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Published in:
Organic letters

DOI:
10.1021/ol800698t

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2008

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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

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Received March 27, 2008

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. To synthesize these important building blocks in enantiopure form, methods for asymmetric epoxidation of olefins and kinetic resolution of epoxides have been developed, mainly based on the use of chiral catalysts containing transition metals. Biocatalytic methods are also available, including monoxygenase-mediated epoxidation and kinetic resolution of racemic epoxides by epoxide hydrolases. Recently, halohydrin dehalogenases have been explored in the latter conversions. These bacterial enzymes catalyze the reversible conversion of vicinal halohalcohols 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 halohydrin dehalogenases, several nucleophiles were tested. The results of these studies are summarized in Table 1.

halohydrin dehalogenase catalyzed ring opening of epoxides, we have discovered that cyanate (OCN$^-$) is accepted as a nucleophile.\textsuperscript{9} Due to cyanate (OCN$^-$) is accepted as a nucleophile.

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.\textsuperscript{12,13} Dyen and Swern\textsuperscript{14} reported that reaction of potassium cyanate with epoxides in DMF/H$_2$O 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.\textsuperscript{15} In 2005, Bartoli et al. reported a method for the synthesis of enantiopure 5-substituted oxazolidinones by Co$^{II}$salen-catalyzed kinetic resolution, using urethane as the nucleophilic reagent.\textsuperscript{16} Despite the established relevance of oxazolidinones in synthetic chemistry, their preparation by other means has been limited to a few methods.\textsuperscript{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.\textsuperscript{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.\textit{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\textsuperscript{18} to be accepted by HheC when CN$^-$ is the nucleophile was tested in these experiments (Figure 1).

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).\textsuperscript{19} Previously, the biocatalytic potential of HheC has been explored by using spectrophotometric assays based on reaction of p-nitrostyrene oxide with different nucleophiles.\textsuperscript{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 ambient 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 are unstable compounds and undergo isomerization to isocyanate at room temperature.\textsuperscript{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

![Scheme 1. Halohydrin Dehalogenase Catalysed Ring Opening Reactions](Image)

![Figure 1. Epoxides tested as substrates in cyanate-mediated ring opening reaction catalysed by HheC.](Image)

![Scheme 2](Image)
epoxides 1a–1e were converted employing catalytic amounts of purified enzyme (2–5% w/w) in Tris-SO₄ buffer (0.5 M, pH 7.5). Reactions were carried out at room temperature and stopped when substrate conversion approached 50% (entries 1a–1e). Reactions were carried out at room temperature and stopped when substrate conversion approached 50% (entries 1a–1e). Substrate was completely consumed; 100% conversion.

Table 1. Conversion of Epoxides 1a–1e with Cyanate Catalysed by HheC

<table>
<thead>
<tr>
<th>entry</th>
<th>compd</th>
<th>t (h)</th>
<th>eeₐ (%)</th>
<th>product</th>
<th>yield (%)</th>
<th>eeₚ (%)</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1a</td>
<td>5</td>
<td>78</td>
<td>(R)-2a</td>
<td>47⁺</td>
<td>80</td>
<td>21</td>
</tr>
<tr>
<td>2</td>
<td>1b</td>
<td>2.5</td>
<td>96</td>
<td>(R)-2b</td>
<td>44⁺</td>
<td>97</td>
<td>200</td>
</tr>
<tr>
<td>3</td>
<td>1c</td>
<td>1</td>
<td>90</td>
<td>(S)-2c</td>
<td>46⁺</td>
<td>93</td>
<td>85</td>
</tr>
<tr>
<td>4</td>
<td>1d</td>
<td>1</td>
<td>95</td>
<td>(S)-2d</td>
<td>47⁺</td>
<td>98</td>
<td>200</td>
</tr>
<tr>
<td>5</td>
<td>1e</td>
<td>3</td>
<td>/</td>
<td>(S)-2e</td>
<td>54⁻</td>
<td>69</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>(S)-1e</td>
<td>1.5</td>
<td>/</td>
<td>(S)-2e</td>
<td>77⁻</td>
<td>96</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: For reaction conditions and procedures see Supporting Information. Isolated yield. Determined by chiral GC analysis. 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. Conversion was 49–50%. Substrate was completely consumed; 100% conversion.

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-SO₄ 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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References:
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.](image)

A comparison of optical rotation values with literature data revealed the absolute configuration of the products (R)-2a\(^\text{16}\) 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.

**Acknowledgment.** This work was supported by the Croatian Ministry of Science, Education and Sports (project 0982933-2908).

**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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\(^{(23)}\) Cahn-Ingold-Prelog rule.

\(^{(24)}\) For more details, see Supporting Information.