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Preparative scale Achmatowicz and aza-Achmatowicz rearrangements catalyzed by *Agrocybe aegerita* unspecific peroxygenase†

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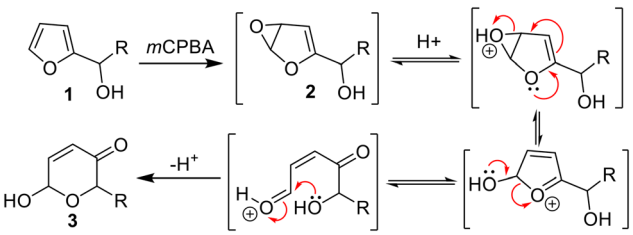
The unspecific peroxygenase (UPO) from *Agrocybe aegerita* (r*Aae*UPO-PaDa-I-H) is an effective and practical biocatalyst for the oxidative expansion of furfuryl alcohols/amines on a preparative scale, using the Achmatowicz and aza-Achmatowicz reaction. The high activity and stability of the enzyme, which can be produced on a large scale as an air-stable lyophilised powder, renders it a versatile and scalable biocatalyst for the preparation of synthetically valuable 6-hydroxypyranones and dihydropiperidinones. In several cases, the biotransformation out-performed the analogous chemo-catalysed process, and operates under milder and greener reaction conditions.

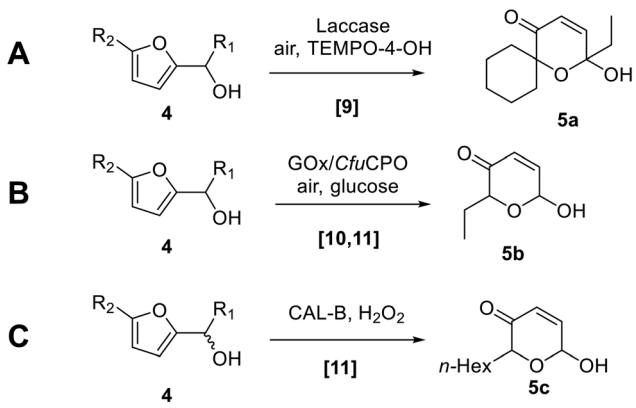
Introduction

The oxidative rearrangement of furyl-alcohols to dihydropyranones – generally termed the Achmatowicz reaction[1] – is one of the most important and widely used furan transformations in organic chemistry (*e.g.* **1** → **3**, Scheme 1).[2,3] Most synthetic Achmatowicz reactions rely on the use of strong stoichiometric chemical oxidants[4,5] such as *m*CPBA, which in this case promotes the reaction *via* an initial epoxidation (to form **2**) and subsequent rearrangement as shown (Scheme 1). Various other Achmatowicz-type methods are also known using other oxidants, *e.g.* employing electrochemistry,[6] singlet oxygen[7] and photocatalysis.[8] Apart from the latter, catalytic methods performed under ambient conditions are rare.

Biocatalysis offers significant advantages compared with chemical methods, to enable milder and more sustainable Achmatowicz reactions (Scheme 2). The first enzymatic Achmatowicz reaction reported was developed by Beifuss *et al.*, and is based on the use of laccase from *Trametes versicolor* and hydroxy-TEMPO as a redox mediator (*e.g.* **4** → **5a**, Scheme 2A).[9] After this, Deska *et al.* reported that *C. fumago* chloroperoxidase (*Cfu*CPO), catalyzes the Achmatowicz reaction on a range of substrates and with moderate enantioselectivity.[10] A subsequent study by the same group utilized *Cfu*CPO, assisted by glucose oxidase (GOx), for the generation of peracid from an acetic acid buffer, for Achmatowicz reactions of the type **4** → **5b** (Scheme 2B).[11] The authors also demonstrated that *C. antarctica* lipase B (CAL-B) can also be used to generate per-



Scheme 1 The Achmatowicz reaction promoted by *meta*-chloroperbenzoic acid.



Scheme 2 Biocatalytic Achmatowicz protocols.

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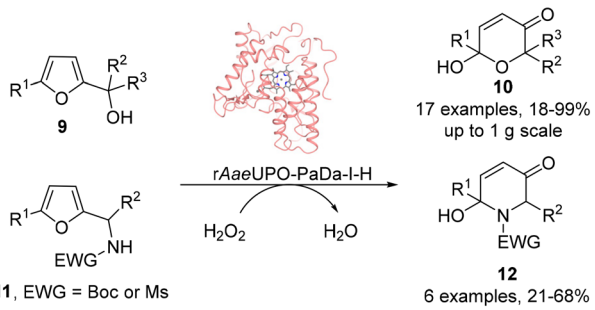
acetic acid from the acetate present in the buffer and the added H_2O_2 (*e.g.* **4** → **5c**; Scheme 2C).[11] Each of these reactions should proceed through an initial oxidation of the furan and subsequent rearrangement, *via* a mechanism similar to that depicted in Scheme 1.

