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Peroxygenase-Catalysed Sulfoxidations in Non-Aqueous Media

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Chiral sulfoxides are valuable building blocks in asymmetric synthesis. However, the biocatalytic synthesis of chiral sulfoxides is still challenged by low product titres. Herein, we report the use of peroxygenase as a catalyst for asymmetric sulfoxidation under non-aqueous conditions. Upon covalent immobilisation, the peroxygenase showed stability and activity under neat reaction conditions. A large variety of sulfides was converted into chiral sulfoxides in very high product concentration with moderate to satisfactory optical purity (e.g. 626 mM of (*R*)-methyl phenyl sulfoxide in approx. 89% ee in 48 h). Further polishing of the ee value via cascading methionine reductase A (MsrA) gave >99% ee of the sulfoxide. The robustness of the enzymes and high product titer is superior to the state-of-the-art methodologies. Gram-scale synthesis has been demonstrated. Overall, we demonstrated a practical and facile catalytic method to synthesize chiral sulfoxides.

Introduction

Chiral sulfoxides are important compounds in organic chemistry serving as chiral building blocks and their use as ligands in asymmetric catalysis.[1] Also a range of pharmaceutically active compounds such as Esomeprazole, Sulmazole or Amodafinil are chiral sulfoxides.[2] Their preparation via stereoselective oxidation of prochiral thioethers is well documented in the literature using simple oxidants such as H_2O_2 or organic hydroperoxides.[3,4]

Also enzymes have been evaluated for stereoselective sulfoxidation reactions. Prominent examples are monooxygenase-catalysed sulfoxidations.[5] Flavin-dependent monooxygenases for example hold much promise for the stereoselective oxyfunctionalisation of prochiral thioethers.[6,7] Monooxygenase-catalysed transformations, however, are notoriously plagued by their cofactor dependency and the O_2-dependency of the transformation. Therefore, H_2O_2-dependent peroxygenases (also called unspecific peroxygenases, UPOs) have been in the centre of attention for a long time.[8] One major advantage of UPOs over monooxygenases is their mechanistic simplicity, which directly translates in simpler practical applicability (use of H_2O_2 instead of NAD(P)H together with the required *in situ* regeneration systems, Scheme 1).

As already pointed out by Klibanov and coworkers in the 1980s, peroxygenases can be used in non-aqueous media if substituting H_2O_2 by organohydroperoxides.[9] Principally, this opens up the possibility to perform UPO-catalysed sulfoxidation reactions under non-aqueous conditions and thereby circumvent the low aqueous solubility of most reagents of interest. Unfortunately, so far, this potential has not been realised and typical sulfoxide concentrations seldom exceed the 10 mM

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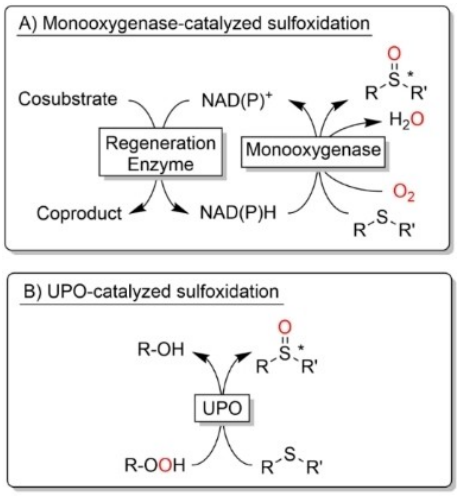

Scheme 1. Comparison of the general reaction systems using monooxygenases (A) and peroxygenases (B) for enantioselective sulfoxidation reactions.

range, causing large quantities of waste water and requiring additional steps such as solvent extraction. To increase product titres, cosolvents have been evaluated[10] but reactions devoid of additional solvents would be most desirable (the best solvent is no solvent).[11]

