



UvA-DARE (Digital Academic Repository)

Chemoenzymatic Halocyclization of 4-Pentenoic Acid at Preparative Scale

Höfler, G.T.; But, A.; Younes, S.H.H.; Wever, R.; Paul, C.E.; Arends, I.W.C.E.; Hollmann, F.

DOI

[10.1021/acssuschemeng.9b07494](https://doi.org/10.1021/acssuschemeng.9b07494)

Publication date

2020

Document Version

Final published version

Published in

ACS Sustainable Chemistry & Engineering

License

CC BY-NC-ND

[Link to publication](#)

Citation for published version (APA):

Höfler, G. T., But, A., Younes, S. H. H., Wever, R., Paul, C. E., Arends, I. W. C. E., & Hollmann, F. (2020). Chemoenzymatic Halocyclization of 4-Pentenoic Acid at Preparative Scale. *ACS Sustainable Chemistry & Engineering*, 8(7), 2602-2607. <https://doi.org/10.1021/acssuschemeng.9b07494>

General rights

It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: <https://uba.uva.nl/en/contact>, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

Chemoenzymatic Halocyclization of 4-Pentenoic Acid at Preparative Scale

Georg T. Höfler, Andrada But, Sabry H. H. Younes, Ron Wever, Caroline E. Paul, Isabel W. C. E. Arends, and Frank Hollmann*

Cite This: *ACS Sustainable Chem. Eng.* 2020, 8, 2602–2607

Read Online

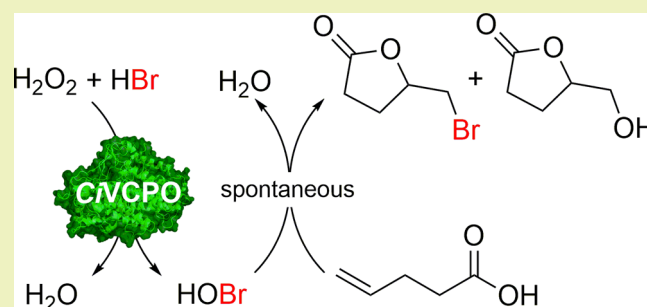
ACCESS |

Metrics & More

Article Recommendations

Supporting Information

ABSTRACT: The scale-up of chemoenzymatic bromolactonization to 100 g scale is presented, together with an identification of current limitations. The preparative-scale reaction also allowed for meaningful mass balances identifying current bottlenecks of the chemoenzymatic reaction.



KEYWORDS: Chemoenzymatic halocyclization, Preparative scale, Haloperoxidase

INTRODUCTION

Activated, electrophilic halogens are common oxidants in organic synthesis.^{1–3} Their high reactivity, however, poses challenges when used as stoichiometric agents. Due to their high instability, their application comes along with considerable safety issues. Furthermore, stoichiometric amounts of salt waste formed during the reaction (or during workup) pose an environmental issue. Finally, undesired side reactions are often observed. The latter issue is frequently met using halogen precursors such as *N*-bromosuccinimide (NBS) which gradually release the activated halide species into the reaction. In this case, the problem of waste formation is even more pronounced.

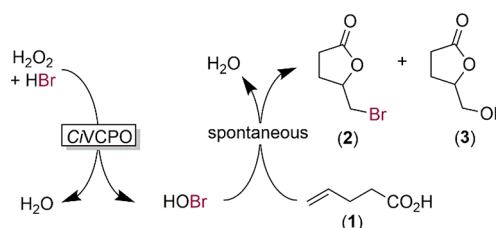
An alternative approach is to generate hypohalites *in situ* (i.e., in the reaction mixture) from the corresponding halide and hydrogen peroxide. Common catalysts comprise chalcogens,^{4–7} transition metal catalysts,⁸ and enzymatic methods.¹ The use of haloperoxidases for the *in situ* generation of hypohalites is gaining interest in organic synthesis.¹ Haloperoxidases catalyze the clean, H₂O₂-dependent oxidation of halides to the corresponding hypohalites. Specifically, the vanadium-dependent chloroperoxidase from *Curvularia inaequalis* (CVCPO) excels by its extraordinary robustness and its exceptional catalytic activity.^{9–16}

The oxidative halolactonization of γ,δ -unsaturated carboxylic acids is a popular application of activated halides, among others in natural product synthesis.^{2,17–19} Very recently, we have demonstrated that CVCPO is an efficient catalyst to initiate the chemoenzymatic halolactonization of γ,δ -unsaturated carboxylic acids (Scheme 1).⁹ The resulting halo-

functionalized lactones may be interesting building blocks for functionalizable polyesters.

