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Research Paper

Combining biocatalytic oxyfunctionalisation and organocatalytic aldol reaction to access chiral β-hydroxy ketones

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ABSTRACT

This study explores a chemoenzymatic cascade to synthesise chiral β-hydroxy ketones by integrating the selective oxyfunctionalisation capabilities of peroxygenases with the carbon-carbon bond-forming progress of organocatalysts. Initial results with simple organocatalysts demonstrated poor performance due to mutual inactivation of the biocatalyst and organocatalyst. However, the use of more complex prolinamide derivatives improved the reaction efficiency and enantioselectivity, enabling a one-pot, one-step synthesis process. This methodology was further optimised to produce high yields of enantiomerically pure aldol products and was shown to be extendable to other substituted toluenes and aldol donors.

Introduction

Chemoenzymatic syntheses hold great potential to complement one another by mutually enhancing their strengths and compensating for each other's weaknesses [1–3]. For example, biocatalytic, aldolase-catalysed C—C bond formation is often highly selective but is significantly limited in terms of donor and acceptor scope [4]. In contrast, organocatalytic aldol reactions offer a much broader substrate range [2]. On the other hand, selective oxyfunctionalisation even of activated C—H bonds still represents a dream reaction on traditional chemistry and can be considered as 'playground' of biocatalysis [5].

Inspired by some recent cascades utilising alcohol oxidase- [6] or laccase/TEMPO-catalysed [7] *in situ* aldehyde formation coupled to organocatalytic C-C-bond formation we sought to start from non-functionalised benzylic CH_3 groups generating benzaldehydes to be submitted to the organocatalytic aldol reaction (Scheme 1).

In particular, we employed the peroxygenase from *Agrocybe aegerita* (*Aae*UPO) [8] as its expression-engineered PaDa-I variant [9–11].

As organocatalyst, we initially concentrated on prolinamide as proposed by Wu and coworkers [12,13]. Finally, we focussed on *p*-nitro toluene as starting material and acetone as donor.

Results and discussion

We initiated our studies by establishing the peroxygenase-catalysed oxyfunctionalisation of *p*-nitro toluene (**1a**). Due to the poor water solubility of substituted toluenes we decided for a non-aqueous reaction system, in which substituted toluenes and acetone act not only as substrates but also solvents. This necessitated immobilising the biocatalyst for which we chose the conditions previously developed by Kara and coworkers [14,15].

As shown in Fig. 1, the conversion of **1a** into the desired *p*-nitro benzaldehyde (**3a**) proceeded smoothly to product concentrations of up to 60 mM within a 30 h time frame. The intermediate *p*-nitro benzyl

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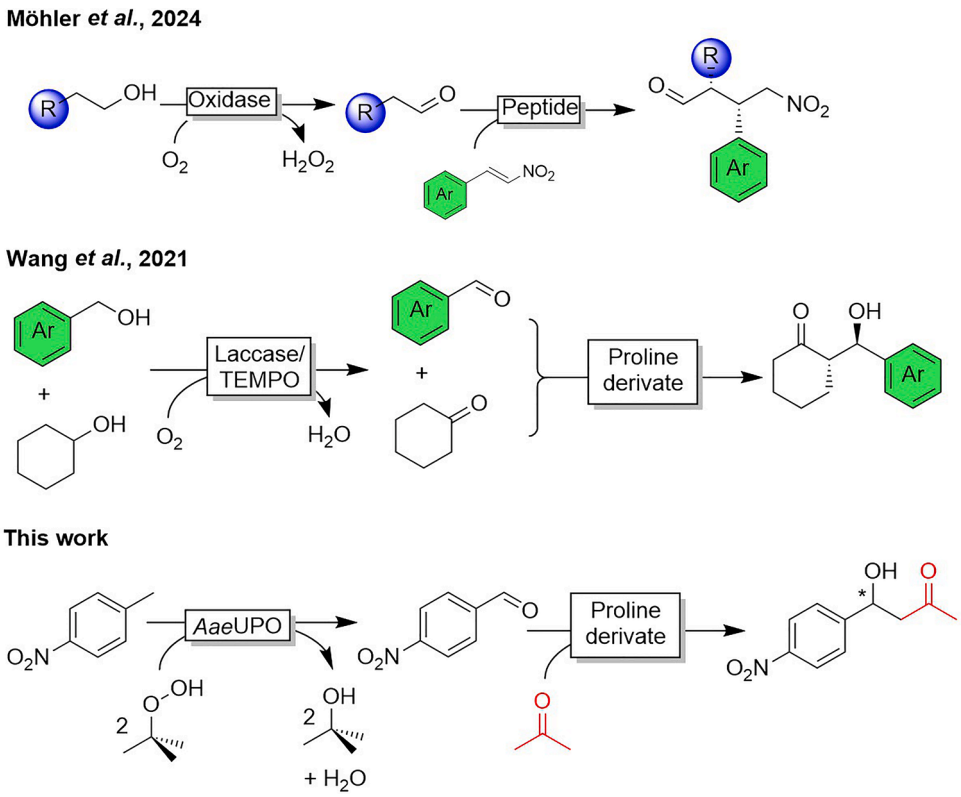