We therefore set out to evaluate the potential of UPO-catalysed sulfoxidation under non-aqueous reaction conditions, aiming at more sustainable production of chiral sulfoxides in terms of less process waste emission. As UPO we chose the PaDa-I mutant[12] archetypal UPO from *Agrocybe aegerita* (*Aae*UPO)[13] recombinantly expressed in *Pichia pastoris*.[14] To make *Aae*UPO applicable in non-aqueous media, we immobilised the enzyme on a glutardialdehyde-activated amino-functionalised resin following the protocol of Kara and co-workers (SI for further information).[15]

Results and Discussion

Having the immobilised *Aae*UPO (imm-*Aae*UPO) at hand, we applied it for the sulfoxidation of thioanisole (**1 a**) by dispersing imm-*Aae*UPO in neat **1 a** and slowly adding *tert*-butyl hydroperoxide (^{t}BuOOH) as organosoluble H_2O_2 equivalent. Pleasingly, already a first trial under arbitrarily chosen conditions yielded 626 mM of (*R*)-**1 b** in approx. 89% *ee* in 48 h. This optical purity is practically identical with the value reported previously by Monti and coworkers.[8f]

As shown in Figure 1A, the enantioselective sulfoxidation of **1 a** proceeded smoothly for at least 72 h yielding the desired (*R*)-**1 b** in greater than 78% *ee*. The sulfone over-oxidation product (**1 c**) was not observed. Upon prolonged reaction times, both, the rate of productivity as well as the enantioselectivity decreased slightly (from 13 mM h^{-1} and 89% *ee* to 10.8 mM h^{-1} and 78% *ee*), which we attribute to inactivation of the biocatalyst and an increased contribution of the non-catalysed background sulfoxidation. Therefore, we tested the rate of the background sulfoxidation in the absence of biocatalyst (empty carrier material, Table S3). Indeed, the spontaneous sulfoxidation by ^{t}BuOOH was negligible within the first 24 h (i.e. [^{t}BuOOH] < 288 mM). Throughout the enzymatic reactions, ^{t}BuOOH was practically not detectable (due to the fast *Aae*UPO-catalysed conversion) which is why we conclude that the *in situ* concentration of ^{t}BuOOH in the enzymatic reactions was too low to contribute significantly to the overall sulfoxide formation. This assumption is further supported by the high optical purity of the product corresponding to literature values.[8f]

A similar observation was made upon variation of the biocatalyst concentration (Figure 1B). Using a comparably low *Aae*UPO concentration of 1.3 μM (100 mg$_{\text{imm-}Aae\text{UPO}}$ mL^{-1}) both the product concentration as well as the optical purity were rather low (267 mM and 54.2% *ee*, respectively). Most probably, the enzyme activity added was not sufficient to consume all ^{t}BuOOH added leading to an accumulation of ^{t}BuOOH in the reaction medium. This would result in peroxide-mediated inactivation of the biocatalyst and, consequently, further accumulation of ^{t}BuOOH; both favouring the non-selective sulfoxidation. Increasing the biocatalyst loading (4.7 μM) resulted in significantly increased product and optical purity (626 mM and 89% *ee*, respectively). For the following experiments we chose for 2.35 μM *Aae*UPO as under these conditions the background activity was seemingly well-suppressed (as judged by the high optical purity of the product). We also systematically varied the ^{t}BuOOH addition rate (Figure 1C). Doubling the addition rate from 6 mM h^{-1} to 12 mM h^{-1} also approx. doubled the sulfoxide