The aim of this study was to scale up bromolactonization of 4-pentenoic acid from laboratory scale (1 mL, <50 mM substrate) to preparative scale and to assess the environmental impact. To determine the environmental impact of the reaction, the E⁺-factor,²⁰ a recent extension of Sheldon's E-

Scheme 1. Chemoenzymatic Bromolactonization of 4-Pentenoic Acid (1) to 5-(Bromomethyl)dihydrofuran-2(3H)-one (2) and (Undesired) 5-(Hydroxymethyl)dihydrofuran-2(3H)-one (3) Using V-Dependent Chloroperoxidase from *Curvularia inaequalis* (CVCPO) as Catalyst for *in situ* Generation of Hypobromite from Bromide and H₂O₂



Received: December 16, 2019

Revised: January 24, 2020

Published: January 31, 2020

factor^{21,22} that takes energy-related CO₂ emissions into account, was used.

RESULTS AND DISCUSSION

First, the substrate loading was increased in order to reduce the amount of wastewater formed in the reaction.²³ Increasing the substrate concentration from 40 to 500 mM, however, resulted in low product formation (7 mM total product, Figure S2). Further experiments revealed that the biocatalyst CiVCPO was prone to a pronounced substrate inhibition (Figure 1). In the

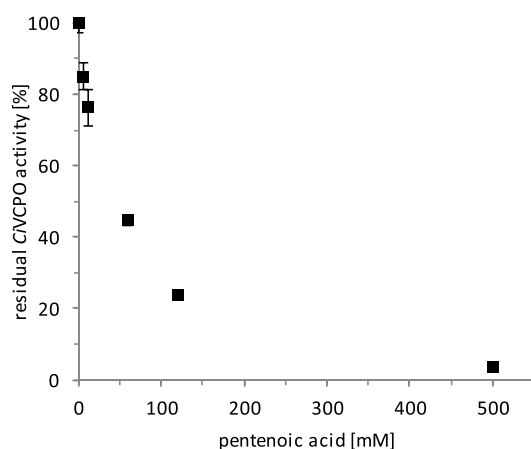


Figure 1. Inhibition of CiVCPO by the starting material (4-pentenoic acid). A spectrophotometric assay based on the bromination of monochlorodimedone (MCD) was used. Assay conditions: [KBr] = 5 mM, [H₂O₂] = 5 mM, [CiVCPO] = 38 nM, [MCD] = 50 μM, [Na₃VO₄] = 100 μM in 100 mM citrate buffer (pH 5), T = 25 °C; after mixing of all reagents in a cuvette, the absorption at 290 nm was followed for 1 min. For activity determinations, a molar extinction coefficient of 20.2 mM⁻¹ cm⁻¹ of the depleting starting material was used.²⁶

presence of 60 mM 4-pentenoic acid, the biocatalyst's activity was reduced to less than half of its maximal value. At 500 mM, there was almost no enzyme activity detectable. Comparative experiments using 4-pentenoic acid gave similar results indicating that high concentrations of carboxylic acids (possibly via coordination to the V-prosthetic group)^{24,25} inhibit CiVCPO.

Besides the inhibition by 4-pentenoic acid, it is known that bromide inhibits the enzyme.²⁷ Further, the undesired spontaneous reaction between hypohalites and H₂O₂ yielding ¹O₂ calls for gradual addition of H₂O₂.¹⁵ We therefore decided to apply a fed-batch strategy adding 4-pentenoic acid together with Br⁻ and H₂O₂ over time and limit their concentrations (and inhibitory effects). At the same time, to reduce the (undesired) spontaneous hydrolysis of the bromolactone product into the corresponding acid,²⁸ an *in situ* product removal strategy was used. For this, we chose the well-known two liquid phase system (2LPS) approach wherein a water-immiscible organic solvent serves as the product sink. Several organic solvents were evaluated with respect to their effect on the stability of the biocatalyst (Figure 2).