Scheme 1. Chemoenzymatic cascades comprising biocatalytic aldehyde formation to provide the reactive intermediate for organocatalytic C—C-bond formation. While the previous examples start from activated alcohol starting materials [6,7] we evaluated non-functionalised benzylic CH_3 functionalities as starting materials.

alcohol (**2a**) accumulated to some extend during the reaction but was consumed in the course of the reaction (Fig. 1a). The initial product accumulation rate was independent from the biocatalyst concentration applied, indicating that the biocatalytic **1a** oxyfunctionalisation reaction was not overall rate-limiting. The product formation rate (9.7 mM h^{-1} of **2a** and **3a**) was somewhat lower than the tert-butyl hydroperoxide (TBHP) feed rate (12 mM h^{-1}). Most likely, the known catalase activity of *Aae*UPO [16] accounts for this observation. We observed an *Aae*UPO--concentration dependency of the overall robustness. Using a TBHP feed rate of 12 mM h^{-1} and 6.5 μM of *Aae*UPO, the product accumulation ceased already after 10 h whereas the product accumulation was more robust at higher biocatalyst concentrations. We attribute this observation to the undesired accumulation of TBHP in the presence of comparably low *Aae*UPO concentrations resulting in oxidative inactivation of the heme cofactor [17].

Next, we investigated the organocatalytic aldol reaction (Fig. 1b). Initially, we utilised L-prolinamide (**OC-1**), which however under our reaction conditions only enabled significant conversions and optical purities of the desired (*R*)-4-(4-nitrophenyl)-4-hydroxybutan-2-one if applied in the higher mol-% concentration range. Using 20-mol% of the organocatalyst near full-conversion of the initial **3a** and an enantiomeric access of approx. 35 % were observed being in line with the observations made by Wu and coworkers [12].

Hence, we proceeded combining both reaction steps (Fig. 2). Performing the cascade in a one-pot fed batch fashion resulted in very mediocre results (Fig. 2, red). The enzymatic reaction rate was reduced by more than 50 % and also the organocatalytic reaction rate was severely impaired, being barely faster than the non-catalysed aldol reaction. As a result the aldol product formed was essentially racemic (ee = 8 %).

In contrast, performing the reaction in a two-pot fed batch fashion gave more satisfying results (Fig. 2, green) with the performances of the individual catalysts resembling the activities determined above (Fig. 1).

We suspected a mutual inactivation of the bio- and organocatalysts to account for this observation, which was confirmed by independent control reactions demonstrating the mutual inactivation (Figure S33). Upon using prolinamide as substrate for *Aae*UPO we qualitatively observed a decrease of its characteristic NMR signals (Figure S34) suggesting that prolinamide is oxidatively converted by *Aae*UPO to a yet unknown product.

We therefore turned our attention to sterically more demanding prolinamide derivates such as *N*-(1-Hydroxy-4-methyl-1,1-diphenylpentan-2-yl) pyrrolidine-2-carboxamide (**OC-2**) [12,17]. We hypothesised that the larger **OC-2** may not bind close enough to CpdI preventing **OC-2** from conversion and inactivation. Following the literature synthesis the two enantiomers of **OC-2** were obtained in two steps and reasonable overall yield (Scheme 2, SI).

