Chemoenzymatic Halocyclization of γ,δ-Unsaturated Carboxylic Acids and Alcohols


A chemoenzymatic method for the halocyclization of unsaturated alcohols and acids by using the robust V-dependent chloroperoxidase from Curvularia inaequalis (CVCP) as catalyst has been developed for the in situ generation of hypohalites. A broad range of halolactones and cyclic haloethers are formed with excellent performance of the biocatalyst.

Halolactonization reactions are well-established in organic synthesis.[1] The established synthetic routes use a variety of catalysts and halide sources. N-Bromosuccinimide, for example, is commonly used.[2] The resulting byproduct, however, is often difficult to recover from the reaction mixture and ends up as waste. Moreover, elementary halides are used, which poses questions of safety and corrosion.[3] Recently, Oxone was proposed as an alternative means of producing electrophilic bromine species from bromide.[4] Although this method avoids organic waste, it still produces significant amounts of inorganic salts (sulfates) as waste. Other catalytic methods to generate BrO⁻ rely on catalysts such as organic tellurides,[5] selenides,[6] or Cu catalysts.[7] Haloetherification of alkenols is similarly difficult to achieve.[8]

Haloperoxidases (E.C. 1.11.1.1) represent an interesting alternative to the aforementioned chemical means to generate electrophilic halide species from halides and hydrogen peroxide. These catalytic cycles.

First, we evaluated the influence of several reaction parameters, such as pH and reagent concentration, on the efficiency of the bromolactonization of 4-pentenoic acid. In accordance with our previous findings,[10, 11, 13] the reaction proceeded optimally at pH 5 (with more than 80% activity at both pH 7 and pH 4; Table 1). Although this behavior can most likely be attributed to the pH-dependency of the biocatalyst, the protonation stage of the carboxylate group may also play a role here. Reactions in non-buffered media were less efficient, most probably owing to the alkalization of the reaction medium in the course of the reaction. The concentrations of bromide and H₂O₂ both directly influenced the conversion of the reaction. Performing the reaction in the absence of the biocatalyst did not result in any significant conversion within the timeframe of the experiment.

A typical time course of the chemoenzymatic bromolactonization is shown in Figure 1. Very pleasingly, CVCP performed more than 5 catalytic cycles per second and at least 325 000 catalytic cycles.

Next, we further evaluated the product scope of the chemoenzymatic halolactonization reaction (Table 2). Pleasingly, all starting materials were converted with good to excellent conversions into the corresponding halolactones. In particular, the cyclohexene-derived (enantiothermally pure) products may
serve as building blocks for a range of natural products.\textsuperscript{[14]} The selectivity of the reaction was generally satisfactory with the corresponding hydroxy lactone as the sole byproduct.\textsuperscript{[15]}

The relative configuration for product 10a was established based on coupling constants and NOE experiments. The NOE correlation between H-5 (m, d\textsubscript{H} 4.51–4.48) and H-6b (dd, J\textsubscript{1,2} = 12.7, 5.0 Hz, 1H) suggested the same orientation of H-5 and H-6b. The NOE correlations between H-5 and the H-4b (m, d\textsubscript{H} 1.97–1.89), as well as the methyl group at 1.25 ppm indicated protons located in the same orientation (see the Supporting Information, Figures S7 and S8).

To demonstrate the preparative feasibility, we performed the chloro-, bromolactonization of 4-pentenoic acid and bromolactonization of 2-methyl-4-pentenoic acid at 10 mmol scale. 0.9, 1.4, and 1.15 g of the desired chloro- and bromolactone products were isolated corresponding to 70, 80, and 60% yields, respectively, as well as 0.58 g (30%) of hydroxy lactone in the case of bromolactonization of 2-methyl-4-pentenoic acid.

One apparent drawback of the current chemoenzymatic halolactonization reaction lies with the nonselective chemical step producing racemic lactones. We therefore envisioned complementing the halolactonization reaction with a hydrolyase-catalyzed kinetic resolution step (Scheme 2). In total, 9 commercial and self-made hydrolases were screened. However, none of the enzymes exhibited an enantioselectivity high enough for efficient kinetic resolution (Figures S56 and S57).

It is ongoing to obtain a more enantioselective and hence, practical catalyst.

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### Table 1. Influence of pH and reagent concentration on the chemoenzymatic bromolactonization of 4-pentenoic acid.

