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A Biocatalytic Aza-Achmatowicz Reaction

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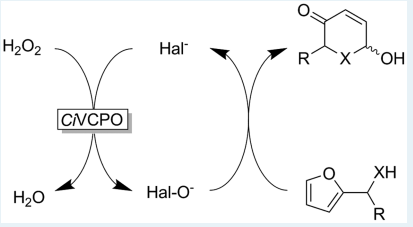
Supporting Information

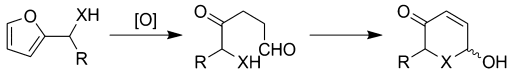
ABSTRACT: A catalytic, enzyme-initiated (aza-) Achmatowicz reaction is presented. The involvement of a robust vanadium-dependent peroxidase from *Curvularia inaequalis* allows the simple use of H_2O_2 and catalytic amounts of bromide.



KEYWORDS: *Achmatowicz reaction, biocatalysis, hypohalogenites, oxidation, peroxidase*

The Achmatowicz reaction[1] over the years has demonstrated its usefulness in the conversion of furan rings into heterocyclic scaffolds containing multiple functional handles for further synthetic transformations. Key in the Achmatowicz process is an oxidative activation of the furan ring, giving rise to a reactive dicarbonyl intermediate, which cyclizes to give the corresponding pyranone (X = O)[2,3] or piperidinone (X = N-EWG) structure (Scheme 1).[4]

Scheme 1. Achmatowicz Reaction[a]



[a]X = O for the Achmatowicz reaction, and X = N-EWG for the aza-Achmatowicz reaction.

Despite frequent application of the Achmatowicz protocol in the synthesis of pharmaceutically relevant building blocks[5−7] and natural products,[8] the oxidative rearrangement is usually conducted using stoichiometric amounts of an oxidative reagent such as *m*-CPBA, and catalytic methods are scarce.

Recently, however, Deska and co-workers reported an enzymatic version of the Achmatowicz reaction using the well-known chloroperoxidase from *Caldariomyces fumago* (*Cf*CPO).[9] One of the major challenges using *Cf*CPO (as with any heme-dependent enzyme) is its poor resistance against the oxidant H_2O_2. Though this challenge can be met by *in situ* generation of H_2O_2,[10−13] the resulting reaction schemes are more complex than necessary.[14]

Furthermore, most *in situ* H_2O_2 generation methods yield additional byproducts such as gluconic acid that not only negatively influences the atom economy of the overall reaction but also may complicate the reaction scheme. In addition, the chemoenzymatic process seems restricted to Achmatowicz reactions, while the corresponding aza-Achmatowicz products are synthetically equally relevant. Therefore, we decided to follow up on the seminal contribution by Deska using the vanadium-dependent peroxidase from *Curvularia inaequalis* [*Ci*VCPO (Scheme 2)].[15−20]

The major advantage of *Ci*VCPO over *Cf*CPO lies in the prosthetic group used (vanadate as compared to heme iron) and the resulting high robustness of the peroxidase in the presence of H_2O_2.[16] Essentially, this renders any *in situ* H_2O_2 generation redundant and allows for simple and clean (yielding H_2O as the sole byproduct) reaction schemes.

Pleasingly, already in initial aza-Achmatowicz experiments under arbitrarily chosen reaction conditions, full conversion of **1a** was observed within 24 h. It is worth noting here that in the absence of either of the catalysts (*Ci*VCPO or Br^-) or the oxidant (H_2O_2), no significant conversion was observed.

Encouraged by these promising results, we set out to determine the parameters influencing the efficiency of the chemoenzymatic aza-Achmatowicz reaction. Acidic pH values appeared to be necessary to achieve full conversion (Figure 1).

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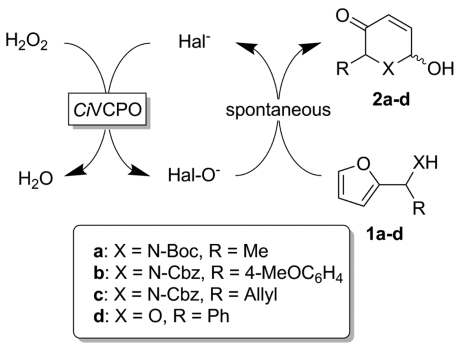
Scheme 2. Simplified Reaction Scheme for the *Ci*VCPO-Mediated (aza-)Achmatowicz Reaction[a]



[a]*In situ*-formed, freely diffusible hypohalogenites (OBr^- and OCl^-) presumably account for the (aza-)Achmatowicz conversion of starting materials **1a–d**. Abbreviations: Boc, *tert*-butyloxycarbonyl; Cbz, carboxybenzyl.