Substituting prolinamide by **OC-2** proofed beneficial in various aspects: Compared to prolinamide, **OC-2** was significantly more active and stereodicriminating (Fig. 3). Already in the presence of 1 mol% of **OC-2**, excellent conversion and enantioselectivities for the organocatalytic aldol reaction were observed [18].

Furthermore, using **OC-2** as organocatalyst also enabled a simple one-pot fed batch cascade reaction (Fig. 4). Interestingly, the formation rate of (*R*)-**4a** in the one-pot tandem reaction (Fig. 4, □) was almost identical to the formation rate of **3a** in the one-pot stepwise reaction (Fig. 4, ●). Also the enantioselectivity of the aldol reaction in both approaches was near-perfect. Hence we concluded that, in contrast to prolinamide and *Aae*UPO, **OC-2** and *Aae*UPO are compatible.

With some optimisation this combination enabled us to produce up

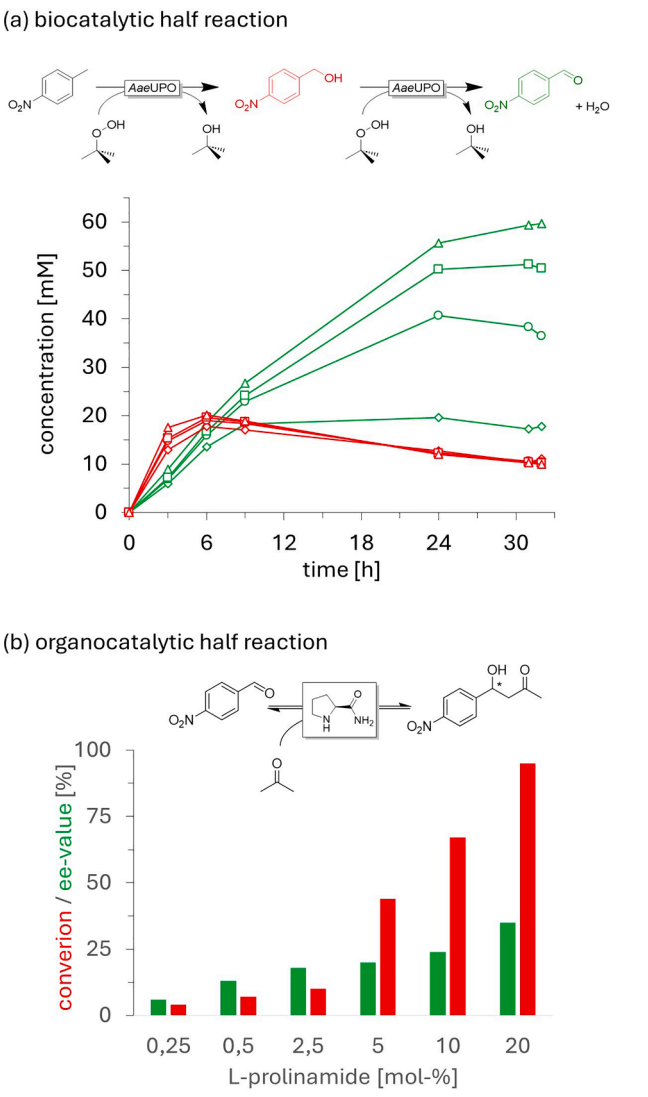


Fig. 1. Individual evaluation of the biocatalytic (a) and the organocatalytic (b) half-reactions: (a) *Aae*UPO-catalysed oxyfunctionalisation of 1a to the corresponding 2a (red) and 3a (green): 1a 375 mg (2.95 mmol), acetone 125 μL (1.8 mmol), $AaeUPO_{imminilised}$: 50-200 mg corresponding to [AaeUPO] = 3.25 nmol (⋄), 6.5 nmol (○), 9.75 nmol (□), 13 nmol (△), TBHP feeding rate = 12 mM h^{-1}; (b) organocatalytic aldol reaction between 3a and acetone catalysed by OC-1: reaction time: 24 h. General conditions: 30 °C, 800 rpm. N = 1.