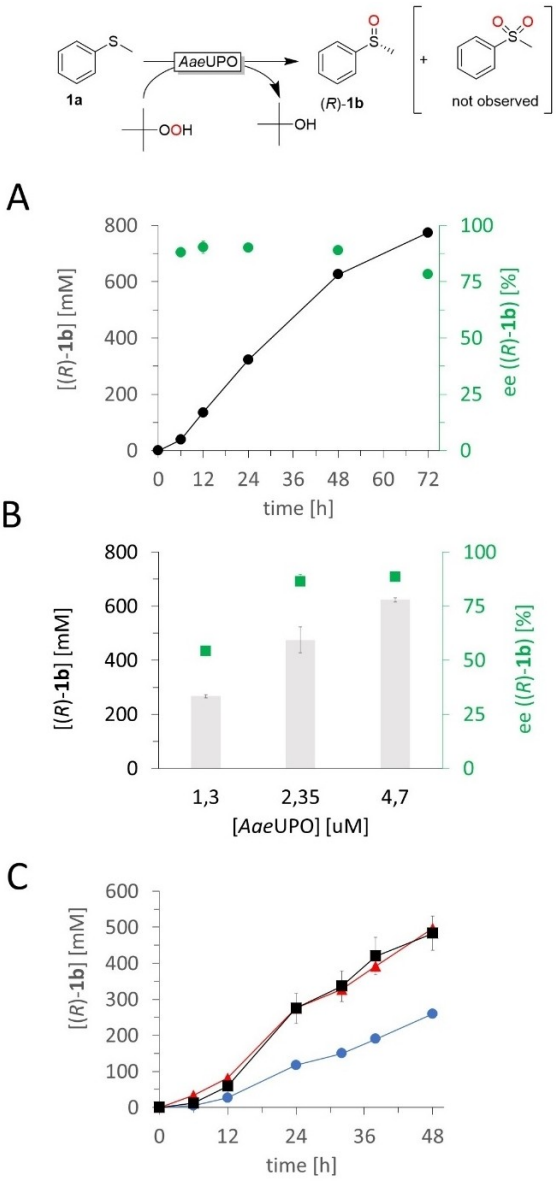

Figure 1. Optimisation of *Aae*UPO catalysed asymmetric sulfoxidation. A: Representative time-course for the sulfoxidation of **1 a**. B: Influence of biocatalyst concentration on product-yield and -optical purity of **1 a**. C: Influence of peroxide dosage on the asymmetric sulfoxidation. Reaction conditions unless specifically stated: [**1 a**] = 4.25 mmol (0.5 mL), [imm-*Aae*UPO] = 200 mg (4.7 μM), [^{t}BuOOH] = 12 mM h^{-1} (5 μL h^{-1}), 30 °C, 800 rpms, 72 h; **B**: [imm-*Aae*UPO] = 50 - 200 mg (1.3 - 4.7 μM), 48 h; **C**: [imm-*Aae*UPO] = 100 mg (2.35 μM), [^{t}BuOOH] = 6 (●), 12 (▲), 15 (■) mM h^{-1}, 48 h. All reactions were performed in duplicate. The concentration was determined by GC (calibration curve) and the *ee* values was determined by chiral HPLC.

formation rate. Further increase of the ^{t}BuOOH addition rate (to 15 mM h^{-1}) did not result in further acceleration of the product formation rate. We interpret these findings as the *Aae*UPO-catalysed sulfoxidation (in the presence of 2.35 μM of biocatalyst) being rate-limited by the ^{t}BuOOH availability until an addition rate of 12 mM h^{-1}. At higher addition rates the enzyme probably was saturated with the peroxide and further rate-increases not leading to higher enzymatic conversion rates. From this, to a catalytic turnover of *Aae*UPO of 1.2 s^{-1} (over 48 h of reaction time) or a specific activity of 0.9 U mg^{-1} for *Aae*UPO was estimated.

Using the partially optimised reaction conditions, we further explored the thioether scope of the *Aae*UPO-catalysed sulfoxidation reaction (Figure 2 and Figures S2-S21). Liquid starting materials were generally converted smoothly yielding product concentrations comparable to those obtained for **1b**. For the solid starting materials **4a**, **7a**, **8a**, **17a**, **18a**, **19a** and **22a**, we applied a two liquid phase system comprising 80% (v/v) of acetone in PBS buffer with the starting materials dissolved in the organic layer at 50 mM. Conversions ranged between 8 and 52%. Most likely, phase transfer diffusion limitations impeded the overall reaction rate. A range of *o*-, *m*- and *p*-substituted thioanisole derivates were well accepted. In case of bulkier *o*-substituents the enantioselectivity of the sulfoxidation was somewhat diminished. Apart from these, the majority of starting materials were converted in satisfactory to good enantioselectivity (up to 94.2% ee). An interesting observation was made upon varying the methyl substituent to ethyl (**15a**) and vinyl (**16a**). While the first sulfoxidation was highly enantioselective, the latter gave very poor optical purities. Additionally, various side products (presumably originating from the double bond oxidation) were observed (Figure S21). Pharmaceutically relevant 1-thiochroman-4-one-derived sulfoxides[16] were generated in moderate enantioselectivity. Even the bulky-bulky thioether **22a** was converted in satisfactory enantioselectivity (89% ee, even though using wt-*Aae*UPO the undesired Esomeprazole enantiomer was obtained).