The aza-Achmatowicz reaction for the transformation of aza-analogues such as **6** into products **8** is another important synthetic transformation, with a general reaction depicted in Scheme 3A.[12] Biocatalytic aza-Achmatowicz reactions are rare however, although Hollman and co-workers have reported successful examples using the vanadium-dependent peroxidase from *C. inaequalis* (*Cin*VPO) in the presence of H_2O_2 and KBr in aqueous media with ethanol.[13] In this case, the reactive oxidant is likely to be hypohalous acid generated biocatalytically from KBr (Scheme 3B). Notably, reactions such as the formation of **5d** were performed at up to 100s milligram scale, although relatively limited scope was demonstrated to date. The CAL-B-mediated *in situ* peracetic acid generation system developed by the Deska group can also be used to perform aza-Achmatowicz reactions in limited cases.[11]

Unspecific Peroxygenases (UPOs) have recently been shown to possess catalytic characteristics bridging those of cytochromes P450 (P450s) and haloperoxidases such as *Cfu*CPO, as well as exhibiting superior stability and catalytic activity.[14–17] In contrast to P450s however, these heme dependent enzymes are able to catalyze oxygenation reactions at the expense of only hydrogen peroxide as the external oxidant. These attributes, coupled with advances in heterologous expression,[18–20] improved protocols for protein engineering[21–23] and also methods for gradual hydrogen peroxide delivery that reduce oxidative stress on the enzyme,[24–26] have established them as attractive biocatalysis for scalable oxygenation reactions. We reasoned therefore that UPOs could also be good biocatalysts for both Achmatowicz and aza-Achmatowicz reactions initiated by epoxidation, especially as UPOs have been shown previously to be capable of promoting the epoxidation of a range of alkenes aliphatic and alicyclic alkenes[27] and styrene derivatives.[28] Therefore, in this study, both reaction classes were explored, using a variant of the PaDa-I mutant of *Agrocybe aegerita* UPO originally described by Alcalde and co-workers,[18,19] which was expressed in *Pichia pastoris* using a modified vector and previously used by our groups as r*Aae*UPO-PaDa-I-H.[29–32] Our studies confirm that r*Aae*UPO-PaDa-I-H is an effective biocatalyst for both transformations (Scheme 4). Both reaction series gave high synthetic yields and were readily scaled-up. The UPO reactions show an improved substrate scope over *Cfu*CPO-catalyzed reactions, with 23 preparative scale reactions (up to 1 g scale) successfully demonstrated and further expand our knowledge of the ever-growing promiscuous activity of UPOs.

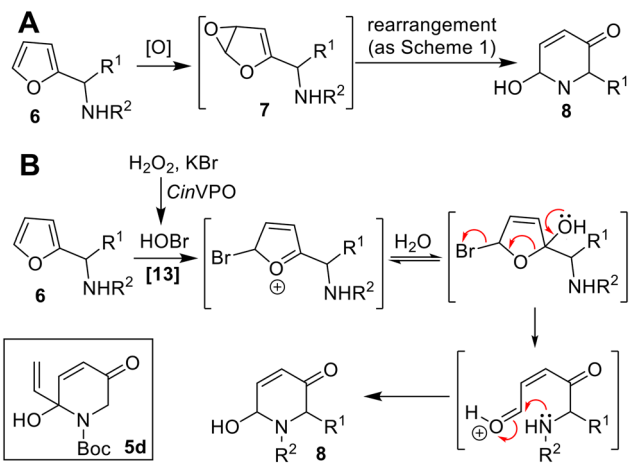


Scheme 4 Preparative scale Achmatowicz and aza-Achmatowicz rearrangements catalyzed by r*Aae*UPO-PaDa-I-H in this study.

Results and discussion

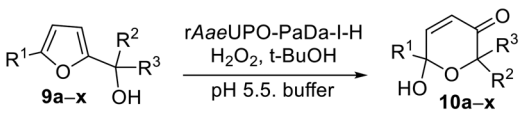
r*Aae*UPO-PaDa-I-H was expressed and lyophilized using our reported method.[29] The 2-furylcarbinol substrates **9a–v** used in this study were prepared using standard methods, with full synthetic details described in the ESI (ESI, section 7†). Synthetic standards for the Achmatowicz products **10a–i** were also prepared from the 2-furylcarbinols *via* oxidation with *N*-bromosuccinimide (NBS) or potassium hypobromite (ESI, section 7†).