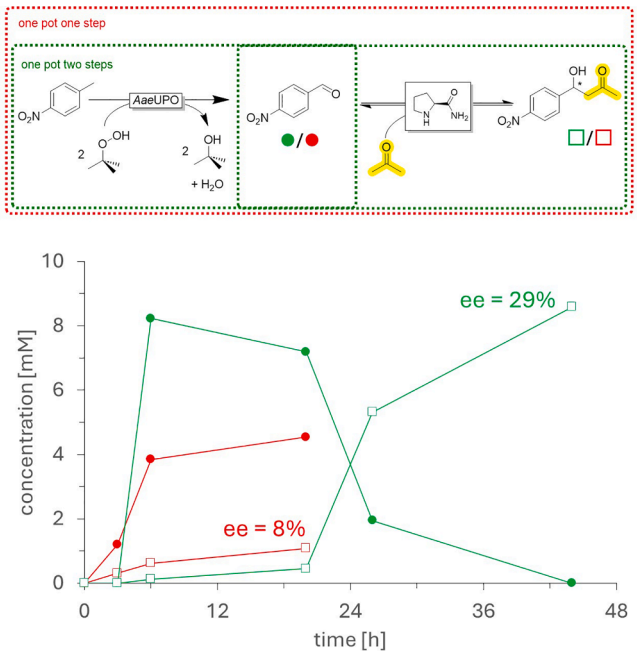


Fig. 2. Combining the bio- and organocatalytic half reactions in either one-pot tandem (red) or two-pot (green) fed batch fashion. One-pot: 1a 375 mg (2.95 mmol), acetone 125 μL (1.8 mmol), $AaeUPO_{imminilised}$ corresponding to [AaeUPO] = 3.25 nmol, TBHP fed rate: 12 mM h^{-1} for 6 h, L-prolinamide: 1 μmol in 1.5 mL glass vials, reactions were performed at 25 °C, 800 rpm for 20 h. (3a (●), 4a (□)) Two-pot: 1a 375 mg, acetone 125 μL, $AaeUPO_{imminilised}$ corresponding to [AaeUPO] = 3.25 nmol, TBHP fed rate: 12 mM h^{-1} for 6 h, in 1.5 mL glass vials, reactions were performed at 25 °C, 800 rpm for 20 h, then L-prolinamide 1 μmol was added and reacted for another 24 h, (3a (●), 4a (□)) N = 1.

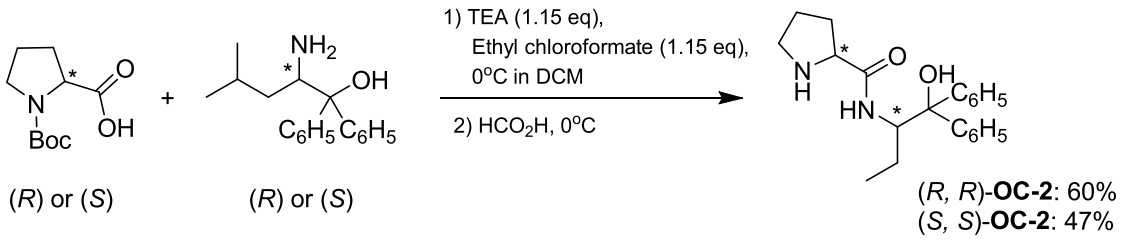
to 100 mM (ca. 21 g L^{-1}) of the essentially enantiopure aldol product (Figure S36).

Also the principal extensibility of the chemoenzymatic cascade to further toluene-based starting materials was investigated (Table 1, Table S2). Quite disappointingly, only the nitro-substituted toluenes **1a** and **1b** considerable amounts of the desired **4a** and **4b** were obtained. While the peroxygenase-catalysed formation of the intermediate aldehyde products (**3a-g**) gave satisfactory product titres and enzyme turnovers, the organocatalytic aldol reaction was rather inefficient in case of the non-nitro substituted starting materials (as evident from the high concentrations of the intermediate aldehydes and the low concentrations of the aldol products). We therefore performed a range of control aldol experiments starting from commercially available aldehyde standards (Table S2). Also here in most cases, the yield of the aldol product was low. This likely stems from the higher reactivity due to the electron-withdrawing nitro group, which enhances the electrophilicity of the carbonyl carbon. In contrast, other substrates, being less reactive, yielded lower product amounts, likely due to kinetic limitations. Further studies will be conducted to better understand and address this issue.