To assess the preparative usefulness of the proposed neat sulfoxidation system the conversion of **1a** was conducted at 90 mL scale. After 51 h of reaction time and chromatographic purification approx. 7.95 g of the desired (*R*)-**1b** (85% *ee*) were obtained (Table S2), corresponding to a TON of 211900 $mol_{Product}\,mol^{-1}{}_{AaeUPO}$ and an average TOF of 1.2 s^{-1} over 51 h for *Aae*UPO.

Admittedly, the conversion in these experiments (< 10%) was far from being satisfactory. We therefore explored possibilities to increase the conversion of **1a** to (*R*)-**1b**. As enzyme inactivation at present still represents the main bottleneck of the reaction, we performed one experiment replacing the biocatalyst every 48 h (Figure 3A). Indeed, under these conditions, the product titre was increased considerably to ca. 2.5 M. Nevertheless, the conversion was still only around 27% and excessive extraction and chromatographic purification was necessary to separate **1b** from the starting material (**1a**). Therefore, we decided to evaluate acetone as solvent for **1a** (Figure 3B). Indeed using this setup, full conversion of **1a** into the desired **1b** was achieved. Another advantage of using lower starting material concentrations was that extraction was not necessary.

Therefore, we conclude that (at least at present stage) a solvent-free reaction (exhibiting incomplete conversion) is less advantageous as compared to one using a cosolvent but enabling full conversion. This assumption is also confirmed by an E-factor comparison of both reactions (Table S7). While in case of the solvent-free reaction system more than 1000 $g_{waste}\,g^{-1}{}_{product}$ were formed, this value was reduced to only 15 in case of acetone as solvent. In both cases the solvents constituted the lion' share. 'No solvent' is not always the best solvent and future process optimization will focus on solvent-based systems in which full conversion can be achieved.

Obviously, the optical purity obtained for e.g. **1b** is not satisfactory for e.g. API synthesis where *ee* of higher than 99.5% are mandatory. More enantioselective variants of PaDa-I are currently engineered in our lab. In the meanwhile, we thought about polishing the optical purity of the product by submitting the crude product to an enantioselective (*S*)-sulfoxide reduction catalysed by methionine reductase A (MrsA).[17] Starting from 2 g of the raw product ((*R*)-**1b**, 85% *ee*) dissolved in 940 mL of buffer supplemented with MsrA and DTT as stoichiometric reductant, the *ee* of the raw product continuously increased from 85% to more than 99% within 14 h of reaction time (Figure 4). Overall, 1.5 g of the enantiomerically pure product (*R*)-**1b** was obtained after isolation.

Conclusions

In summary, we developed a biosynthetic methodology for preparing chiral sulfoxides via *Aae*UPO catalysed the oxidation of thioethers under non-aqueous conditions. *Aae*UPO is a promising catalyst for this transformation even though the wild-type enzyme used so far with most thioether starting materials exhibited only modest enantioselectivity. Further engineering of *Aae*UPO will certainly overcome this current issue[18] and will circumvent the MrsA-catalysed polishing step. Also further optimisation of the immobililsation protocol will be needed to attain scalable reaction schemes. Characterisation of the kinetic parameters of the immobilised enzyme and further optimisation of the reaction are currently underway. Such optimised reaction schemes will then also put the basis for more detailed life cycle assessment-based environmental impact evaluations.