We started by examining the reactions of 2-furylcarbinols **9a–i** on small scale. The substrates (10 mM) were reacted with H_2O_2 (3.3 mM in a single portion, or 10 mM added in three portions at 10 min intervals) in the presence of r*Aae*UPO-PaDa-I-H, on 1 mL scale in a pH 5.5 buffer solution, with 10% *t*-BuOH as co-solvent at RT[33] (Table 1). The reaction extracts were analysed by GC-FID. The results of biotransformations of the first substrates tested were somewhat disappointing; for example, the simplest furyl-carbinol **9a** (entry 1), was not converted into the Achmatowicz product **10a** and a complex mixture of side products was obtained. Substitution with an ethyl group on the furan 5-position (**9b**) resulted in a simpler product mixture, but aldehyde **13** was the only product identified (entries 2 and 3). 2-Furylcarbinol **9c** was not oxidized by r*Aae*UPO-PaDa-I-H (entry 4). In this regard, r*Aae*UPO-PaDa-I-H is similar to CPO as that enzyme was also reported not to convert a 2,5-disubstituted substrate in the original report.[10]



Scheme 3 Biocatalytic aza-Achmatowicz reaction.

Table 1 GC conversions for the rAaeUPO-PaDa-I-H-catalyzed Achmatowicz reaction. Products were identified by comparing their retention times to authentic standards



Entry	Substrate	$[H_2O_2]$/mM	Product[a]
1	**9a**	3.3	**10a**, 0%
2	**9b**	3.3	**13**, 29%
3		10	**13**, 85%
4	**9c**	3.3	**10c**, 0%
5	**9d**	3.3	**10d**, 13%
6		10	**10d**, 80%
7	**9e**	3.3	**10e**, 20%
8		10	**10e**, 59%
9	**9f**	3.3	**10f**, 27%
10		10	**10f**, 70%
11	**9g**	3.3	**10g**, 21%
12		10	**10g**, 59%
13	**9h**	3.3	**10h**, 16%
14		10	**10h**, 47%
15	**9i**	3.3	**10i**, 30%
16		10	**10i**, 84%[b]

Reaction conditions: 10 mM substrate, 4.4 U mL^{-1} *r*AaeUPO or 20 U mL^{-1} CPO, 1 mL total volume, 10% v/v ^{t}BuOH, 50 mM sodium citrate buffer (pH 5.5) at RT.[33] Reactions were extracted with 2 × 1 mL EtOAc, dried over $MgSO_4$ and analysed by GC-FID (HP-5MS column). [a] Yields based on analysis by GC-FID. [b] H_2O_2 was added dropwise by a syringe pump over 7 h. Control reactions, to which no enzyme was added, gave no conversion.[34]

However, more promisingly, r*Aae*UPO-PaDa-I-H successfully converted monosubstituted 2-furylcarbinols **9d–g** into the analogous pyranones **10d–g** (entries 5–16; the products were positively identified using authentic standards). This initial screen showed that less bulky furyl-carbinols are transformed more readily by the enzyme, with similar results obtained in published studies using *Cfu*CPO as the biocatalyst.[11] It is also noteworthy that, using the same H_2O_2 addition method, in our hands *Cfu*CPO failed to convert any of 2-furylcarbinols **9d–i** at 3.3 mM and 10 mM H_2O_2 loading (data not shown). This may be due to the instability of *Cfu*CPO in the presence of the comparatively higher peroxide loadings used, and serves as a useful illustration of the practicality and robustness of r*Aae*UPO-PaDa-I-H. The >50% conversions observed in several cases show that the UPO is capable of converting both enantiomers of the chiral 2-furylcarbinols tested, at least to some degree. This observation, coupled to near-zero specific rotation measurements for the products (*vide infra*), suggests that achieving an enantioselective r*Aae*UPO-PaDa-I-H-catalysed Achmatowicz reaction through kinetic resolution of racemic 2-furylcarbinols is unlikely to be feasible using this enzyme.

Next, we set out to optimize the reaction conditions prior to increasing the reaction scale. First, a pH screen was carried out using 1-(2-furyl)-ethanol **9d**. A pH between 5.0 and 7.0 was found to be optimal for its conversion into **10d** (Fig. 1), hence a pH 5.5 citrate buffer was taken forward and used in the preparative reactions.[24] The effect of different co-solvents was also examined, with acetone identified as the best co-solvent based solely on conversion, especially at higher concentrations (Fig. 2).