Most solvents did not significantly influence the robustness of CiVCPO; only methyl isobutyl ketone (MIBK) seemed to have a negative effect on the enzyme, as upon prolonged incubation time (24 h) the activity was reduced by more than 60%. All other solvents tested had no or even a slightly beneficial effect. For further experiments, we chose ethyl

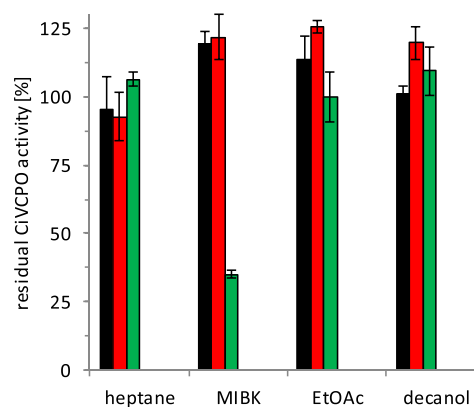


Figure 2. Solvent stability of CiVCPO in biphasic system. Incubation conditions: [CiVCPO] = 700 nM in 100 mM citrate buffer (pH 5), [Na₃VO₄] = 100 μM, and T = 30 °C in the presence of one aliquot of the organic solvent. The mixtures were shaken at 500 rpm in a thermoshaker. Directly after mixing (black bar) and after 4 h (red bar) and 24 h (green bar), samples were taken from the aqueous layer and analyzed as described in Figure 1. Values shown represent the ratio of the residual activity of CiVCPO in the presence of solvent to the residual activity of CiVCPO in buffer only under otherwise identical incubation conditions.

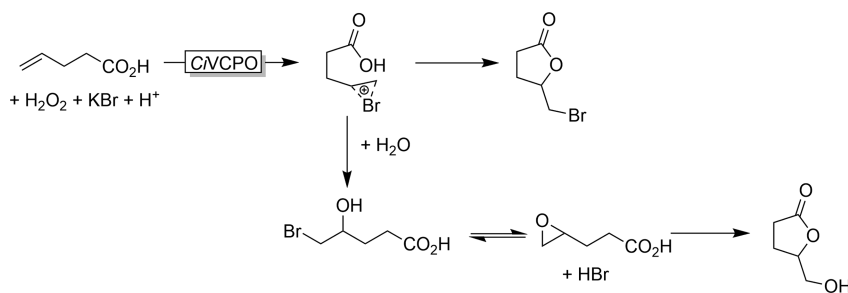
acetate (EtOAc), since it is generally considered as “green”²⁹ and—more importantly for us—showed good solubilization properties for the product. Ethyl acetate exhibits the lowest boiling point of all solvents tested, an important parameter in the recovery process (by distillation) of the product and the recycling of the organic phase.

Further optimizations of some reaction parameters such as feed rates were performed at 500 mL scale. In summary, feeding the neat substrate (9.8 M) at 4 mL h⁻¹ together with a feed rate of 37.5 mL h⁻¹ of KBr (1.2 M) and H₂O₂ (2M), respectively, turned out to give reasonable productivities of more than 2.3 mM h⁻¹, corresponding to an average turnover frequency of CiVCPO of more than 10 s⁻¹ (Figure S3). It should be mentioned that in these experiments a significant amount of product 3 (hydroxylactone), accounting for approximately one-third of the total product, was produced. Comparative experiments showed that the bromolactone (2) was stable under the reaction conditions; even upon prolonged incubation in a buffer, no conversion of 2 into 3 was observed. We hypothesize that 3 originates from hydrolysis of the intermediate bromonium ion to the corresponding hydroxyl bromide product; the latter is in equilibrium with the corresponding epoxide¹¹ from which through intramolecular attack by the carboxylate the hydroxylactone may be formed (Scheme 2). Further experiments will clarify the origin of the (seemingly undesired) side reaction.

Also, rather unexpectedly considering the high boiling temperature of 4-pentenoic acid of greater than 180 °C, in these experiments, we observed substrate evaporation, which could be solved by using a condenser cooled to approximately 5 °C.

