost equivalent (entry 6 *vs.* entry 7), and 15 g cdw L^{-1} of cells was selected for the ARO reaction in the following experiments.

After obtaining the optimum reaction conditions, the reaction courses at the substrate concentrations from 5 to 60 mM were investigated. The yields were analyzed by HPLC at 6, 12 and 24 h for each reaction. The highest yield was set

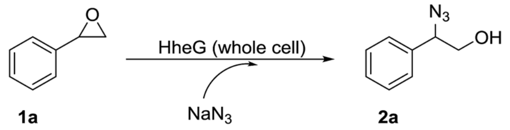
Table 1 Investigation of the pH/buffer and temperature of ARO reaction



Entry	pH/buffer[a]	*T* (°C)	NaN_3 : **1a**	Conc. **1a** (mM)	Cell conc. (g cdw L^{-1})	Time (h)	Relative yield **2a** [b] (%)
1	5.0/HAc–NaAc	30	1 : 1	5	5	6	11
2	6.0/HAc–NaAc	30	1 : 1	5	5	6	76
3	6.0/PBS	30	1 : 1	5	5	6	32
4	7.0/PBS	30	1 : 1	5	5	6	100
5	7.5/PBS	30	1 : 1	5	5	6	86
6	8.0/PBS	30	1 : 1	5	5	6	82
7	7.0/Tris–H_2SO_4	30	1 : 1	5	5	6	88
8	8.0/Tris–H_2SO_4	30	1 : 1	5	5	6	96
9	9.0/Gly–NaOH	30	1 : 1	5	5	6	72
10	7.0/PBS	25	1 : 1	5	5	6	98
11	7.0/PBS	35	1 : 1	5	5	6	53
12	7.0/PBS	40	1 : 1	5	5	6	44
13	7.0/PBS	45	1 : 1	5	5	6	49

[a] Buffer concentration: 50 mM; PBS = Na_2HPO_4–KH_2PO_4. [b] The relative yields were calculated by HPLC analysis. The highest peak area of **2a** was set to 100% yield. Conc. = concentration.

Table 2 Investigation of the ratio of NaN_3 to **1a** and cell density of ARO reaction



Entry	pH/buffer[a]	T (°C)	NaN_3 : **1a**	Conc. **1a** (mM)	Cell conc. (g cdw L^{-1})	Time (h)	Relative yield **2a** [b] (%)
1	7.0/PBS	30	1 : 1	5	5	6	70
2	7.0/PBS	30	1.5 : 1	5	5	6	83
3	7.0/PBS	30	2 : 1	5	5	6	80
4	7.0/PBS	30	3 : 1	5	5	6	72
5	7.0/PBS	30	1.5 : 1	5	10	6	84
6	7.0/PBS	30	1.5 : 1	5	15	6	99
7	7.0/PBS	30	1.5 : 1	5	20	6	100

[a] Buffer concentration: 50 mM; PBS = Na_2HPO_4–KH_2PO_4. [b] The relative yields were calculated by HPLC analysis. The highest peak area of **2a** was set to 100%. Conc. = concentration.

to 100% to calculate the relative yields. The results depicted in Fig. 2 indicated the reactions at the substrate concentration of 5–40 mM were almost finished at 12 h. Prolonging the reaction time to 24 h did not caused notable increase in yields. For a higher substrate concentration of 60 mM, the yield obtained at 6 h was as same as that obtained from the lower concentrations. It should be noted that the yield didn't increase by extending reaction time, which might be caused by product inhibition at high substrate concentration. The ARO reaction was established to react on 40 mM of epoxides for 12 h.

Finally, several structurally different aromatic epoxides **1a–1k** were selected as substrates to investigate the substrate scope of the ARO reaction. All the reactions were carried out on the optimal conditions with 40 mM of epoxides **1**. DMSO (1 vol%) was used as co-solvent to solubilize the epoxides in the aqueous reaction medium. From the results of Table 3, we could find that the HheG showed a broad substrate range in the ARO reaction. The 2-azido-2-aryl-1-ols **2a–2k** were synthesized from the corresponding epoxides **1a–1k** with the high yields up to 96%. The epoxide substrates with substituent group at *p*-position generated relative higher yield than that with substituent group at *m*-position (entries 6–9 *vs.* entries 2–5). The azidolysis of *p*-cyanophenyl-substituted epoxide **1j** and *p*-*tert*-butyl-substituted epoxide **1k** also gave the corresponding azidoalcohols with moderate yields of 65% and 76% respectively (entry 10 and entry 11). All the isolated yields were obtained from 40 mM of epoxides after reaction for 12 h, which pointed to a good enzyme activity in such biocatalytic ring-opening process. More importantly, the ARO reaction exhibited highly α-position regioselectivity in the ring-opening of epoxides **1a–1k**, which indicated the HheG was an excellent α-position regioselective HHDH. To our knowledge, this was the first time that 2-azido-2-aryl-1-ols **2a–2k** were synthesized by an enzymatic catalysis.