Conclusion

Overall, in the current contribution we have demonstrated a chemoenzymatic cascade reaction to activate and valorise simple toluene derivates into β-keto alcohols. Using a 'first generation', simple organocatalyst resulted in poor catalytic performance due to mutual inactivation. We hypothesise that reactive intermediates (such as hemiaminals obtained from *Aae*UPO-catalysed α-hydroxylation of the small prolineamide) may account for this. Further studies will be necessary to validate this hypothesis. Switching to a more complex organocatalyst circumvented these issues and allowed for one pot one step syntheses.

Admittedly, the present work represents not much more than a proof-of-concept study and several issues such as the currently poor performance of the organocatalytic aldol reaction need to be addressed in future investigations. Also the demonstration of its preparative value remains to be performed. However, we are convinced that this study provides a solid basis for such characterisation and optimisation.



Scheme 2. Synthesis of the 2nd generation organocatalyst OC-2.

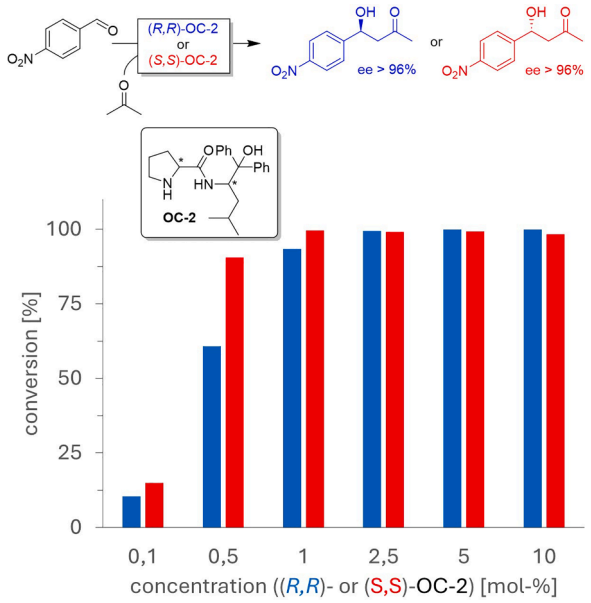


Fig. 3. Evaluation of OC-2 for the stereoselective aldol reaction. Conditions: 1a: 375 mg (2.95 mmol), acetone 125 µL (1.8 mmol), 3a 20 µmol, OC-2: 0.02-2 µmol (0.1-10 mol%) in 1.5 mL glass vials, reactions were performed at 25 °C, 800 rpm for 24 h. N = 1.