However, *t*-BuOH performed similarly at 10% and 20% (v/v), and it was later found that product purification in the

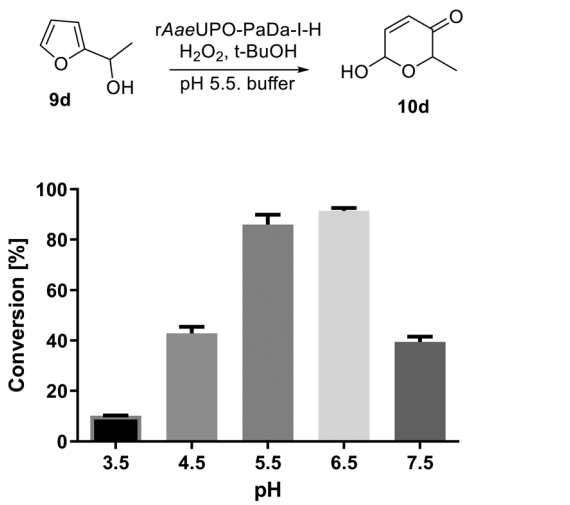


Fig. 1 pH profile of the r*Aae*UPO-PaDa-I-H catalysed transformation of **9d** into **10d**. Reactions were performed in 150 μL total volume, extracted with 2 × 200 μL EtOAc and analysed by GC-FID on a HP-5MS column (30 m) at RT.[33] Citrate buffers were used between pH 3.50 and 5.50 (50 mM) and above this KPi buffers (50 mM) were employed. Error bars show ±one standard deviation from the mean.

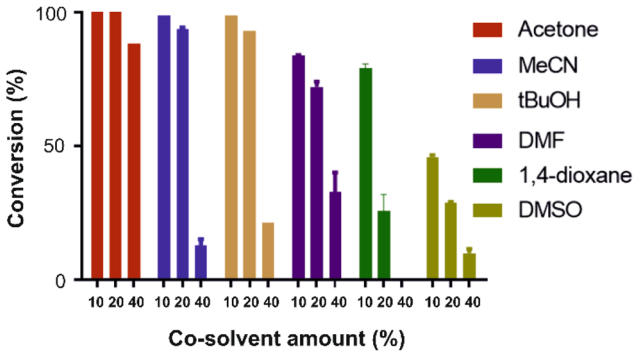


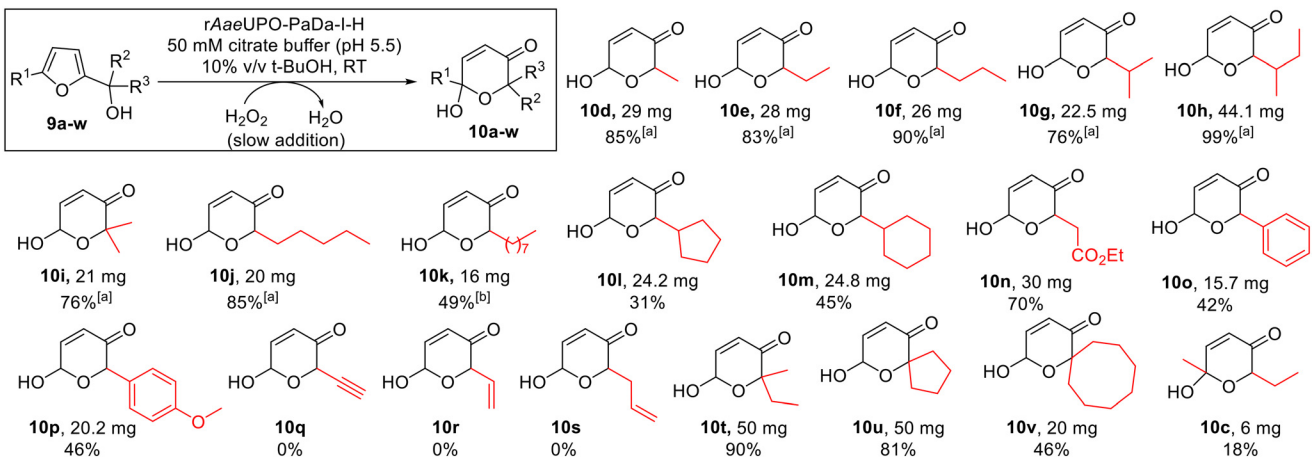
Fig. 2 Effect of co-solvent on the r*Aae*UPO-PaDa-I-H catalysed transformation of **9d** into **10d**. Tests were performed in 150 μL total volume in sodium citrate buffer (pH = 5.50, 50 mM) at 10 mM substrate concentration at RT.[33] Reactions were extracted with 2 × 200 μL EtOAc after 15 min and the extract was directly analysed by GC-FID (30 m HP-5MS column). Error bars indicate ±one standard deviation. MeCN – acetonitrile; DMF – dimethylformamide; DMSO – dimethylsulfoxide.

preparative reactions was simpler from reactions containing *t*-BuOH compared with those containing acetone. Therefore, *t*-BuOH was preferred for the preparative studies that follow (*vide infra*, Scheme 5).