This observation is in line with the previously determined preference of *Ci*VCPO for slightly acidic pH values.[15−20]

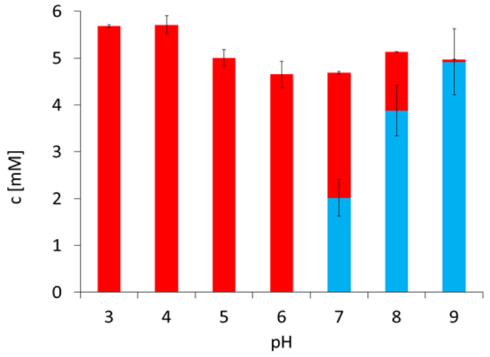


Figure 1. Influence of pH on the chemoenzymatic aza-Achmatowicz reaction: blue for *c*(**1a**) and red for *c*(**2a**). General conditions: solvent, ethanol with 100 mM universal B&R (Britton−Robinson) buffer [1:1 (v/v)]; *c*(**1**) = 5 mM substrate; *c*(KBr) = 10 mM; $c(H_2O_2)$ = 10 mM; *c*(*Ci*VCPO) = 0.1 μM (52 units mL^{-1}); *T* = 30 °C; *t* = 24 h.

To ensure the high catalytic efficiency of *Ci*VCPO while minimizing the acidolytic degradation of the reagents, we performed all subsequent reactions at pH 5.

Next we turned our attention to the influence of the catalysts. Obviously, reducing the concentration of the biocatalyst (*Ci*VCPO) is desirable, but substoichiometric amounts of bromide would avoid possible inhibitory effects on the enzyme.[21,22] Pleasingly, both catalyst concentrations could be reduced very significantly without impairing the apparent activity of the overall chemoenzymatic aza-Achmatowicz reaction (Figures 2 and 3).

Reducing the biocatalyst concentration from 0.1 to 0.01 μM resulted in a somewhat reduced reaction rate (Figure 2), indicating that below 0.05 μM the enzymatic oxidation of Br^- becomes overall rate-limiting.

As shown in Figure 3, a decrease in the bromide concentration from 10 mM to 50 μM (1 mol %) had no apparent influence on the conversion of **1a** into **2a**. The corresponding Achmatowicz reaction of alcohol **1d**, however, exhibited a very distinct dependency on the bromide concentration applied (Figure 3, dark blue bars). Within the time frame of these experiments, full conversion of the starting

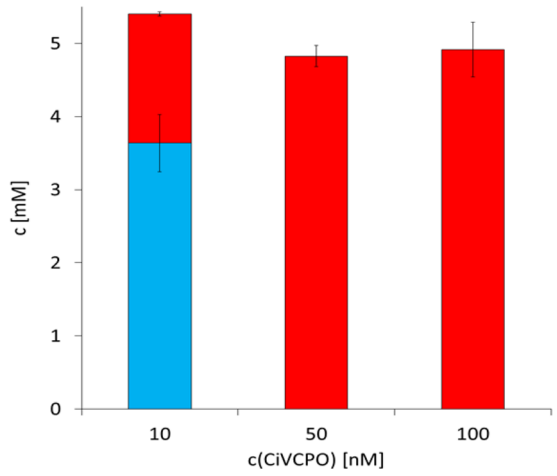


Figure 2. Influence of *c*(*Ci*VCPO) on the chemoenzymatic aza-Achmatowicz reaction: blue for *c*(**1a**) and red for *c*(**2a**). General conditions: solvent, ethanol with 0.1 M citrate at pH 5 [1:1 (v/v)]; *c*(**1**) = 5 mM; $c(H_2O_2)$ = 10 mM; *c*(KBr) = 0.05 μM; *T* = 30 °C; *t* = 24 h.