Experimental Section

Preparation of the biocatalysts

Preparation of AaeUPO. The expression and preparation of unspecific peroxygenase from *A. aegerita* (PaDa-I variant) in *P. pastoris* was performed following the original protocol by Alcalde and coworkers.[12b]

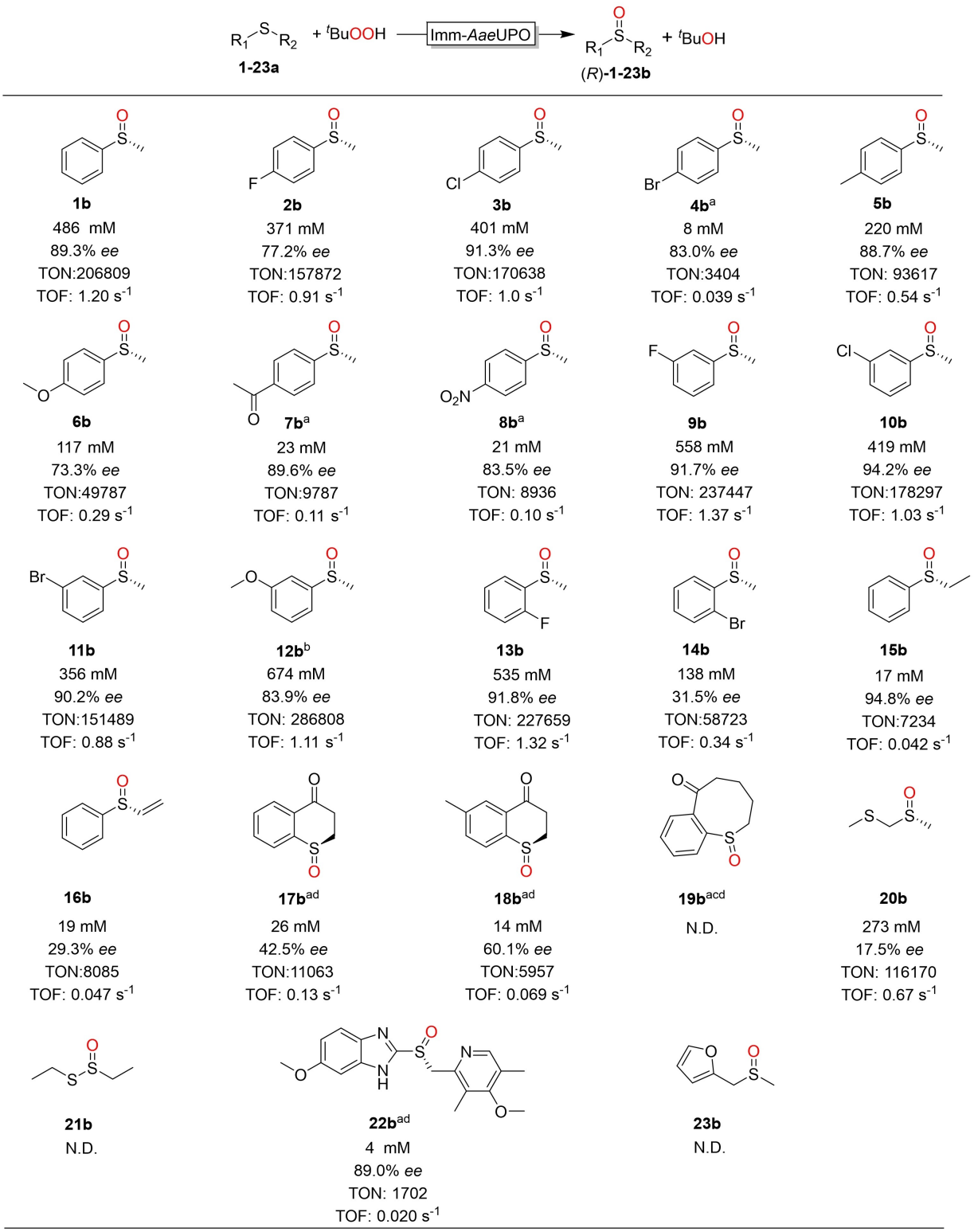

Figure 2. Substrate scope of imm-*Aae*UPO catalysed asymmetric sulfoxidation. Reaction conditions unless specifically stated: [substrates] = 0.5 mL, [imm-*Aae*UPO] = 100 mg (2.35 μM), [^{t}BuOOH] = 12 mM h^{-1} (5 μL h^{-1}), 30 °C, 800 rpm, 48 h. Concentrations was determined by GC, *ee* values were determined by chiral HPLC. [a] [substrate] = 25 μmol, 400 μL acetone, 100 μL PBS (50 mM, pH 6.5), [^{t}BuOOH] = 3 mM h^{-1} (1.25 μL h^{-1}), 24 h. [b] [substrates] = 0.5 mL, 72 