The optimised reaction conditions were then tested at preparative scale. A range of monosubstituted 2-furylcarbinols **9d–v** was explored (Scheme 5), with a syringe pump used to control slow H_2O_2 addition at a rate of 5 mM h^{-1}, at 10 mM substrate concentration. In all cases the reactions were run for 2 h at room temperature. Pleasingly, the preparative results for 2-furylcarbinols **9d–i** were in line with the small scale experiments, with pyranones **10d–i** all being formed and isolated in good to excellent yields. Longer chain homologues **10j** and **10k** were also prepared in the same way. In the case of **10k** the co-solvent was switched to acetonitrile, as the pyranone product was found to be too poorly soluble when using *t*-BuOH. Notably, such bulky substrates were not reported to be transformed by *Cfu*CPO in previous studies.[11]

Products containing bulkier cyclic hydrocarbon substituents (**10l** and **10m**) and aryl (**10o** and **10p**) were also obtained in acceptable yields, while ester-containing pyranone **10n** was isolated in 70% yield. Conversely, alkyne and alkene containing starting materials **9q–9s** were not converted into the expected products (**10q–s**), with unreacted 2-furylcarbinol starting material recovered in each case and no epoxidation of the double bond detected during the reactions. We postulated that this may be a consequence of enzyme inhibition by these substrates, and indeed, this notion was supported by the observation that 2-furylcarbinols **9r** and **9s** significantly reduced the conversion of **9e** into **10e** in inhibition studies (ESI section 4, Fig. S1†). However, a mechanism for this inhibition was not clear from these experiments. Bulky tertiary alcohol containing starting materials were well-tolerated, with pyranones **10t–v** isolated in good yields using the standard protocol. Finally, pyranone **10c** was obtained from 2,5-disubstituted starting material **9c**, although the isolated yield was modest in this case (18%). This example is notable as **9c** was not converted in the initial small-scale screen. This highlights the synthetic advantages that can be realised by using the slow H_2O_2 addition method.

Notably, in our hands, these biocatalytic Achmatowicz reactions using r*Aae*UPO-PaDa-I-H tended to give similar or superior yields to those obtained for the analogous reactions promoted by *N*-bromosuccinimide (NBS, ESI section 5†). The biocatalytic method also generally gave rise to much cleaner reaction mixtures; indeed, column chromatographic purification was not required in several cases. As expected, all of the pyranone products formed from chiral alcohols were obtained as mixtures of diastereoisomers (dr between 1 : 1 and 3 : 1; see ESI†). The dr of the products produced by the enzymatic method was the same as in the material synthesised using NBS, strongly indicating that these are simply thermodynamic



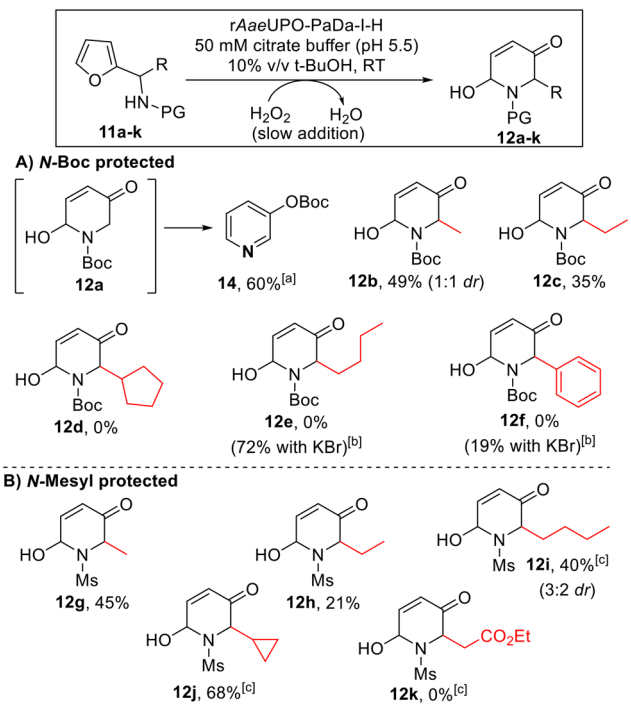
Scheme 5 Achmatowicz reactions promoted by r*Aae*UPO-PaDa-I-H and H_2O_2. Reaction conditions as shown in the box above (see ESI section 7† for more detail). 10 mM substrate concentration was used, with H_2O_2 supplied *via* syringe pump at 5 mM h^{-1} (2 h reaction time in total) at RT.[33] [a] No chromatographic purification was needed in these cases. [b] Performed with 10 v/v % MeCN in place of *t*-BuOH.

outcomes associated with the relative energies of the two equilibrating diastereoisomers. It is therefore very unlikely that other UPOs would affect the dr of the products formed. However, it remains possible that the application of sequence diverse UPOs or engineered enzymes may be capable of delivering enantiomerically enriched products, *via* the kinetic resolution of chiral starting materials.

