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Formation of Enantiopure 5-Substituted Oxazolidinones through Enzyme-Catalysed Kinetic Resolution of Epoxides

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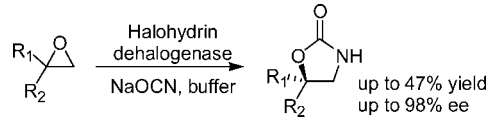
ABSTRACT



Halohydrin dehalogenase from *Agrobacterium radiobacter* catalyzed the enantioselective ring opening of terminal epoxides with cyanate as a nucleophile, yielding 5-substituted oxazolidinones in high yields and with high enantiopurity (69−98% ee). This is the first example of the biocatalytic conversion of a range of epoxides to the corresponding oxazolidinones.

Epoxides are highly valuable compounds in organic synthesis, because the oxirane ring can be chemically transformed into numerous intermediates.[1] To synthesize these important building blocks in enantiopure form, methods for asymmetric epoxidation of olefins[2] and kinetic resolution of epoxides[3] have been developed, mainly based on the use of chiral catalysts containing transition metals. Biocatalytic methods are also available,[4] including monooxygenase-mediated epoxidation[5] and kinetic resolution of racemic epoxides by epoxide hydrolases.[6,7] Recently, halohydrin dehalogenases have been explored in the latter conversions.[8] These bacterial enzymes catalyze the reversible conversion of vicinal haloalcohols to epoxides, and their natural role is the metabolism of compounds that possess a vicinal halohydrin group or of substrates that are degraded via intermediates that carry such a functionality.

In the epoxide ring opening reactions mediated by the HheC-type halohydrin dehalogenase from *Agrobacterium radiobacter*, various small anionic nucleophiles can be accepted (Scheme 1). In a recent study on the scope of

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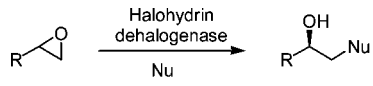
Scheme 1. Halohydrin Dehalogenase Catalysed Ring Opening Reactions



Nu = Cl^-, Br^-, I^-, N_3^-, NO_2^-, CN^-, OCN^-, SCN^-, $HCOO^-$

halohydrin dehalogenase catalyzed ring opening of epoxides, we have discovered that cyanate (OCN^-) is accepted as a nucleophile.[9] Due to cyclization, the products that are formed undergo conversion to 2-oxazolidinones, which are interesting building blocks for the preparation of pharmaceutical intermediates[10] and have wide application as chiral auxiliaries and ligands.[11]

Although chemical ring opening reactions of epoxides with different nucleophiles have been studied extensively, little work has been done on the reaction with inorganic cyanates and the transformation of the products to 2-oxazolidinones.[12,13] Dyen and Swern[14] reported that reaction of potassium cyanate with epoxides in DMF/H_2O produced 2-oxazolidinones in moderate yield. This method is applicable to terminal alkyl epoxides only. The main obstacle for the direct conversion of epoxides to oxazolidinones is the weak nucleophilicity of the cyanate ion.[15] In 2005, Bartoli et al. reported a method for the synthesis of enantiopure 5-substituted oxazolidinones from racemic epoxides by Co^{III}salen-catalyzed kinetic resolution, using urethane as the nucleophilic reagent.[16] Despite the established relevance of oxazolidinones in synthetic chemistry, their preparation by other means has been limited to a few methods.[17,11] Of these, an indirect procedure involving preparation of amino alcohols and subsequent cyclization emerged as a versatile route, in part because of the availability of substrates in both racemic and enantiomerically pure form.[11]

Here, we report that cyanate ion can act as a nucleophile in the ring opening of epoxides catalyzed by halohydrin dehalogenase to form highly enantioenriched 5-substituted 2-oxazolidinones.

The *A. radiobacter* halohydrin dehalogenase (HheC) was explored as a biocatalyst in the reaction of epoxides with OCN^-. A range of epoxides that are known from previous studies[18] to be accepted by HheC when CN^- is the nucleophile was tested in these experiments (Figure 1).