<table>
<thead>
<tr>
<th>pH</th>
<th>KBr [mM]</th>
<th>H\textsubscript{2}O\textsubscript{2} [mM]</th>
<th>Conversion [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>160</td>
<td>170</td>
<td>39</td>
</tr>
<tr>
<td>3</td>
<td>160</td>
<td>170</td>
<td>40</td>
</tr>
<tr>
<td>4</td>
<td>160</td>
<td>170</td>
<td>80</td>
</tr>
<tr>
<td>5</td>
<td>160</td>
<td>170</td>
<td>99</td>
</tr>
<tr>
<td>7</td>
<td>160</td>
<td>170</td>
<td>90</td>
</tr>
<tr>
<td>9</td>
<td>160</td>
<td>85</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>80</td>
<td>170</td>
<td>40</td>
</tr>
<tr>
<td>9\textsuperscript{[c]}</td>
<td>160</td>
<td>170</td>
<td>–</td>
</tr>
</tbody>
</table>

General conditions: c(4-pentenoic acid) = 40 mM; 100 mM citrate buffer (pH 5); c(CiVCPO) = 100 mM; T = 25°C; t = 24 h. Other buffers used: acetate (pH 3), citrate (pH 4), potassium phosphate (pH 7) and Tris buffer (pH 9); a: double distilled water, unbuffered; b: reaction performed in the absence of CiVCPO.

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### Table 2. Preliminary product scope of the chemoenzymatic halolactonization of \( \gamma,\delta \)-unsaturated carboxylic acids.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Product</th>
<th>Conversion [%][a] (Selectivity [%])</th>
</tr>
</thead>
<tbody>
<tr>
<td>[\text{a: } X = \text{Br} ]</td>
<td>1</td>
<td>( &gt; 99 ) (67) ( &gt; 99 ) (67)</td>
</tr>
<tr>
<td>[\text{b: } X = \text{Cl} ]</td>
<td>2</td>
<td>( &gt; 99 ) (64) ( &gt; 99 ) (65)</td>
</tr>
<tr>
<td>3</td>
<td>9, a, b</td>
<td>( &gt; 99 ) (72) ( &gt; 99 ) (68)</td>
</tr>
<tr>
<td>4</td>
<td>10, a, b</td>
<td>( &gt; 99 ) (57) ( &gt; 99 ) (56)</td>
</tr>
<tr>
<td>5</td>
<td>11, a, b</td>
<td>( &gt; 99 ) (56) ( &gt; 99 ) (70)</td>
</tr>
<tr>
<td>6</td>
<td>12, a, b</td>
<td>( &gt; 99 ) (70) ( &gt; 99 ) (62)</td>
</tr>
<tr>
<td>7</td>
<td>13, a, b</td>
<td>( &gt; 99 ) (80) ( 86 ) (82)</td>
</tr>
<tr>
<td>8</td>
<td>14, a, b</td>
<td>( &gt; 99 ) (79) ( 80 ) (87)</td>
</tr>
</tbody>
</table>

General conditions: c(substrate) = 40 mM; c(H\textsubscript{2}O\textsubscript{2}) = 100 mM; c(KX) = 160 mM; 100 mM citrate buffer (pH 5); c(CiVCPO) = 100 mM; T = 25°C; t = 24 h. [a] determined by NMR spectroscopy (see the Supporting Information for spectra and further details).
Finally, we investigated the possibility of performing halo-etherification reactions in the current setup. Assuming the intermediate halonium ion is sufficiently stable under the aqueous conditions, we reasoned that intramolecular etherifications should be feasible (Scheme 3).

The proof-of-concept reaction proceeded smoothly to full conversion (Figure 2). Overall 36 mm of 2-(bromomethyl)tetrahydro-2H-pyran were obtained within 24 h, corresponding to a turnover number of more than 360 000 for the biocatalyst.

Indeed, with all commercially available alkenols tested, we found significant formation of the expected cyclic ethers (Scheme 4). As in case of the lactonization reactions, the sole byproducts observed in these reactions were the hydroxyethers (X=OH). The relative configuration of compound 19a was determined depending upon NOE correlations. Based on the structure of the starting material (−)-carveol, the NOE correlation of CH3-2 and H-3 indicated the positioning of these functional groups on the same side (Figure S27).

Preparative scale reactions of some selected alkenols were performed at 10 mmol scale. For example (−)-carveol and (±)-citronellol were converted almost quantitatively, albeit at lower selectivity than shown in Scheme 4. After 24 h, the desired products were isolated in 60 and 50% yield, respectively.