UPO-catalyzed aza-Achmatowicz reactions

To our knowledge, there have been few reports of biocatalytic aza-Achmatowicz reactions reported in the literature,[11,13] and those examples relied on the formation of a diffusible oxidants by either lipase-catalysed perhydrolysis of acetate[11] or the vanadium-dependent peroxidase from *Curvularia inequalis* (*Cin*VPO).[13] Based on our results with the standard Achmatowicz reaction, we reasoned that the active site of r*Aae*UPO-PaDa-I-H may permit the binding and direct oxidation of aza-Achmatowicz substrates within the enzyme active site. To probe this, we challenged the enzyme with a series of *N*-protected furan-2-ylmethanamine substrates (**11a–f**, Scheme 6A), on preparative scale, using the method established for the Achmatowicz reaction of 2-furylcarbinols (ESI section 5†).

Substrates bearing a *tert*-butyloxycarbonyl (Boc) protecting group in the amine (**11a–f**) were explored first (Scheme 3A).



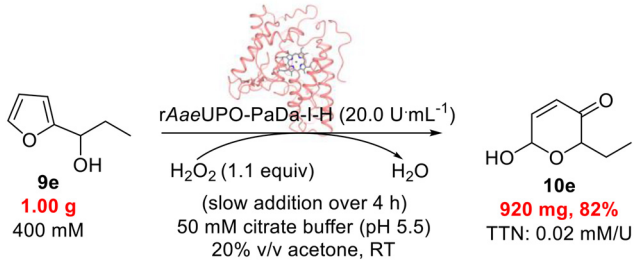
Scheme 6 Aza-Achmatowicz reactions promoted by r*Aae*UPO-PaDa-I-H and H_2O_2. Reaction conditions as shown in the box above. 10 mM substrate concentration was used, with H_2O_2 supplied using a syringe pump at a rate of 5 mM h^{-1} at RT.[33] [a] **12a** could not be isolated, due to its propensity to rearrange into *tert*-butyl pyridine-3-yl carbonate product **14**. [b] KBr (100 mM) was added to the buffer. [c] 1 mM h^{-1} H_2O_2 addition rate was used.

Pleasingly, the smaller substrates in this series were accepted by r*Aae*UPO-PaDa-I-H. However, in the case of the smallest substrate in this series (**11a**, R = H), while we believe that the aza-Achmatowicz took place, the product **12a** could not isolated as it was observed to be unstable and to convert into pyridine **14**, which was isolated in 60% yield. Pleasingly, substituted analogues **12b** and **12c** were more stable, and using the standard method, the expected aza-Achmatowicz products were obtained in acceptable yields. To the best of our knowledge, these represent the first biocatalytic aza-Achmatowicz reactions to be performed without using diffusible oxidants. Substrate **11c** apparently represents the limit of the *N*-Boc series with respect to size however, as bulkier substrates **11d–f** were not converted into the respective products **12d–f**. This result was not wholly surprising, especially considering the additional steric imposition of the Boc protecting group.

Nonetheless, in these more challenging cases, we found that a biocatalytic aza-Achmatowicz can still be achieved, demonstrated by the successful synthesis of **12e** and **12f**, by simply adding KBr to the reaction medium. Presumably, these modified examples operate *via* enzyme-catalysed oxidation of the added bromide to form hypobromite, which then diffuses from the enzyme active site to promote the oxidative rearrangement. This discovery is useful in expanding the scope the method to include bulkier substrates. However, this is be balanced against the strong likelihood that the oxidation takes place outside of the UPO active site when KBr is used, and hence the opportunity for the enzyme to impart stereocontrol on the reaction is lost.

N-Mesyl protected substrates **11g–k** (Scheme 6B) were also examined, in the hope that the switch to a smaller protecting group would allow more flexibility to incorporate bulkier substituents. Although yields from substrates **11g** and **11h** gave results comparable to analogous *N*-Boc protected substrates **11b** and **11c**, in the case of substrate **11i**, this was transformed with 40% yield, whereas the *N*Boc-protected analog **11e** was not. No conversion was observed when trying to convert the larger ester containing substrate **11k** however. In most cases (**12b** and **12i** being the exceptions) the products were isolated as single diastereoisomers. It is not always clear whether the 2,6-*cis* or 2,6-*trans* diastereoisomer will predominate in aza-Achmatowicz reactions, although it is relatively common for there to be a thermodynamic preference for one diastereoisomer.[2] Based on specific rotation measurements, all products **12b–j** appear to be racemic, indicating little or no kinetic resolution of the chiral amine precursors **11b–j**, as was the case for the 2-furylcarbinol substrates.

