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A biocatalytic aza-Achmatowicz reaction

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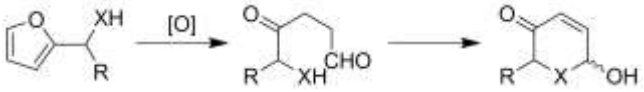
ABSTRACT: A catalytic, enzyme-initiated (*aza*-) Achmatowicz reaction is presented. The involvement of a robust vanadium-dependent peroxidase from *Curvularia inaequalis* enables simple use of H_2O_2 and catalytic amounts of bromide.

KEYWORDS: Achmatowicz reaction, Biocatalysis, Hypohalogenites, Oxidation, Peroxidase.

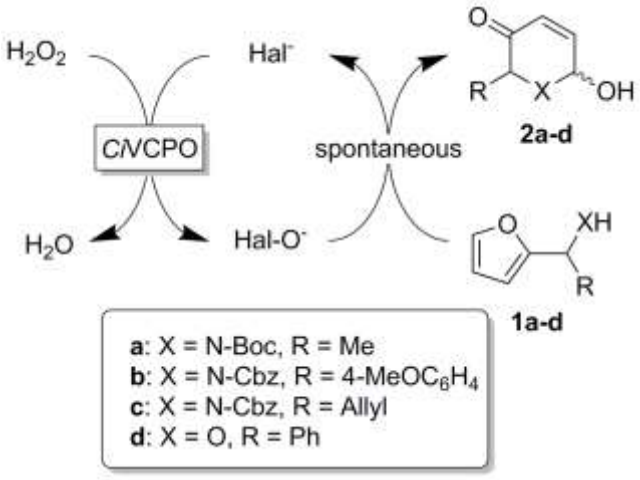
The Achmatowicz reaction[1] over the years has demonstrated its usefulness in the conversion of furan rings into heterocyclic scaffolds containing multiple functional handles for further synthetic transformations. Key in the Achmatowicz process is an oxidative activation of the furan ring, giving rise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



Scheme 1. The Achmatowicz reaction. X = O: Achmatowicz reaction; X = N-EWG: 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 carried out using stoichiometric amounts of an oxidative reagent such as *m*-CPBA and catalytic methods are scarce.

Recently, however, Deska and coworkers 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]



Scheme 2. Simplified reaction scheme for the *Ci*VCPO-mediated (aza-)Achmatowicz reaction. *In situ* formed, freely diffusible hypohalogenites (OBr^-, OCl^-) presumably account for the (aza-)Achmatowicz conversion of starting materials **1a-d**. Boc: *tert*-butyloxycarbonyl; Cbz: Carboxybenzyl.

Furthermore, most *in situ* H_2O_2 generation methods yield additional by-products 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 sole by-product) reaction schemes.

Pleasingly, already in initial aza-Achmatowicz experiments under arbitrarily chosen reaction conditions full conversion of **1a** was observed within 24 h reaction time. It is worth noting here that in the absence of either one 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 necessary to achieve full conversion (Figure 1). This observation is in line with the previously determined preference of *Ci*VCPO for slightly acidic pH values.[15-20]

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

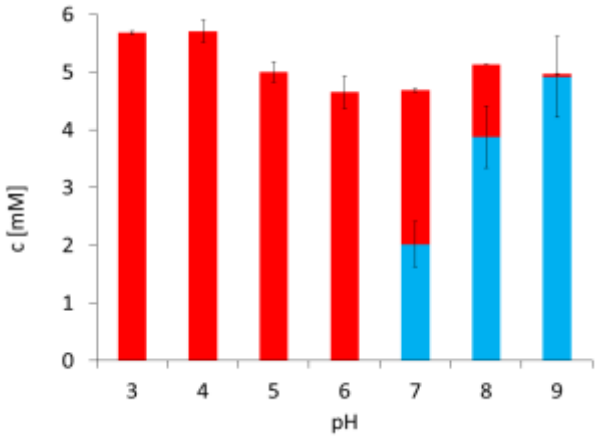


Figure 1. Influence of pH on the chemoenzymatic aza-Achmatowicz reaction. blue: c(**1a**), red: c(**2a**); General conditions: solvent: ethanol - 100 mM universal B&R (Britton–Robinson) buffer (1:1 vol/vol), c(**1**) = 5 mM substrate, c(KBr) = 10 mM, c(H_2O_2) = 10 mM, c(*Ci*VCPO) = 0.1 μM (52 U ml^{-1});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

Next we drew our attention to the influence of the catalysts. Obviously, reducing the concentration of the biocatalyst (*Ci*VCPO) is desirable but also sub-stoichiometric 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 μM 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.