In the current contribution, we have expanded the scope of CiVCPO as a biocatalyst for organic synthesis. A semiquantitative comparison \[17\] of the proposed chemoenzymatic halolactonization reaction.
nalization and haloetherification reaction with established protocols demonstrates its potential environmental benefits (Table 3). The mass intensities of the chemical and chemoenzymatic reactions are comparable. However, the quality of the reaction mixture was extractable by ethyl acetate (3 × 100 mL), dried over anhydrous Na2SO4. The combined organic layers were concentrated under reduced pressure. The products were purified by flash column chromatography on silica gel (EtOAc/hexanes, 1:2). 1.38 g of 7-(bromomethyl)-4,7-dimethyl-6-oxabicyclo[3.2.1]oct-3-ene was isolated with 70, 80, and 60% yield, respectively, as well as hydroxylactone in 30% yield in the case of bromolactonization of 2-methyl-4-pentenoic acid and analyzed by NMR spectroscopy.

Preparative-scale chloro- and bromolactonization reactions

The reaction was performed in a 100 mL Erlenmeyer flask at room temperature with stirring. The reaction medium consisted of 0.1 M citrate buffer (pH 5, final volume of 50 mL) with 160 mM of KBr or KCl, 4-pentenoic acid or 2-methyl-4-pentenoic acid (10 mmol), and 100 nM CVCPO. The reaction was started by the addition of 100 mM of H2O2. After 24 h the reaction mixture was acidified, extracted with dichloromethane (3 × 100 mL), and dried over anhydrous Na2SO4. The combined organic layers were concentrated under reduced pressure. The reaction product contained 70, 80, and 60% yield, respectively, as well as hydroxylactone in 30% yield in the case of bromolactonization of 2-methyl-4-pentenoic acid and analyzed by NMR spectroscopy.

Preparative-scale synthesis of 7-(bromomethyl)-4,7-dimethyl-6-oxabicyclo[3.2.1]oct-3-ene (19a)

The reaction was performed in a 100 mL Erlenmeyer flask at room temperature with stirring. The reaction medium consisted of 0.1 M citrate buffer (pH 5, final volume of 50 mL) with 160 mM of KBr, 10 mmol carveol and 100 nM CVCPO. The reaction was started by the addition of 100 mM of H2O2. After 24 h the reaction mixture was extracted by ethyl acetate (3 × 100 mL), dried over anhydrous Na2SO4. The combined organic layers were concentrated under reduced pressure. The reaction product contained 70, 80, and 60% yield, respectively, as well as hydroxylactone in 30% yield in the case of bromolactonization of 2-methyl-4-pentenoic acid and analyzed by NMR spectroscopy.

Preparative-scale of 2-(2-bromopropan-2-yl)-5-methyloxepane (20a)

The reaction was performed in a 10 mL Erlenmeyer flask at room temperature with stirring. The reaction medium consisted of 0.1 M citrate buffer (pH 5, final volume of 50 mL) with 160 mM of KBr, 10 mmol (+)-4-pentenoic acid and 100 nM CVCPO. The reaction was started by the addition of 100 mM of H2O2. After 24 h the reaction mixture was extracted by ethyl acetate (3 × 100 mL), dried over anhydrous Na2SO4. The combined organic layers were concentrated under reduced pressure. The reaction product contained 70, 80, and 60% yield, respectively, as well as hydroxylactone in 30% yield in the case of bromolactonization of 2-methyl-4-pentenoic acid and analyzed by NMR spectroscopy.

Table 3. Semiquantitative comparison of the mass intensity of the chemical and the chemoenzymatic bromolactonization reaction.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Chemical process</th>
<th>Chemoenzymatic process</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH2Cl2</td>
<td>29.9</td>
<td>35.2</td>
</tr>
<tr>
<td>H2O</td>
<td>citrate</td>
<td>0.67</td>
</tr>
<tr>
<td>NBS</td>
<td>1</td>
<td>0.12/0.67</td>
</tr>
<tr>
<td>mol. sieve</td>
<td>0.05</td>
<td>0.00016</td>
</tr>
</tbody>
</table>

Agents and waste products differ significantly. In the case of chemical synthesis, methylene chloride as solvent is questionable, especially compared to simple citric acid buffer. Furthermore, stoichiometric amounts of succinimide, the recycling of which necessitates further down-stream processing (DSP) steps, is formed as a byproduct in the chemical process, whereas the chemoenzymatic process yields water (and unreacted bromide) as byproduct. Finally, the catalyst consumption of both processes also differs significantly.