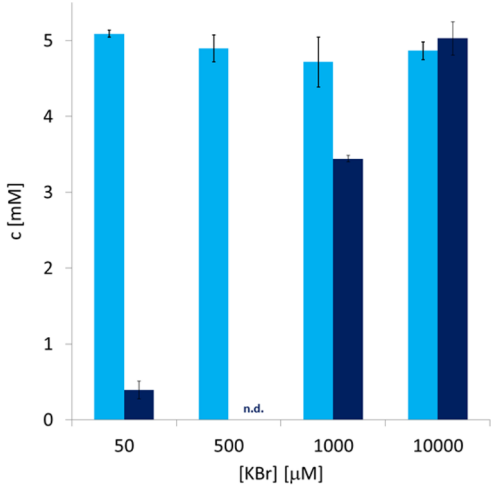


Figure 3. Influence of *c*(KBr) on the chemoenzymatic Achmatowicz (dark blue) and aza-Achmatowicz (light blue) reactions. General conditions: solvent, ethanol with 100 mM citrate buffer at pH 5 [1:1 (v/v)]; *c*(**1a** or **1d**) = 5 mM substrate; $c(H_2O_2)$ = 10 mM; *c*(*Ci*VCPO) = 0.1 μM (52 units mL^{-1}); *T* = 30 °C; *t* = 24 h.

material (**1d**) into product (**2d**) was observed only in the presence of (super)stoichiometric amounts of KBr. One possible explanation for this observation may be a low reactivity of **1d** with OBr^-. The accumulating OBr^- reacts with hydrogen peroxide, giving rise to the formation of singlet oxygen,[16] therefore necessitating higher *in situ* concentrations of the latter. Nevertheless, catalytic amounts of bromide were feasible.

Encouraged especially by the high efficiency of the chemoenzymatic aza-Achmatowicz reaction in the presence of low bromide concentrations, we also evaluated seawater as a reaction medium and source of bromide (Figure 4). The overall rate of the chemoenzymatic aza-Achmatowicz reaction fell significantly behind the rate in defined buffers. Instead of being complete within maximally 30 min, full conversion was achieved only within approximately 2.5 h (Figure 4).

Most probably, the reduced reaction rate can be attributed to the huge molar surplus of chloride (~550 mM) over bromide (<1 mM) present in seawater, leading to a predominant

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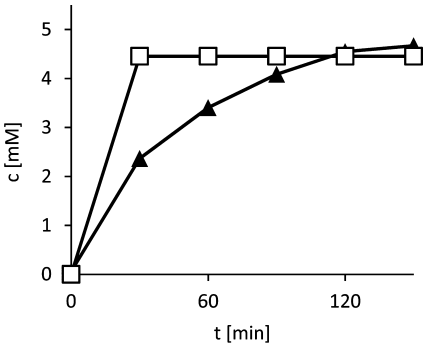


Figure 4. Time courses of the chemoenzymatic aza-Achmatowicz reaction using seawater (▲) and 50 μM KBr (□) as the halide source. General conditions: ethanol with aqueous medium [0.1 M citrate (pH 5) and 0.05 mM KBr] or seawater [0.1 M citrate (pH 5), 1:0.74 (v/v)]; c(**1**) = 5 mM; $c(H_2O_2)$ = 10 mM; c(*Ci*VCPO) = 0.1 μM (52 units mL^{-1}); T = 30 °C.

formation of hypochlorite over hypobromite. Indeed, control experiments in defined buffers and KCl as halogen resulted in only 46% conversion after 24 h under otherwise identical conditions. Alternatively, substrate inihibition of *Ci*VCPO by chloride may also contribute to the reduced overall activity observed in seawater.[21] Further experiments clarifying the chemical reactivities are currently ongoing in our laboratories. Nevertheless, the experiments mentioned above suggest that simple (and cheap) seawater may serve as the reaction medium and source of catalysts for chemoenzymatic aza-Achmatowicz reactions.

Admittedly, the characterization experiments reported above are not suitable for preparative-scale application of the proposed biocatalytic (aza-)Achmatowicz reaction. Therefore, we set out to perform reactions at more practical reagent concentrations [100 mM, approximately 25 g L^{-1} (Figure 5)].