Finally, having demonstrated the feasibility of both the r*Aae*UPO-PaDa-I-H-catalyzed Achmatowicz and aza-Achmatowicz reactions, we wanted to further demonstrate the scalability of the protocol by performing the oxidation of **9e** on a 1 g scale (Scheme 7). To do this, the concentration of the substrate was raised radically to 400 mM. The enzyme tolerated this change well, but it was necessary to switch the co-solvent to acetone better solubilise **9e**. The use of acetone as co-solvent introduces the possibility of unwanted dimethyl-



Scheme 7 r*Aae*UPO-PaDa-I-H catalyzed oxidation of **9e** on a 1 g scale at RT.[33]

dioxirane (DMDO – potentially explosive) being formed in the reaction, although the slow addition of H_2O_2 and the quick enzymatic reaction both minimise this risk. Under these conditions, 0.92 g of the product **10e** was isolated, which corresponds to 82% isolated yield. This transformation showcases well the robustness of r*Aae*UPO-PaDa-I-H under challenging biocatalytic conditions.

Conclusions

We have demonstrated that r*Aae*UPO-PaDa-I-H is an easy-to-use catalyst for Achmatowicz and aza-Achmatowicz reactions. A simple, slow-addition H_2O_2 delivery method has been established for preparative scale reactions, and the lyophilized enzyme is practical, easy to use and is stable for years when stored at −20 °C. The transformations are performed under mild reaction conditions, and are generally highly chemoselective – the biocatalytic method often out-performed the standard chemo-promoted method (NBS), and in several cases afforded products that were sufficiently clean that column chromatography was not needed. The scope of the transformations is relatively broad, with substrate specificity primarily influenced by steric factors. Notably, the scalability of the r*Aae*UPO-PaDa-I-H catalyzed Achmatowicz reaction was further demonstrated by performing the oxidation of 1-(2-furyl) propan-1-ol **9e** at 1 g scale, with the desired product **10e** isolated in 82% yield. This study expands the reaction scope of UPOs and provides further substantial evidence of practicality and ever-growing practical synthetic utility as green oxidants.

Author contributions

All authors contributed to the design and execution of experiments. BP, WPU and GG wrote the manuscript, with contributions also from ADR.

Data availability

The data supporting this article have been included as part of the ESI.†

Conflicts of interest

There are no conflicts to declare.

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References

1 O. Achmatowicz, P. Bukowski, B. Szechner, Z. Zwierzchowska and A. Zamojski, *Tetrahedron*, 1971, **27**, 1973–1996.
2 A. K. Ghosh and M. Brindisi, *RSC Adv.*, 2016, **6**, 111564–111598.
3 L. Liang, L.-D. Guo and R. Tong, *Acc. Chem. Res.*, 2022, **55**, 2326–2340.
4 M. P. Georgiadis, E. A. Couladouros, M. G. Polissiou, S. E. Filippakis, D. Mentzafos and A. Terzis, *J. Org. Chem.*, 1982, **47**, 3054–3058.
5 T. Shono and Y. Matsumura, *Tetrahedron Lett.*, 1976, **17**, 1363–1364.
6 X. Liu, B. Li, G. Han, X. Liu, Z. Cao, D.-e. Jiang and Y. Sun, *Nat. Commun.*, 2021, **12**, 1868.
7 G. C. M. Lee, E. T. Syage, D. A. Harcourt, J. M. Holmes and M. E. Garst, *J. Org. Chem.*, 1991, **56**, 7007–7014.
8 M. B. Plutschack, P. H. Seeberger and K. Gilmore, *Org. Lett.*, 2017, **19**, 30–33.
9 C. Asta, D. Schmidt, J. Conrad, B. Förster-Fromme, T. Tolasch and U. Beifuss, *RSC Adv.*, 2013, **3**, 19259–19263For a review on laccase mediated organic transformations, see:J. Jayakumar, D. Priyadarshini, A. Parthasarathy and S. R. Reddy, *Asian J. Org. Chem.*, 2022, **12**, e202200564.
10 D. Thiel, D. Doknić and J. Deska, *Nat. Commun.*, 2014, **5**, 5278.
11 D. Thiel, F. Blume, C. Jäger and J. Deska, *Eur. J. Org. Chem.*, 2018, 2717–2725.
12 F. van der Pijl, F. L. van Delft and F. P. J. T. Rutjes, *Eur. J. Org. Chem.*, 2015, 4811–4829.
13 E. Fernández-Fueyo, S. H. H. Younes, S. v. Rootselaar, R. W. M. Aben, R. Renirie, R. Wever, D. Holtmann, F. P. J. T. Rutjes and F. Hollmann, *ACS Catal.*, 2016, **6**, 5904–5907.
14 M. Hofrichter, H. Kellner, M. J. Pecyna and R. Ullrich, in *Monooxygenase, Peroxidase and Peroxygenase Properties and Mechanisms of Cytochrome P450*, ed. E. G. Hrycay and S. M. Bandiera, Springer International Publishing, Cham, 2015, pp. 341–368.
15 Y. Wang, D. Lan, R. Durrani and F. Hollmann, *Curr. Opin. Chem. Biol.*, 2017, **37**, 1–9.