Following the established method, the present procedure entailed extraction of the products with dichloromethane, which obviously is questionable from an environmental point-of-view. Therefore, future efforts will concentrate on the substitution of CH2Cl2 with more acceptable alternatives, such as ethyl acetate. A particular focus will lie on the intensification of the reaction, that is, increasing the substrate loading (and consequently also the product concentration). This will reduce the relatively large E-factor contribution of the solvent.

Overall, we are convinced that the proposed chemoenzymatic method for halocyclization represents a promising alternative to established chemical procedures. Further upscaling and characterization of the reaction is currently ongoing in our laboratory.

Experimental Section

A detailed description of the biocatalyst preparation and purification as well as a complete description of the experimental and analytical procedures can be found in the Supporting information.

Halocyclization of γ,δ-unsaturated carboxylic acids and alcohols

The halocyclization reactions were performed by using 1 mL glass vials containing 40 mM unsaturated acids, and/or alcohols in 0.1 M citrate buffer (pH 5) with 160 mM KBr and 100 nM CVCPO. Reactions were started by the addition of 100 mM of H2O2 and stirred by a magnetic bar at 500 rpm for 24 h. The reaction mixtures were extracted with ethyl acetate (1 mL; containing 5 mM acetonaphone as an internal standard), dried over anhydrous MgSO4, and analyzed by GC (Shimadzu; see Table S1).

Preparative-scale chloro- and bromolactonization reactions

The reaction was performed in a 100 mL Erlenmeyer flask at room temperature with stirring. The reaction medium consisted of 0.1 M citrate buffer (pH 5, final volume of 50 mL) with 160 mM of KBr or KCl, 4-pentenoic acid or 2-methyl-4-pentenoic acid (10 mmol), and 100 nM CVCPO. The reaction was started by the addition of 100 mM of H2O2. After 24 h the reaction mixture was acidified, extracted with dichloromethane (3 × 100 mL), and dried over anhydrous Na2SO4. The combined organic layers were concentrated under reduced pressure. The chloro- and bromolactone products were purified by flash column chromatography on silica gel (EtOAc/hexanes, 1:2). 1.38 g of 7-(bromomethyl)-4,7-dimethyl-6-oxabicyclo[3.2.1]oct-3-ene (19a) was isolated with 60% yield and analyzed by NMR spectroscopy.

Preparative-scale synthesis of 7-(bromomethyl)-4,7-dimethyl-6-oxabicyclo[3.2.1]oct-3-ene (19a)

The reaction was performed in a 100 mL Erlenmeyer flask at room temperature with stirring. The reaction medium consisted of 0.1 M citrate buffer (pH 5, final volume of 50 mL) with 160 mM of KBr, 10 mmol carveol and 100 nM CVCPO. The reaction was started by the addition of 100 mM of H2O2. After 24 h the reaction mixture was extracted by ethyl acetate (3 × 100 mL), dried over anhydrous Na2SO4. The combined organic layers were concentrated under reduced pressure. The reaction product contained 70, 80, and 60% yield, respectively, as well as hydroxylactone in 30% yield in the case of bromolactonization of 2-methyl-4-pentenoic acid and analyzed by NMR spectroscopy.

Preparative-scale of 2-(2-bromopropan-2-yl)-5-methyloxepane (20a)

The reaction was performed in a 10 mL Erlenmeyer flask at room temperature with stirring. The reaction medium consisted of 0.1 M citrate buffer (pH 5, final volume of 50 mL) with 160 mM of KBr, 10 mmol (+)-4-pentenoic acid and 100 nM CVCPO. The reaction was started by the addition of 100 mM of H2O2. After 24 h the reaction mixture was extracted by ethyl acetate (3 × 100 mL), dried over anhydrous Na2SO4. The combined organic layers were concentrated under reduced pressure. The reaction product contained 70, 80, and 60% yield, respectively, as well as hydroxylactone in 30% yield in the case of bromolactonization of 2-methyl-4-pentenoic acid and analyzed by NMR spectroscopy.

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Conflict of interest

The authors declare no conflict of interest.

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You had me at “halo”: A chemoenzymatic method for the halocyclization of unsaturated alcohols and acids by using a robust V-dependent chloroperoxidase as catalyst has been developed for the in situ generation of hypohalites. A broad range of halolactones and cyclic haloethers are formed with excellent biocatalyst performance.