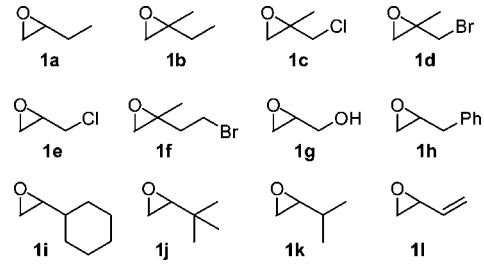
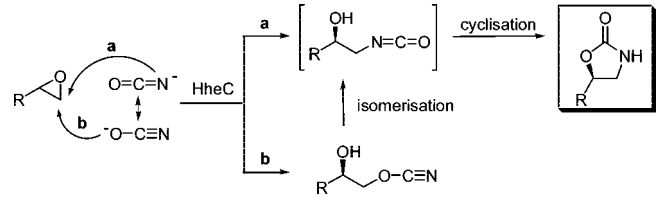


Figure 1. Epoxides tested as substrates in cyanate-mediated ring opening reaction catalysed by HheC.

Enzymatic conversions were first performed on analytical scale. Reactions were monitored by extracting products from the aqueous phase, followed by GC analysis. We found that only five substrates (**1a**−**1e**) were converted at a reasonable rate. Only very low or no activity was observed toward substrates **1f**−**1l** (less than 0.2 μmol•min^{-1}•mg^{-1}). For example, no reaction of NaOCN with **1i** or **1k** took place, even though formation of the cyanoalcohols 3-cyclohexyl-3-hydroxypropionitrile and 3-hydroxy-4-methylpentanenitrile in a CN^--mediated ring opening reaction with the same substrates occurred at a good rate (1.1 and 0.8 μmol•min^{-1}•mg^{-1}, respectively).[19] Previously, the biocatalytic potential of HheC has been explored by using spectrophotometric assays based on reaction of *p*-nitrostyrene oxide with different nucleophiles.[20] It was observed that the enzyme does not catalyze a reaction between this substrate and OCN^-, whereas Br^-, Cl^-, N_3^-, NO_2^-, and CN^- were accepted as nucleophiles, affording the corresponding ring opening products. These results indicate that HheC has a more restricted substrate range when OCN^- is the nucleophile than in azide- and cyanide-mediated ring opening reactions.

Due to the ambident nature of the OCN^-, ring opening of epoxides can proceed *via* nitrogen (**a**) or oxygen (**b**) attack, leading to two isomeric products, β-hydroxy isocyanate and β-hydroxycyanate, respectively (Scheme 2). Organic cyanates

Scheme 2



are unstable compounds and undergo isomerization to isocyanate at room temperature.[21] The reaction is faster in polar than in nonpolar solvents. On the other hand, β-hydroxy isocyanates cannot be isolated, because they spontaneously cyclize to oxazolidinones.

To identify products formed, semipreparative scale reactions (0.50 g, 100−250 mM) were performed (Table 1). Racemic

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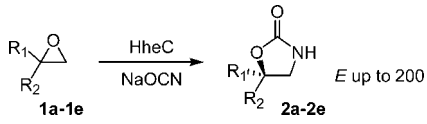
Table 1. Conversion of Epoxides **1a**−**1e** with Cyanate Catalysed by HheC[a]



entry	compd	t (h)	ee_s (%)	product	yield (%)[b]	ee_p (%)[c]	*E*
1	**1a**	5	78	(*R*)-**2a**	47[e]	80	21
2	**1b**	2.5	96	(*R*)-**2b**	44[e]	97	>200
3	**1c**	1	90	(*S*)-**2c**[d]	46[e]	93	85
4	**1d**	1	95	(*S*)-**2d**[d]	47[e]	98	>200
5	**1e**	3	/	(*S*)-**2e**[d]	54[f]	69	-
6	(*S*)-**1e**	1.5	/	(*S*)-**2e**[d]	77[f]	96	-

[a] For reaction conditions and procedures see Supporting Information. [b] Isolated yield. [c] Determined by chiral GC analysis. [d] Apparent inversion of configuration in a series of halogen-containing epoxides compared to alkyl epoxides is due to different substituent priority according to CIP rule, not to a different stereochemical preference of the enzyme. [e] Conversion was 49−50%. [f] Substrate was completely consumed; 100% conversion.