Next, we proceeded to a 10 L-scale (in a 15 L reactor) reaction using 5 L each of the aqueous reaction mixture (d₂O) and of the ethyl acetate organic phase. For safety reasons, to eliminate the possibility of an explosion arising from O₂/ethyl acetate mixtures, the headspace was constantly flushed with a gentle stream of N₂ gas. The time course of this up-scaled reaction is shown in Figure 3. To compensate for the

Scheme 2. Hypothesized Mechanism for Formation of Hydroxylactone



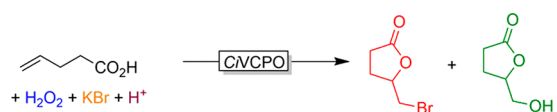
expected pH increase, we used a pH control (acetic acid) to maintain the optimal operational pH of CiVCPO (pH 5).

In total, 1425 mmol of 4-pentenoic acid (1) and equimolar amounts of KBr were fed to the reaction and were converted into 1291 mmol products 2 and 3 (molar ratio approximately 2:1), corresponding to more than 90% yield. The yield in H₂O₂ was less impressive (26%), which we attribute to the undesired reaction of H₂O₂ with hypobromite discussed above. In the course of the reaction, we observed some inactivation of CiVCPO, which was compensated by supplementation with fresh CiVCPO. As CiVCPO generally is a very robust enzyme,^{13,27} we suspect the rigorous stirring as a possible reason for the reduced stability of CiVCPO, but further in-depth studies will be necessary to fully understand the reasons for the reduced CiVCPO stability. Nevertheless, a total turnover number of the enzyme of more than 715,000 [$\text{mol}_{\text{product}} \times \text{mol}^{-1}_{\text{CiVCPO}}$] was achieved corresponding to the production of more than 770 g of product 2 per gram of CiVCPO.

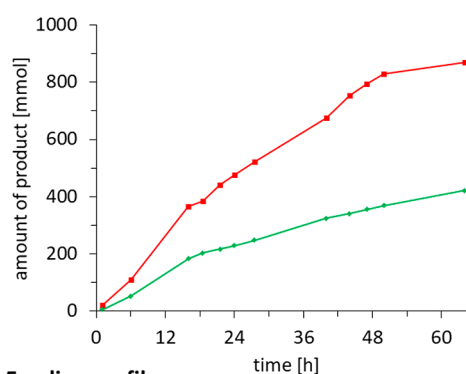
The product was isolated *via* separation of the organic phase from the reaction phase by centrifugation, drying of MgSO₄, and concentration under reduced pressure. The crude product contained acetic acid and the (undesired) hydroxylactone (3). Both could be largely removed by treatment with caustic water followed by drying. Overall, 81.4 g of bromolactone (2) was obtained with this procedure.

Having quantitative data from the 15 L-scale fermentation of CiVCPO as well as the 10 L-scale bromolactonization at hand, we estimated the environmental impact of the proposed chemoenzymatic production of 2. Sheldon's E-factor is a very common, simple approach to assess the environmental impact of lab-scale reactions if the data basis does not allow for a full life cycle assessment.^{21,22} The classical E-factor, however, does not take into account CO₂ emissions caused by energy consumed in the process. We therefore used the recently proposed E⁺-factor²⁰ and also measured the electricity used whereas possible. To estimate the electricity-related CO₂ emissions, we used the average European CO₂ footprint (i.e., 404 g CO₂ kWh⁻¹).³⁰ The E⁽⁺⁾-factor contributions of the single fermentation and reaction components are listed in Tables 1 and 2, respectively.

At first sight, the E⁺-factor of CiVCPO is shockingly high, as, for example, more than 30 tons of CO₂ per kg of enzyme have been emitted. Also the water consumption is very high. Several factors, however, should be considered here. On the one hand, the overexpression of CiVCPO is very low (less than 50 mg L⁻¹ fermentation broth); optimized expression systems will certainly yield higher enzyme titers and lower E⁺-factors. Purification also greatly contributed to the overall waste generation, which is why we will consider whole *E. coli* cells in