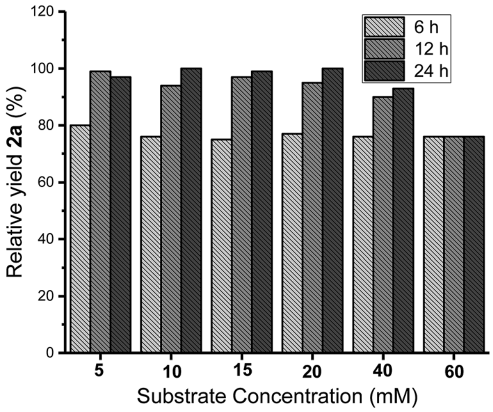
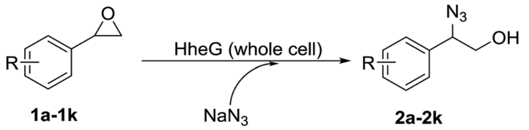


Fig. 2 Investigation of ARO reaction courses at the substrate concentrations from 5 to 60 mM.

Table 3 Substrate scope of the ARO reaction



Entry[a]	Substrate	R	Product	Yield[b] (%)
1	**1a**	H	**2a**	96
2	**1b**	*m*-F	**2b**	73
3	**1c**	*m*-Cl	**2c**	69
4	**1d**	*m*-Br	**2d**	66
5	**1e**	*m*-CH_3	**2e**	76
6	**1f**	*p*-F	**2f**	80
7	**1g**	*p*-Cl	**2g**	91
8	**1h**	*p*-Br	**2h**	76
9	**1i**	*p*-CH_3	**2i**	95
10	**1j**	*p*-CN	**2j**	65
11	**1k**	*p*-$C(CH_3)_3$	**2k**	76

[a] Reaction condition: epoxide **1** conc. 40 mM in PBS (50 mM, pH 7.0), NaN_3 conc. 60 mM, cells conc. 15 g cdw L^{-1}, 30 °C, 12 h. [b] Isolated yields.

Experimental

Strains culture and enzymes preparation

The HheC gene (GenBank accession number: AF397296.1) and HheG gene (GenBank accession number: AP012057.1, 3979394–3980182) were synthesized after codon optimization and ligated into pET-28a(+) plasmid using restricted enzymes *Nco*I and *Xho*I. The recombinant plasmid pET-28-HheC and pET-28-HheG were transformed into *Escherichia coli* BL21 (DE3), respectively. The kanamycin antibiotic was used in whole culture process to a final concentration of 50 μg mL^{-1}.

A single recombinant *E. coli* colony was taken from the agar plate and inoculated in Luria-Bertani (LB) medium. The culture was grown overnight at 37 °C, 200 rpm, for 16 h. A large flask (1000 mL) containing 100 mL LB was inoculated with 5 mL of the overnight culture. The flask culture was grown overnight at 37 °C, 200 rpm, until the OD$_{600}$ up to 0.6–0.8. Then, culture was induced with IPTG (final concentration 0.1 mM) and further shaken at 200 rpm, 28 °C, for 12–14 h. The cultures were centrifuged and harvested to obtain recombinant *E. coli* cells.

General procedure for the synthesis of 2a–2k

To a 20 mL suspension of 15 g cdw L^{-1} recombinant *E. coli* cells (HheG) in 50 mM PBS buffer (pH 7.0), NaN$_3$ was added to a concentration of 60 mM. Then the epoxides **1** was added to a concentration of 40 mM using 200 μL of dimethyl sulfoxide (DMSO) as co-solvent. The mixture was stirred at 250 rpm and 30 °C. After reaction for 12 h, the mixture was extracted using ethyl acetate (2 × 20 mL). The organic phase was separated, dried over anhydrous Na$_2$SO$_4$, filtered, and concentrated by rotary evaporation. The residue was purified by flash column chromatography on silica gel (petroleum ether/ethyl acetate = 10 : 1–5 : 1) to afford the desired product **2**.

2-Azido-2-phenylethanol (2a). Light yellow oil, 125 mg, 96% yield, ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.44–7.31 (m, 5H), 4.68 (t, J = 6.4 Hz, 1H), 3.74 (t, J = 6.0 Hz, 2H), 2.24 (t, J = 6.1 Hz, 1H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 136.3, 129.1, 128.8, 127.3, 68.0, 66.6.

2-Azido-2-(3-fluorophenyl)ethanol (2b). Light yellow oil, 82.9 mg, 73% yield, ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.39–7.32 (m, 1H), 7.13–7.01 (m, 3H), 4.66 (dd, J = 7.9, 4.7 Hz, 1H), 3.81–3.66 (m, 2H), 2.57 (s, 1H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 163.0 (d, J = 246.0 Hz), 138.9 (d, J = 7.0 Hz), 130.6 (d, J = 8.0 Hz), 122.9 (d, J = 3.0 Hz), 115.7 (d, J = 21.0 Hz), 114.2 (d, J = 22.0 Hz), 67.2 (d, J = 1.0 Hz), 66.4.