epoxides **1a**−**1e** were converted employing catalytic amounts of purified enzyme (2−5% w/w) in Tris-SO_4 buffer (0.5 M, pH 7.5). Reactions were carried out at room temperature and stopped when substrate conversion approached 50% (entries 1−4) or 100% (entries 5 and 6). After usual workup, enantiomerically enriched products **2a**−**2d** were isolated in high yields, 44−47%, whereas some loss of material was observed with **2e** (entries 5 and 6). In control reactions, in which the enzyme was omitted, formation of products was not observed. The structure of products **2a**−**2e** was confirmed by NMR, MS, and IR analysis. All products show an infrared band at around 1740 cm^{-1}, characteristic for the carbonyl in 2-oxazolidinones. To further prove the structure of the products, racemic **2a**−**2d** were synthesized (**2e** was commercially available) and NMR spectra were found to match the products isolated from the enzymatic reactions.

Formation of 2-oxazolidinones (**2a**−**2e**) was a clean and highly regioselective reaction, with no observable formation of byproduct. None of the two possible intermediates (Scheme 2) could be detected by GC. In each case, the corresponding 5-substituted 2-oxazolidinone was the only detectable product and reactions occurred with no evidence of formation of the other regioisomer. The exclusive formation of 5-substituted isomers indicates that attack at the less substituted position of the oxirane ring was highly predominant. This is consistent with our previous observations on the regioselectivity of HheC.[9,18,19]

Ring opening of racemic epoxides **1a**−**1e** resulted in a kinetic resolution. Conversion of **1b**−**1d** proceeds rapidly to 50%, after which it drastically slows down, indicating high enantioselectivity of the biotransformations. Very high ee's of 5-methyl substituted 2-oxazolidinones **2b**−**2d** were obtained (93−98% ee, entries 2, 3, and 4). HheC was less enantioselective toward monosubstituted epoxides. Ring opening of **1a** and **1e** resulted in moderate enantioselectivity (69−80% ee, entries 1 and 5). As previously observed,[19] HheC exhibited very high enantioselectivity toward 2,2-disubstituted epoxides, with *E* values ($k_{cat,R}/K_{m,R}/(k_{cat,S}/K_{m,S})$ from 85 to over 200. This property makes the enzyme a valuable tool for the preparation of enantiomerically pure tertiary alcohols and, in this case, oxazolidinones with quaternary stereocenters. Conversion of racemic epoxides was achieved within 1−5 h, pointing to a good enzyme activity in such a non-natural reaction.

HheC can catalyze a racemization of the enantiomers of epichlorohydrin (**1e**) when Cl^- is present in the incubation mixture.[22] Upon ring opening by halide, epichlorohydrin is converted to prochiral 1,3-dichloro-2-propanol. Racemization is caused by the reversibility of this ring opening reaction, of which the equilibrium is toward the epoxide. Treatment of highly enantioenriched (*R*)-**1e** with HheC led to nearly racemic epoxide (20% ee) within 1 h (Figure 2). Traces of

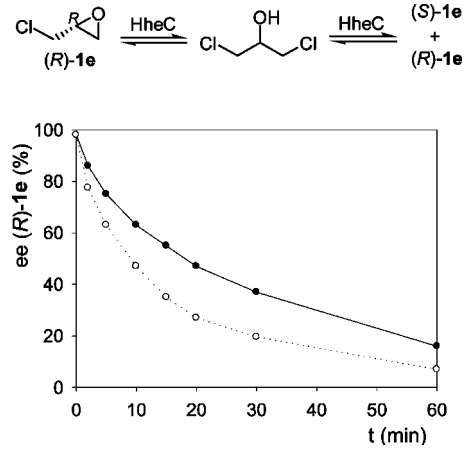


Figure 2. Racemization of (*R*)-**1e** catalysed by HheC. The reaction was carried out with 25 mg (100 mM) of (*R*)-**1e** and 0.75 mg of HheC in 2.5 mL of Tris-SO_4 in the presence (—○—) and absence (—•—) of 25 mM NaCl.