A) Time course



B) Feeding profile

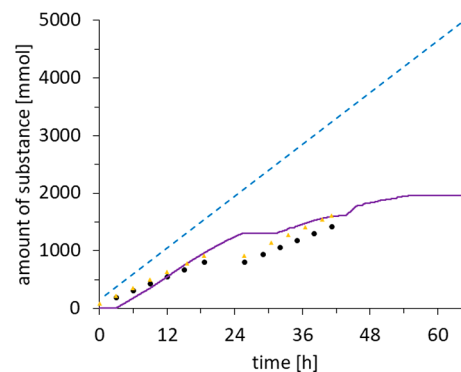


Figure 3. Ten liter-scale bromolactonization of 4-pentenoic acid. (A) Time course showing absolute amounts of 2 (red square) and 3 (green diamond). (B) Substrate feeding profile (cumulative feed): (black circle) pure 4-pentenoic acid 9.798 M, 4.04 mL/h; (yellow triangle) KBr 1.2 M, 37.5 mL/h; (blue dashed line) H₂O₂ 2 M, 37.5 mL/h; pH 5 controlled by pH stat with (purple line) 2 M acetic acid. General conditions: Feeds were started 2 h prior to first CiVCPO addition; biphasic system with 5 L EtOAc and a total end volume of 9.84 L; *T* = 25 °C; 50 rpm after 24 h to 75 rpm; VCPO aliquots added (0.3 μmol) 0, 6, 21.5, 27.5, 41.5, and 64 h. Note that the results shown originate from a single experiment. At the start of the reaction, a malfunction of the pH stat lead to hyperacidification of the reaction medium irreversibly inactivating the biocatalyst present at the start of the reaction. Therefore, at *t* = 2 h, fresh CiVCPO was added (reaction volume = 1.63 L, *c*(4-pentenoic acid) = 49 mM, *c*(KBr) = 55 mM, *c*(H₂O₂) = 92 mM).

future applications of the enzyme. Also, the environmental impact of enzyme fermentation is subject to scaling effects and

Table 1. Estimation of E⁺-Factor for Production of CiVCPO^a

Component	Absolute amount	E ⁽⁺⁾ -factor contribution (g _{waste} g ⁻¹ _{CiVCPO})
Fermentation		
Media ^b	805 g	1.188
H ₂ O	14.000 g	20.650
Cryostat	25.6 kWh (10.3 kg CO ₂)	15.278
Stirring and heating	5.68 kWh (2.3 kg CO ₂)	3.386
Autoclaving	4.51 kWh (1.8 kg CO ₂)	2.689
Centrifugation	16 kWh (6.5 kg CO ₂)	9.541
Sum		52.732
Purification		
Buffer components	25 g	37
H ₂ O	3.320 g	4.900
Total energy ^c	20.87 kWh (8.4 kg CO ₂)	12.445
Sum		17.382
Total E⁺-factor		70.114

^aBased on a total yield of 0.678 g of purified CiVCPO. ^bYeast extracts, sugars, buffers etc. ^cComprising French press breaking of the cells, centrifuges, pumps, and temperature control).

Table 2. Estimation of E⁺-Factor for Production of 2^a

Component	Absolute amount	E-factor contribution (g g ⁻¹ _{product})
Ethyl acetate	4470 g	55
Water	4839 g	59
CiVCPO	0.1 g	86 ^b
Reagents ^c	355 g	4
Cryostat	29.34 kWh (11.9 kg CO ₂)	146
Stirring/pump	0.85 kWh (343 g CO ₂)	4
Sum		354

^aBased on a yield of 81.5 g of 2. ^bTaking the E⁺-factor of CiVCPO (Table 1) into account. ^cNot reacted, byproducts, pH control.

hence may be significantly smaller at industrial scale.^{31,32} Finally, it should be kept in mind that CiVCPO is not the final product but rather the catalyst. Hence, due its excellent performance in the bromolactonization reaction, the immense E⁺-factor of CiVCPO is reduced by 3 orders of magnitude (*vide infra*).

Overall, a waste generation of approximately 354 kg per kg of the desired product (2) was generated. CO₂ thereby comprised approximately two-thirds of the overall waste

generated underlining the importance of taking energy-related emissions (wastes) into account.

CONCLUSION

In this Letter, we demonstrate that chemoenzymatic bromolactonization is indeed a practical alternative to the established chemical methods. From a safety point-of-view, we believe that the *in situ* generation of hypobromite is advantageous over its stoichiometric use or the use of elementary bromine. Admittedly, H₂O₂ is not unproblematic but overall easier to handle than HOBr. Furthermore, *in situ* generation of H₂O₂ is principally feasible and may open new avenues for bromolactonization reactions.^{33,34}

The numbers presented here underline the necessity to add energy considerations to the “classical” E-factor. The CO₂ emissions caused by cooling, stirring, and distillation show that these contributions are in the same order of magnitude as the “simple” mass balances. We also believe that the E⁺-factor can be used as a starting point for further improvements. For example, the contribution of the biocatalyst (its fermentation) underlines the need for better expression systems for CiVCPO as higher enzyme titers will immediately reduce its E⁺-factor (contribution). Further process intensification should aim at increasing the product concentrations, which will lead to drastically reduced environmental impacts.