Starting material **1a** was converted smoothly into **2a** within 4 h, corresponding to an excellent average turnover frequency of *Ci*VCPO of 8.7 s^{-1} over the entire reaction time. This

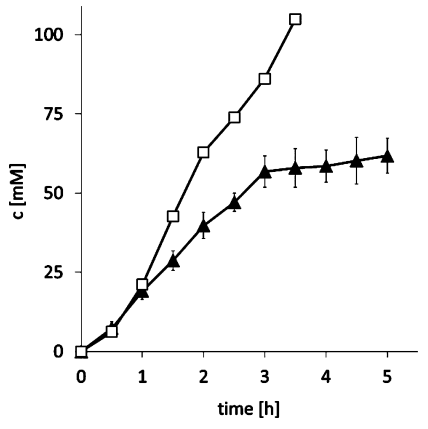
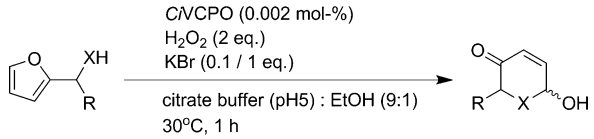


Figure 5. Representative time courses for the (aza-)Achmatowicz reactions of **1a** (□) and **1d** (▲) at semiprepatative scale. General conditions: solvent, ethanol with 100 mM citrate buffer at pH 5 [1:1 (v/v)]; c(**1a** or **1d**) = 100 mM substrate; H_2O_2 added at 30 min intervals in 10 mM_{final} portions; c(*Ci*VCPO) = 0.1 μM (52 units mL^{-1}); c(KBr) = 0.1 mM (**1a**) or 10 mM (**1d**); T = 30 °C; t = 24 h. For the sake of clarity, the corresponding starting material concentrations have been omitted; the mass balances in each reaction were closed.

corresponds to an average specific activity of 7.8 units mg^{-1} (over at least 3 h), which is in good agreement with the specific activity determined previously for this enzyme under initial rate conditions (<30 s).[21] This underlines the high robustness of *Ci*VCPO under operational conditions. The tunover number for *Ci*VCPO in this experiment exceeded 1 million, which should be seen as a minimal value and not as a total turnover number as the reaction proceeded in an almost linear fashion to full conversion [Figure 5 (□)]. In contrast, the accumulation of **2d** (oxo-Achmatowicz reaction) was somewhat slower and yielded less product. It is also worth mentioning that after approximately 3 h product accumulation ceased and formation of a (yet undefined) side product was observed via high-performance liquid chromatography (HPLC) (see the Supporting Information for further information). Table 1 summarizes the semipreparative conversions of starting materials **1a**–**d**.

Table 1. Summary of Semipreparative-Scale Reactions[a]



product	conversion (%)[b]	isolated product[a]	diastereomeric ratio
2a	100	228 mg (69%)	–
2b	100	160 mg (56%)	65:35
2c	100	175 mg (50%)	80:20
2d	100	195 mg (82%)	75:25

[a]See the Supporting Information for further details about the reaction conditions and product isolation and purification. [b]Determined via HPLC.

The aza-Achmatowicz reactions prodeeded smoothly to full conversion of the starting materials into the target products with only trace amounts of byproducts formed (see the Supporting Information for further details), which were removed by a single flash chromatography step. Hence, the moderate isolated yields listed in Table 1 can be assigned to a suboptimal reaction workup and product isolation, which will be further optimized in our laboratories. It should also be mentioned that so far we have no indication of racemization of the chiral center ("furanylic C–H bond") in the course of the reaction. Deska et al.[9] found no racemization under comparable conditions.

In summary, we have presented a chemoenzymatic alternative to the established stoichiometric (aza-)Achmatowicz protocols. *Ci*VCPO is an efficient catalyst for generating hypohalogenites *in situ* under mild reaction conditions from catalytic amounts of halogenides. Because of its high robustness and catalytic activity, excellent turnover numbers and frequencies have been observed, making it a promising catalyst. Next to broadening the scope of the proposed chemoenzymatic protocol, we will particularly focus on mechanistic studies and investigation of the stereochemical outcome.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acscatal.6b01636.

Control experiments detailing experimental information, including full characterization of the products (PDF)

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Notes

The authors declare no competing financial interest.

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