Cl^- present in sample were enough to trigger this reaction. However, the rate of racemization became faster by adding a small amount of Cl^- (Figure 2).

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A ring opening reaction of **1e** with OCN^- resulted in a dynamic kinetic resolution due to the fact that enantioselective ring opening and substrate racemization occurred simultaneously. The reaction of *rac*-**1e** with OCN^- without additional optimization (pH, concentrations) yielded (*S*)-**2e** in 69% ee (Table 1, entry 5) with complete conversion of the epoxide. No NaCl was added to the reaction mixture because the initial amount of Cl^- was sufficient to cause racemization. Besides, by increasing the concentration of Cl^-, the rate of (*S*)-**2e** formation significantly decreased, as OCN^- competes with Cl^-. The low product enantiopurity can be assigned to the modest enantioselectivity of HheC toward **1e**. The low product yields we attribute partially to hydrolytic instability of substrate **1e** but mostly to formation of polymeric material.

Because **1c** and **1d** are homologues of **1e**, a dynamic kinetic resolution could also be performed with these substrates. HheC rapidly racemizes the slower reacting enantiomer (*R*)-**1d**. Surprisingly, in the presence of OCN^-, racemization of (*R*)-**1d** was inhibited. Because of this inhibition of racemization of (*R*)-**1d**, the conversion of *rac*-**1d** presented in Table 1 predominantly has the character of a kinetic resolution. The dynamic character of the resolution improved by adding halide.

Absolute configurations of the faster-reacting epoxide enantiomers were determined using chiral GC by comparing the retention times with standards. The faster reacting enantiomers all have the same relative configuration, but because of the priority switch,[23] the (*R*) enantiomer was the faster reacting one in the case of substrates **1a** and **1b**, and the (*S*) enantiomer was in the case of **1c**−**1e**. These results and our earlier observations[18,19] suggest that the stereochemical preference of the HheC can be described by a general substrate model (Figure 3).

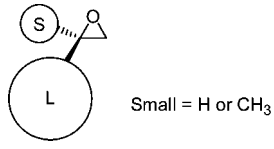

Figure 3. Substrate model for HheC.

A comparison of optical rotation values with literature data revealed the absolute configuration of the products (*R*)-**2a**[16] and (*S*)-**2e**.[13] Additionally, the configuration of oxazolidinone (*S*)-**2e** was confirmed by crystal structure determination.[24] The tentative assignments of absolute stereochemistry were made by analogy within a related series.

A surprising observation that emerged from this study was the narrow epoxide substrate tolerance of HheC when cyanate was used as the nucleophile as compared to when other ring opening anions were used (N_3^-, NO_2^-, CN^-). Because the epoxide-binding catalytic residues, the anion-binding site, and the place where the R-group binds to the enzyme (Scheme 1) can be clearly distinguished in the structure, there is no obvious explanation for this phenomenon. It was also observed that the cyanate anion can cause inhibition of ring opening. One possible explanation could be that cyanate induces an unproductive binding mode similar to the one observed with the nonpreferred (*S*) enantiomer of styrene oxide.[25] Here, the epoxide oxygen is positioned toward the nucleophile instead of the terminal carbon atom of the oxirane ring.

In conclusion, we have found that HheC is a sufficiently active and very enantioselective catalyst for the addition of cyanate to some small terminal epoxides. In this way, 5-substituted 2-oxazolidinones were prepared for the first time by biocatalytic kinetic resolution of epoxides with NaOCN. Preparative scale syntheses could be performed at 15−20 g/L substrate concentrations due to the high substrate concentration tolerance and good enzyme stability. The results further extend the range of nucleophiles that can be used in enzymatic epoxide ring opening reactions, which also includes azide, cyanide and nitrite. Once again, this highly promiscuous halohydrin dehalogenase proves to be valuable new tool for biocatalysis.

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Supporting Information Available: Biocatalytic procedures, analytical methods, chemical synthesis, crystallographic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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