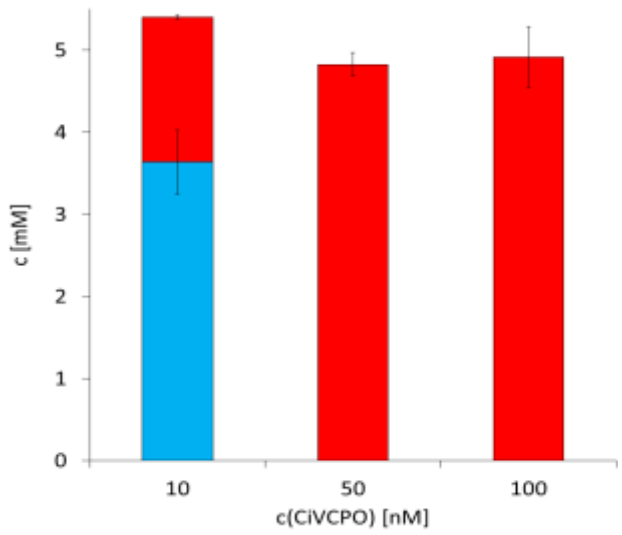


Figure 2. Influence of c(*Ci*VCPO) on the chemoenzymatic aza-Achmatowicz reaction. blue: c(**1a**), red: c(**2a**); General conditions: solvent: ethanol - 0.1 M citrate pH 5 (1:1 vol/vol), c(**1**) = 5 mM, c(H_2O_2) = 10 mM, c(KBr) = 0.05 μM; T = 30 °C, t = 24 h.

As shown in Figure 3, a reduction of 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 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.

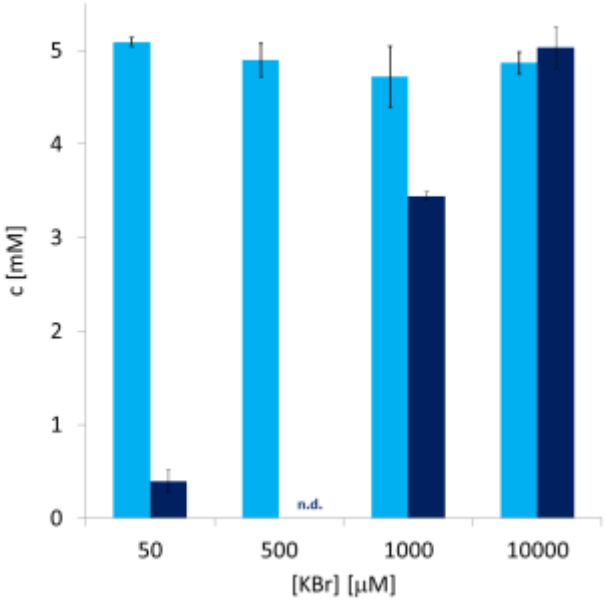


Figure 3. Influence of c(KBr) on the chemoenzymatic Achmatowicz (**dark blue**) and aza-Achmatowicz reaction (**light blue**). General conditions: solvent: ethanol – 100 mM citrate buffer pH 5 (1:1 vol/vol), c(**1a or 1d**) = 5 mM substrate, $c(H_2O_2)$ = 10 mM, c(*Ci*VCPO) = 0.1 µM (52 U ml^{-1}); T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

Encouraged especially by the high efficiency of the chemoenzymatic *aza*-Achmatowicz reaction in the presence of low bromide concentrations, we also evaluated seawater as 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 minutes, full conversion was achieved only within approximately 2.5 h (Figure 4).

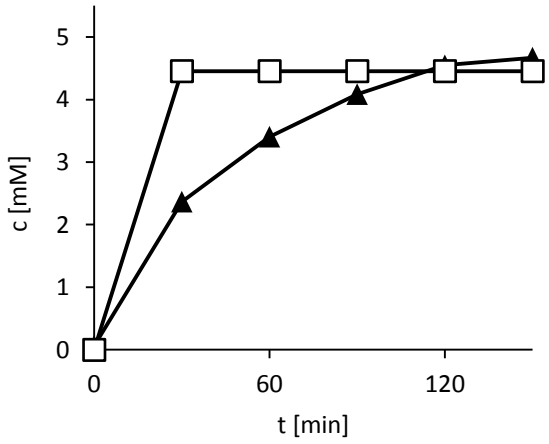


Figure 4. Time-courses of the chemoenzymatic *aza*-Achmatowicz reaction using seawater (▲) and 50 µM KBr (□) as halides source. General conditions: ethanol – aqueous medium (0.1 M citrate pH 5, 0.05 mM KBr) or seawater) (0.1 M citrate pH 5, 1:0.74 vol/vol), c (**1**) = 5 mM, $c(H_2O_2)$ = 10 mM, c(*Ci*VCPO)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

Most probably the reduced reaction rate can be attributed to the huge molar surplus of chloride (approx. 550 mM) over bromide (less than 1 mM) present in seawater leading to a predominant formation of hypochlorite over hypobromite. Indeed control experiments in defined buffers and KCl as halogen resulted in only 46% conversion after 24 hours reaction time 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 above-mentioned experiments suggest that simple (and cheap) seawater may serve as 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 approx. 25 gL^{-1}, Figure 5).