h. [c] [substrate] = 25 μmol, 400 μL acetone, 100 μL PBS (50 mM, pH 6.5), [^{t}BuOOH] = 6 mM h^{-1}, 48 h. Concentrations are based on calibration curves using vauthentic standards. [d] The *ee* values were determined by chiral HPLC. [e] The *ee* values were determined by chiral GC.

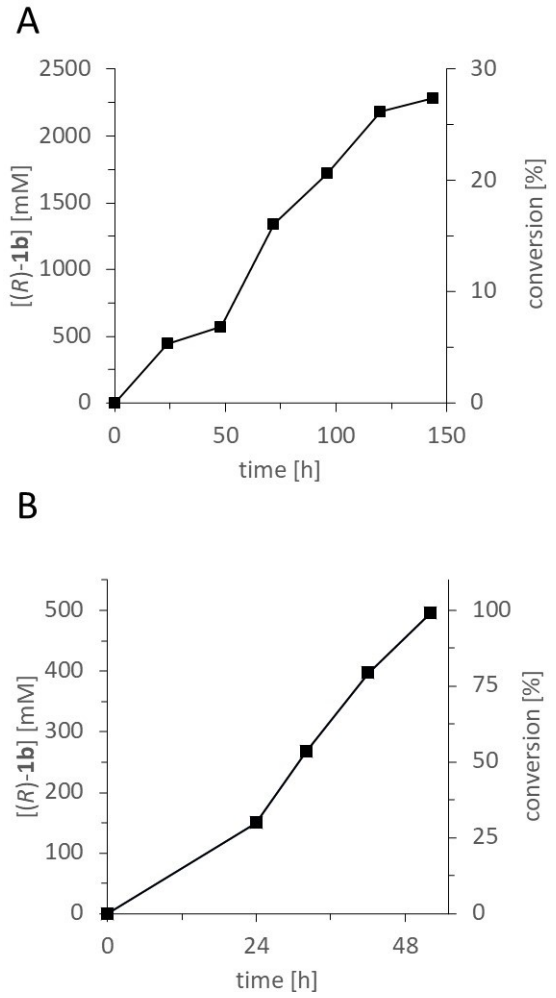

Figure 3. Increasing the conversion of the *Aae*UPO-catalysed sulfoxidation. (a) replacing consumed immo-*Aae*UPO every 48 h and (b) using thioanisol dissolved in acetone. Reaction conditions: (a) [**1 a**] = 765 mmol (90 mL), [imm-*Aae*UPO] = 18 g and replaced by fresh imm-*Aae*UPO every 48 h, [^{t}BuOOH] = 12 mM h^{-1}, 30 °C, 300 rpm, 144 h. (b) [**1 a**] = 250 μmol, 0.5 mL acetone, [imm-*Aae*UPO] = 100 mg, [^{t}BuOOH] = 12 mM h^{-1} (5 μL h^{-1}), 30 °C, 800 rpm, 52 h.