2-Azido-2-(3-chlorophenyl)ethanol (2c). Light yellow oil, 56 mg, 69% yield, ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.33 (d, J = 4.9 Hz, 3H), 7.21–7.21 (m, 1H), 4.64 (dd, J = 7.7, 4.8 Hz, 1H), 3.74–3.70 (m, 2H), 2.30 (s, 1H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 138.5, 134.9, 130.3, 129.0, 127.4, 125.4, 67.2, 66.5.

2-Azido-2-(3-bromophenyl)ethanol (2d). Light yellow oil, 128 mg, 66% yield, ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.48–7.43 (m, 2H), 7.23 (dd, J = 4.4, 0.8 Hz, 2H), 4.60 (dd, J = 7.8, 4.7 Hz, 1H), 3.69 (qd, J = 11.6, 6.3 Hz, 2H), 2.68 (s, 1H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 138.7, 131.8, 130.5, 130.2, 125.8, 123.0, 67.0, 66.3.

2-Azido-2-(3-methphenyl)ethanol (2e). Light yellow oil, 108 mg, 76%, yield, ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.31–7.22 (m, 1H), 7.13 (m, 3H), 4.62 (t, J = 6.4 Hz, 1H), 3.72 (d, J = 6.5 Hz, 2H), 2.36 (s, 3H), 1.24 (s, 1H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 138.9, 136.3, 129.6, 129.0, 127.9, 124.3, 68.0, 66.6, 21.6.

2-Azido-2-(4-fluorophenyl)ethanol (2f). Light yellow oil, 115 mg, 80% yield, ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.31 (dd, J = 8.3, 5.4 Hz, 2H), 7.08 (t, J = 8.6 Hz, 2H), 4.68–4.61 (m, 1H), 3.80–3.65 (m, 2H), 2.32 (s, 1H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 162.9 (d, J = 246.0 Hz), 132.3 (d, J = 4.0 Hz), 129.0 (d, J = 8.0 Hz), 116.0 (d, J = 22.0 Hz), 67.2, 66.5.

2-Azido-2-(4-chlorophenyl)ethanol (2g). White solid, 144 mg, 91% yield, ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.35 (d, J = 8.4 Hz, 2H), 7.25 (d, J = 8.4 Hz, 2H), 4.62 (dd, J = 7.4, 5.1 Hz, 1H), 3.76–3.62 (m, 2H), 2.34 (s, 1H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 134.9, 134.6, 129.2, 128.6, 67.1, 66.4.

2-Azido-2-(4-bromophenyl)ethanol (2h). Light yellow solid, 96 mg, 76% yield, ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.53 (d, J = 8.3 Hz, 2H), 7.21 (d, J = 8.3 Hz, 2H), 4.64 (dd, J = 7.3, 5.1 Hz, 1H), 3.78–3.66 (m, 2H), 1.99 (s, 1H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 135.5, 132.3, 129.0, 122.9, 67.3, 66.5.

2-Azido-2-(p-tolyl)ethanol (2i). Light yellow oil, 136 mg, 95% yield, ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.28 (s, 4H), 4.70 (t, J = 6.4 Hz, 1H), 3.79 (d, J = 6.5 Hz, 2H), 2.62 (s, 1H), 2.43 (s, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 138.7, 133.7, 129.7, 127.2, 67.7, 66.47, 21.2.

2-Azido-2-(4-cyanophenyl)ethanol (2j). Light yellow oil, 90 mg, 65% yield, ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.71–7.69 (m, 2H), 7.48–7.46 (m, 2H), 4.74 (dd, J = 7.7, 4.5 Hz, 1H), 3.77 (dq, J = 19.3, 11.5, 6.1 Hz, 2H), 1.79 (s, 1H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 138.7, 133.7, 129.7, 127.2, 67.7, 66.4, 21.2.

2-Azido-2-(4-(*tert*-butyl)phenyl)ethanol (2k). Light yellow solid, 100 mg, 76% yield, ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.41 (d, J = 8.3 Hz, 2H), 7.25 (d, J = 8.3 Hz, 2H), 4.64 (t, J = 6.4 Hz, 1H), 3.73 (d, J = 6.4 Hz, 2H), 2.56 (s, 9H), 1.32 (s, 9H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 151.8, 133.3, 126.9, 125.9, 67.7, 66.5, 34.7, 31.4.

Conclusions

In summary, we found the HheG exhibited highly α-position regioselectivity for ring-opening of styrene oxide derivatives with azide and developed a biocatalytic approach to 2-azido-2-aryl-1-ols **2a–2k** with good yields. Our results brought a complementary regioselective biotransformation for the HHDH-catalyzed ring-opening of epoxides. We expect that it would broaden the product spectrum of β-functional alcohols synthesized by HHDHs.

Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgements

We are grateful for financial support from the National Natural Science Foundation of China (No. 21662050 and 31800663), Guizhou Science and Technology Department (QKHRC-2016-4029, QKHPTRC-2016-5801, QKHJC-2019-1467 and QKHZC-2019-2579), Program for Outstanding Youth of Zunyi Medical

University (17zy-001), National First-Rate Construction Discipline of Guizhou Province (Pharmacy) (YLXKJS-YX-04) and the Fifth Batch of Talent Base in Guizhou Province (2016).

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