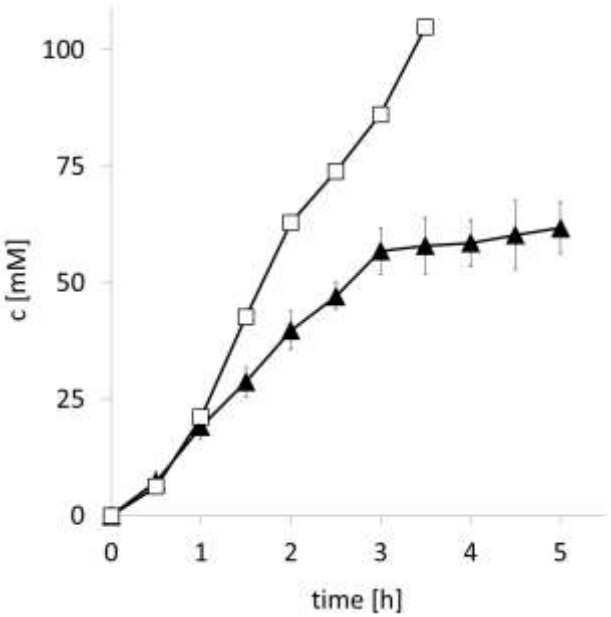
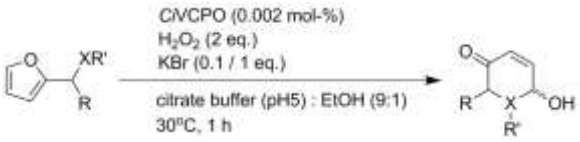


Figure 5. Representative time-courses for the (aza-) Achmatowicz reactions of **1a** (□) and **1d** (▲) at semi-prepatative-scale. General conditions: solvent: ethanol – 100 mM citrate buffer pH 5 (1:1 vol/vol), c(**1a or 1d**) = 100 mM substrate, H_2O_2 was added at 30 minutes intervals in 10 mM_{final}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

The starting material **1a** was converted smoothly into **2a** within 4h corresponding to an excellent average turnover frequency of *Ci*VCPO of 8.7 s^{-1} over the entire reaction time. This corresponds to an average specific activity of 7.8 Umg^{-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 sec).[21] This underlines the high

robustness of *Ci*VCPO under operational conditions. The tunover number for *Ci*VCPO in this experiment exceeded one million, which should be seen as a minimal value and not as a total turnover number as the reaction proceeded almost linear 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 3h product accumulation ceased and formation of a (yet undefined) side product was observed with HPLC (please see SI X for further information). Table 1 summarizes the semi-preparative conversions of starting materials **1a-d**.

Table 1. Summary of semi-preparative scale reactions.[a]



Product	Conversion [%][b]	Isolated product[a]	d.r.
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: Please see SI for further details on 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 by-products formed (see SI for further details), which were removed by a single flash chormmatograpy step. Hence, the moderate isolated yields shown in Table 1 can be assigned to a sub-optimal 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 about 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.

Summarizing, we have presented a chemoenzymatic alternative to the established stoichiometric (aza-)Achmatowicz protocols. *Ci*VCPO is an efficient catalyst to *in situ* generate hypohalogenites under mild reaction conditions from catalytic amounts of halogenides. Thanks to 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 a particular focus will lie on mechanistic studies and investigation of the stereochemical outcome.

ASSOCIATED CONTENT

Supporting Information

Supporting material is available free of charge via the Internet at http://pubs.acs.org. These contain control experiments detailed experimental information including full characterization of the products.

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Notes

The authors declare no competing financial interests.

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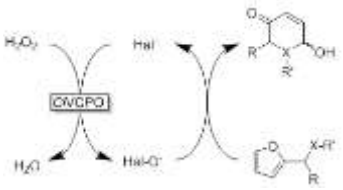

A biocatalytic (aza-)Achmatowicz reaction utilizing a robust chloroperoxidase from *Curvularia inaequalis* is presented.