Concerning the product, we envision utilization as a building block for functionalizable polyesters. Lipase-catalyzed polymerization reactions are currently ongoing in our laboratory. The imperfect selectivity of the lactonization step (yielding bromo- and hydroxylactone) may prove as an advantage from a polymer modification point-of-view as it offers two different functionalities for further modification (Scheme 3).

ASSOCIATED CONTENT

Supporting Information

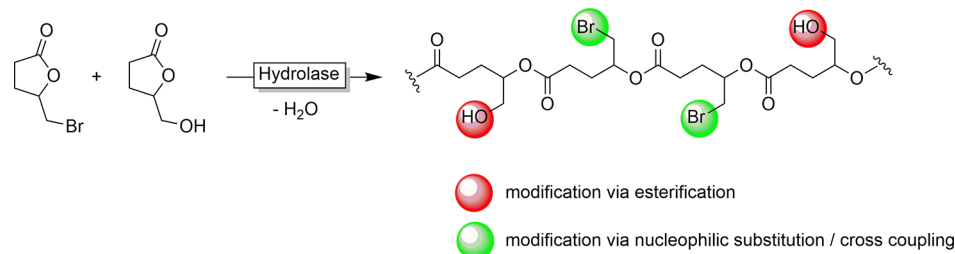
The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acssuschemeng.9b07494>.

Experimental details such as enzyme preparation and purification, detailed description of the reactions, analytical details, and supporting data. (PDF)

AUTHOR INFORMATION

Corresponding Author

Frank Hollmann – Department of Biotechnology, Delft University of Technology, 2629 HZ Delft, The Netherlands; orcid.org/0000-0003-4821-756X; Email: f.hollmann@tudelft.nl

Scheme 3. Envisioned (Enzymatic) Ring-Opening Polymerization of Lactone Products Obtained in This Study to Yield Polyesters with Two Different Functionalities to Synthesize Tailored Comb Polymers

Authors

- Georg T. Höfler** – Department of Biotechnology, Delft University of Technology, 2629 HZ Delft, The Netherlands
Andrada But – Department of Biotechnology, Delft University of Technology, 2629 HZ Delft, The Netherlands
Sabry H. H. Younes – Department of Biotechnology, Delft University of Technology, 2629 HZ Delft, The Netherlands; Department of Chemistry, Faculty of Sciences, Sohag University, 82524 Sohag, Egypt
Ron Wever – Van't Hoff Institute for Molecular Sciences, University of Amsterdam, 1090 GD Amsterdam, The Netherlands
Caroline E. Paul – Department of Biotechnology, Delft University of Technology, 2629 HZ Delft, The Netherlands; orcid.org/0000-0002-7889-9920
Isabel W. C. E. Arends – Faculty of Science, University of Utrecht, 3584 CD Utrecht, The Netherlands

Complete contact information is available at:
<https://pubs.acs.org/10.1021/acssuschemeng.9b07494>

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

Financial support by the European Research Council (ERC Consolidator Grant 648026) is gratefully acknowledged.

ABBREVIATIONS

- NBS = *N*-bromosuccinimide
ClVCPO = chloroperoxidase from *Curvularia inaequalis*
MCD = monochlorodimedone
2LPS = two liquid phase system
MIBK = methyl isobutyl ketone
EtOAc = ethyl acetate