16 M. Hobisch, D. Holtmann, P. Gomez de Santos, M. Alcalde, F. Hollmann and S. Kara, *Biotechnol. Adv.*, 2021, **51**, 107615.
17 D. T. Monterrey, A. Menés-Rubio, M. Keser, D. Gonzalez-Perez and M. Alcalde, *Curr. Opin. Green Sustainable Chem.*, 2023, 100786.
18 P. Molina-Espeja, E. Garcia-Ruiz, D. Gonzalez-Perez, R. Ullrich, M. Hofrichter and M. Alcalde, *Appl. Environ. Microbiol.*, 2014, **80**, 3496–3507.
19 P. Molina-Espeja, S. Ma, D. M. Mate, R. Ludwig and M. Alcalde, *Enzyme Microb. Technol.*, 2015, **73–74**, 29–33.
20 J. Carro, A. González-Benjumea, E. Fernández-Fueyo, C. Aranda, V. Guallar, A. Gutiérrez and A. T. Martínez, *ACS Catal.*, 2019, **9**, 6234–6242.
21 P. Molina-Espeja, M. Cañellas, F. J. Plou, M. Hofrichter, F. Lucas, V. Guallar and M. Alcalde, *ChemBioChem*, 2016, **17**, 341–349.
22 J. Münch, J. Soler, N. Hünecke, D. Homann, M. Garcia-Borràs and M. J. Weissenborn, *ACS Catal.*, 2023, **13**, 8963–8972.
23 P. Gomez de Santos, I. Mateljak, M. D. Hoang, S. J. Fleishman, F. Hollmann and M. Alcalde, *J. Am. Chem. Soc.*, 2023, **145**, 3443–3453.
24 Y. Ni, E. Fernández-Fueyo, A. Gomez Baraibar, R. Ullrich, M. Hofrichter, H. Yanase, M. Alcalde, W. J. H. van Berkel and F. Hollmann, *Angew. Chem., Int. Ed.*, 2016, **55**, 798–801.
25 S. J. Freakley, S. Kochius, J. van Marwijk, C. Fenner, R. J. Lewis, K. Baldenius, S. S. Marais, D. J. Opperman, S. T. L. Harrison, M. Alcalde, M. S. Smit and G. J. Hutchings, *Nat. Commun.*, 2019, **10**, 4178.
26 E. Romero, M. J. Johansson, J. C. Cartwright, G. Grogan and M. A. Hayes, *Angew. Chem., Int. Ed.*, 2022, **61**, e202207831.
27 P. M. Kinne, R. Ullrich, G. Kayser and M. Hofrichter, *Enzyme Microb. Technol.*, 2013, **52**, 370–376.
28 M. C. R. Rauch, F. Tieves, C. E. Paul, I. W. C. E. Arends, M. Alcalde and F. Hollmann, *ChemCatChem*, 2019, **11**, 4519–4523.
29 H. E. Bonfield, K. Mercer, A. Diaz-Rodriguez, G. C. Cook, B. S. J. McKay, P. Slade, G. M. Taylor, W. X. Ooi, J. D. Williams, J. P. M. Roberts, J. A. Murphy, L. Schmermund, W. Kroutil, T. Mielke, J. Cartwright, G. Grogan and L. J. Edwards, *ChemPhotoChem*, 2020, **4**, 45–51.
30 L. Schmermund, S. Reischauer, S. Bierbaumer, C. K. Winkler, A. Diaz-Rodriguez, L. J. Edwards, S. Kara, T. Mielke, J. Cartwright, G. Grogan, B. Pieber and W. Kroutil, *Angew. Chem., Int. Ed.*, 2021, **60**, 6965–6969.
31 B. Pogrányi, T. Mielke, A. Díaz-Rodríguez, J. Cartwright, W. P. Unsworth and G. Grogan, *Angew. Chem., Int. Ed.*, 2023, **63**, e202214759.
32 B. Melling, T. Mielke, A. C. Whitwood, T. J. C. O'Riordan, N. Mulholland, J. Cartwright, W. P. Unsworth and G. Grogan, *Chem. Catal.*, 2024, 100889.
33 Throughout this manuscript, 'RT' refer to the ambient temperature in out laboratory, which is typically ≈21 °C.
34 No reaction occurred in a control reaction in which **9d** was reacted under the same conditions but lacking the enzyme; this confirms that the oxidation does not simply result from the generation of a peracid oxidant derived from the citrate buffer and H_2O_2.