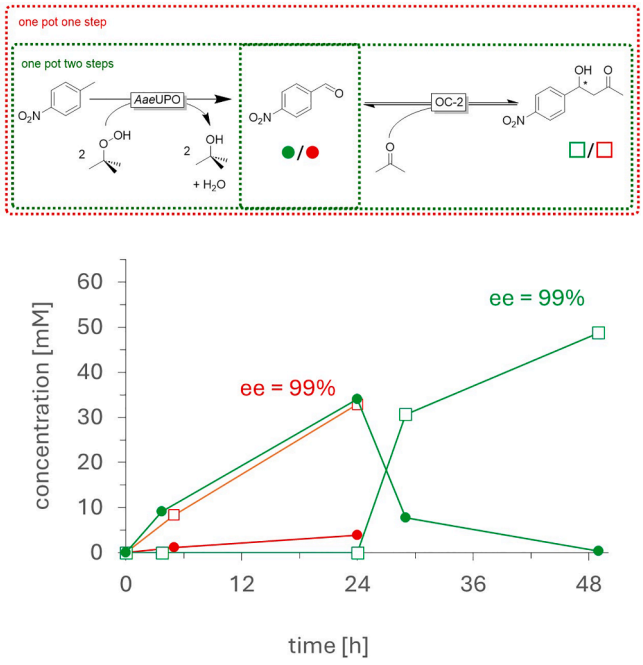
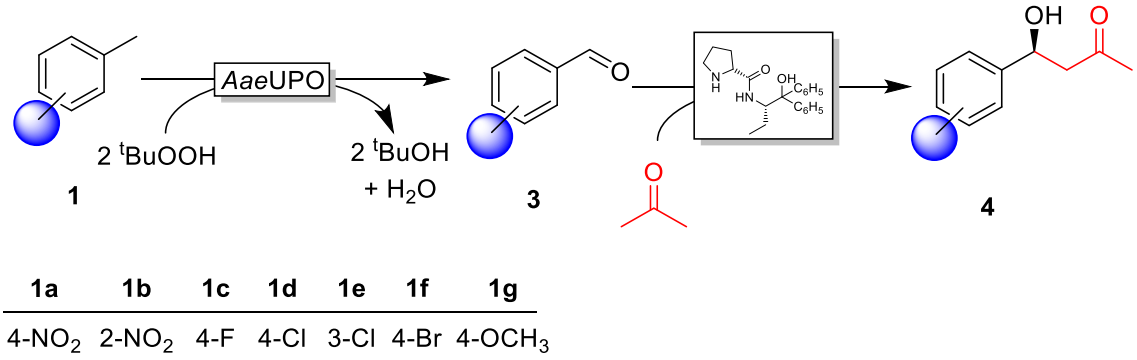


Fig. 4. Combining the bio- and organocatalytic half reactions in either one pot tandem (red) or one pot stepwise (green) fashion. One-pot tandem: 1a: 375 mg (2.95 mmol), acetone: 125 µL (1.8 mmol), $AaeUPO_{imminilised}$: 100 mg ([AaeUPO] 6.5 nmol), TBHP feed rate: 12 mM h^{-1} for 6 h, OC-2 0.2 µmol in 1.5 mL glass vials, reactions were performed at 25 °C, 800 rpm for 20 h. (3a (●), 4a (□)) One-pot stepwise: 1a: 375 mg (2.95 mmol), acetone: 125 µL (1.8 mmol), $AaeUPO_{imminilised}$: 100 mg ([AaeUPO] 6.5 nmol), TBHP feed rate: 12 mM h^{-1} for 6 h, in 1.5 mL glass vials, reactions were performed at 25 °C, 800 rpm for 20 h, then OC-2: 0.4 mM was added and reacted for another 24 h, (3a (●), 4a (□)) N = 1.

Table 1
Evaluating the substrate scope of the chemoenzymatic cascade.



Substrate	c (3) [mM]	TN_{AaeUPO} [mol × mol^{-1}]	c (4) [mM]	ee (4) [%]
1a	7	6923	38	> 80
1b	3	8154	50 ± 4	91
1c	46 ± 1	7077	n.d.	n.d.
1d	54 ± 0.5	8462	< 5	89
1e	50 ± 1	7846	< 5	99
1f	92 ± 35	15077	6 ± 3	80
1g	30 ± 4	4615	n.d.	n.d.

Reaction conditions *Aae*$UPO_{imminilised}$: 100 mg ([*Aae*UPO] = 6.5 nmol), [**OC-2**] = 0.2 µmol, substituted toluene 375 mg (2.95 mmol), acetone 125 µL (1.8 mmol), TBHP 12 mM h^{-1}, 25 °C, 800 rpm, 24 h;

CRediT authorship contribution statement

Yutong Wang: Investigation, Data curation. **Chiara Domestici:** Investigation. **Niklas Teetz:** Investigation. **Dirk Holtmann:** Supervision, Conceptualization. **Miguel Alcalde:** Conceptualization. **Mengfan Wang:** Supervision. **Wei Qi:** Supervision. **Wuyuan Zhang:** Supervision. **Frank Hollmann:** Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.mcat.2024.114515.

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[18] It is important to acknowledge that the current analytical protocols do not achieve baseline separation of the two enantiomers of the product (Figure S7). As a result, the reported optical purities of **4a** should be interpreted with caution.