REFERENCES

- Höfler, G. T.; But, A.; Hollmann, F. Haloperoxidases as catalysts in organic synthesis. *Org. Biomol. Chem.* **2019**, *17* (17), 9267–9274.
- Kristianslund, R.; Tungen, J. E.; Hansen, T. V. Catalytic enantioselective iodolactonization reactions. *Org. Biomol. Chem.* **2019**, *17* (12), 3079–3092.
- Grabarczyk, M.; Winska, K.; Maczka, W. An Overview of Synthetic Methods for the Preparation of Halolactones. *Curr. Org. Synth.* **2019**, *16* (1), 98–111.
- Alberto, E. E.; Muller, A. L.; Detty, M. R. Rate Accelerations of Bromination Reactions with NaBr and H₂O₂ via the Addition of Catalytic Quantities of Diaryl Ditellurides. *Organometallics* **2014**, *33* (19), 5571–5581.
- Alberto, E. E.; Braga, L. A.; Detty, M. R. Imidazolium-containing diselenides for catalytic oxidations with hydrogen peroxide and sodium bromide in aqueous solutions. *Tetrahedron* **2012**, *68* (S1), 10476–10481.
- Detty, M. R.; Higgs, D. E.; Nelen, M. I. Iodination of Organic Substrates with Halide Salts and H₂O₂ Using an Organotelluride Catalyst. *Org. Lett.* **2001**, *3* (3), 349–352.
- Bennett, S. M.; Tang, Y.; McMaster, D.; Bright, F. V.; Detty, M. R. A Xerogel-Sequestered Selenoxide Catalyst for Brominations with Hydrogen Peroxide and Sodium Bromide in an Aqueous Environment. *J. Org. Chem.* **2008**, *73* (17), 6849–6852.
- Ariyaratna, J. P.; Wu, F.; Colombo, S. K.; Hillary, C. M.; Li, W. Aerobic Catalytic Features in Photoredox- and Copper-Catalyzed Iodolactonization Reactions. *Org. Lett.* **2018**, *20* (20), 6462–6466.
- Younes, S. H. H.; Tieves, F.; Lan, D.; Wang, Y.; Süß, P.; Brundiek, H.; Wever, R.; Hollmann, F. Chemoenzymatic Halocyclization of γ,δ -Unsaturated Carboxylic Acids and Alcohols. *ChemSusChem* **2020**, *13* (1), 97–101.
- Xu, X.; But, A.; Wever, R.; Hollmann, F. Towards preparative chemoenzymatic oxidative decarboxylation of glutamic acid. *ChemCatChem* **2020**, *na*, na DOI: 10.1002/cctc.201902194.
- Dong, J. J.; Fernandez-Fueyo, E.; Li, J.; Guo, Z.; Renirie, R.; Wever, R.; Hollmann, F. Halofunctionalization of alkenes by vanadium chloroperoxidase from *Curvularia inaequalis*. *Chem. Commun.* **2017**, *53*, 6207–6210.
- Fernández-Fueyo, E.; Younes, S. H. H.; Rootselaar, S. v.; Aben, R. W. M.; Renirie, R.; Wever, R.; Holtmann, D.; Rutjes, F. P. J. T.; Hollmann, F. A biocatalytic Aza-Achmatowicz reaction. *ACS Catal.* **2016**, *6*, 5904–5907.
- Fernández-Fueyo, E.; van Wingerden, M.; Renirie, R.; Wever, R.; Ni, Y.; Holtmann, D.; Hollmann, F. Chemoenzymatic halogenation of phenols by using the haloperoxidase from *Curvularia inaequalis*. *ChemCatChem* **2015**, *7*, 4035–4038.
- But, A.; Le Nôtre, J.; Scott, E. L.; Wever, R.; Sanders, J. P. M. Selective Oxidative Decarboxylation of Amino Acids to Produce Industrially Relevant Nitriles by Vanadium Chloroperoxidase. *ChemSusChem* **2012**, *5* (7), 1199–1202.
- Renirie, R.; Pierlot, C.; Aubry, J.-M.; Hartog, A. F.; Schoemaker, H. E.; Alsters, P. L.; Wever, R. Vanadium Chloroperoxidase as a Catalyst for Hydrogen Peroxide Disproportionation to Singlet Oxygen in Mildly Acidic Aqueous Environment. *Adv. Synth. Catal.* **2003**, *345* (6–7), 849–858.
- van Schijndel, J. W. P. M.; Vollenbroek, E. G. M.; Wever, R. The chloroperoxidase from the fungus *Curvularia inaequalis* - a novel vanadium enzyme. *Biochim. Biophys. Acta, Protein Struct. Mol. Enzymol.* **1993**, *1161* (2–3), 249–256.
- Jiang, X. J.; Liu, S. H.; Yang, S.; Jing, M.; Xu, L. P.; Yu, P.; Wang, Y. Q.; Yeung, Y. Y. Enantioselective Bromolactonization of Deactivated Olefinic Acids. *Org. Lett.* **2018**, *20* (11), 3259–3262.
- Ding, R.; Lan, L. Y.; Li, S. H.; Liu, Y. G.; Yang, S. X.; Tian, H. Y.; Sun, B. G. A Novel Method for the Chlorolactonization of Alkenoic Acids Using Diphenyl Sulfoxide/Oxalyl Chloride. *Synthesis* **2018**, *50* (13), 2555–2566.
- Nolsoe, J. M. J.; Hansen, T. V. Asymmetric Iodolactonization: An Evolutionary Account. *Eur. J. Org. Chem.* **2014**, *2014* (15), 3051–3065.
- Tieves, F.; Tonin, F.; Fernández-Fueyo, E.; Robbins, J. M.; Bommarius, B.; Bommarius, A. S.; Alcalde, M.; Hollmann, F. Energising the E-factor: The E⁺-factor. *Tetrahedron* **2019**, *75*, 1311–1314.
- Sheldon, R. A. Metrics of Green Chemistry and Sustainability: Past, Present, and Future. *ACS Sustainable Chem. Eng.* **2018**, *6* (1), 32–48.
- Sheldon, R. A. The E factor 25 years on: the rise of green chemistry and sustainability. *Green Chem.* **2017**, *19* (1), 18–43.
- Ni, Y.; Holtmann, D.; Hollmann, F. How green is biocatalysis? To calculate is to know. *ChemCatChem* **2014**, *6* (4), 930–943.
- Brand, S. G.; Hawkins, C. J.; Parry, D. L. Acidity and vanadium coordination in vanadocytes. *Inorg. Chem.* **1987**, *26* (5), 627–629.
- Tracey, A. S.; Gresser, M. J.; Parkinson, K. M. Vanadium(V) oxyanions. Interactions of vanadate with oxalate, lactate and glycerate. *Inorg. Chem.* **1987**, *26* (5), 629–638.
- Wever, R.; Barnett, P. Vanadium chloroperoxidases, the missing link in the formation of chlorinated compounds and chloroform in the terrestrial environment? *Chem. - Asian J.* **2017**, *12*, 1997–2007.
- van Schijndel, J.; Barnett, P.; Roelse, J.; Vollenbroek, E.; Wever, R. The stability and steady-state kinetics of vanadium chloroperoxidase from the fungus *Curvularia inaequalis*. *Eur. J. Biochem.* **1994**, *225* (1), 151–157.
- Kara, S.; Spickermann, D.; Schrittwieser, J. H.; Weckbecker, A.; Leggewie, C.; Arends, I. W. C. E.; Hollmann, F. Access to Lactone Building Blocks via Horse Liver Alcohol Dehydrogenase-Catalyzed Oxidative Lactonization. *ACS Catal.* **2013**, *3*, 2436–2439.
- Jessop, P. G. Searching for green solvents. *Green Chem.* **2011**, *13* (6), 1391–1398.

(30) International Energy Agency. *CO₂ Emissions from Fuel Combustion*; OECD Publishing: Paris, 2017. DOI: 10.1787/co2-fuel-2017-en.

(31) Nielsen, P. H.; Oxenbøll, K. M.; Wenzel, H. Cradle-to-gate environmental assessment of enzyme products produced industrially in Denmark by Novozymes A/S. *Int. J. Life Cycle Assess.* 2007, 12 (6), 432.

(32) Jegannathan, K. R.; Nielsen, P. H. Environmental assessment of enzyme use in industrial production – a literature review. *J. Cleaner Prod.* 2013, 42, 228–240.

(33) Burek, B. O. O.; Bormann, S.; Hollmann, F.; Bloh, J.; Holtmann, D. Hydrogen peroxide driven biocatalysis. *Green Chem.* 2019, 21, 3232–3249.

(34) Tieves, F.; Willot, S. J.-P.; van Schie, M. M. C. H.; Rauch, M. C. R.; Younes, S. H. H.; Zhang, W.; Dong, J.; Gomez de Santos, P.; Robbins, J. M.; Bommarius, B.; Alcalde, M.; Bommarius, A. S.; Hollmann, F. Formate oxidase (FOx) from *Aspergillus oryzae*: one catalyst to promote H₂O₂-dependent biocatalytic oxidation reactions. *Angew. Chem., Int. Ed.* 2019, 58, 7873–7877.