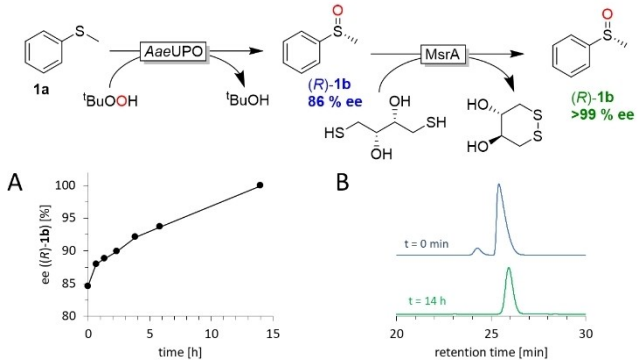

Figure 4. Polishing the optical purity of (R)-1 b by MrsA-catalysed stereoselective reduction of the contaminating (S)-enantiomer. (A) Time course (B) corresponding HPLC chromatograms. Reaction conditions: [(*R*)-**1 b**, 85 % ee] = 15 mM (2 g), [MsrA] = 25 μM (9.4 mL), [DTT] = 56 mM (8.214 g), acetonitrile = [2 % v/v, 19 mL], PBS buffer (50 mM, pH 6.5), total 944 mL, 35 °C, 800 rpm, 14 h.

Preparation of MsrA. Methionine Sulfoxide Reductase A (MsrA) was produced according to a protocol previously established in our lab.[17]

AaeUPO immobilisation

The covalent immobilisation of *Aae*UPO using amino-functionalized resin (LXTE-700) was performed as following: the resin carriers (1 g) were first washed three times with phosphate buffer (pH 8.0, 50 mM) and redispersed in 3 mL water. 320 μL glutaraldehyde aqueous solution (8 %, v/v) was added to the above dispersion and mixed for 1 h in a thermal shaker (220 rpm) at 22 °C. The carriers were recovered by filtration, followed by washing three times with phosphate buffer. The resin carriers were dispersed in 2.5 mL of phosphate buffer and 400 μl of 80 μM *Aae*UPO was added. The mixture was mixed for 3 h in a thermal shaker at 25 °C. The immobilised *Aae*UPO was recovered by filtration, followed by washing three times with phosphate buffer. The enzyme resin was kept at 4 °C for further use. The actual amount of *Aae*UPO on the amino-functionalized resin was about 12 nmol g^{-1}. More detailed information on the carriers, the immobilisation efficiency, etc., are shown in the Supporting Information.

Sulfoxidation reactions

In a typical procedure, the thioether substrate (0.5 mL initial volume), [imm-*Aae*UPO] = 100 mg resin corresponding to 2.35 μM *Aae*UPO were added into a 4 mL transparent glass vial, and tBuOOH in decane of 1.2 M was continuously supplied at a rate of 12 mM h^{-1} (5 μL h^{-1}) by a syringe-pump (total 360 μL). The reaction mixture was incubated in a thermo shaker (30 °C, 800 rpm) for 72 h with a continuous supply of ^{t}BuOOH. To determine the concentration and conversion of the reactions and optical purity, GC and HPLC were used for detection at regular intervals.

Supporting Information

The authors have cited additional references in the Supporting Information (Ref. [12a,17,19]).

Acknowledgements

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords: Asymmetric sulfoxidation · Biocatalysis · Cascade · Enzyme Immobilisation · Peroxygenase

[1] a) E. Wojaczynska, J. Wojaczynski, *Chem. Rev.* **2010**, *110*, 4303–4356; b) I. Fernandez, N. Khiar, *Chem. Rev.* **2003**, *103*, 3651–3706.
[2] E. Wojaczyńska, J. Wojaczyński, *Chem. Rev.* **2020**, *120*, 4578–4611.
[3] a) W. Dai, J. Li, B. Chen, G. Li, Y. Lv, L. Wang, S. Gao, *Org. Lett.* **2013**, *15*, 5658–5661; b) M. Mba, L. J. Prins, G. Licini, *Org. Lett.* **2007**, *9*, 21–24.
[4] a) S. Liao, I. Čorić, Q. Wang, B. List, *J. Am. Chem. Soc.* **2012**, *134*, 10765–10768; b) C. Drago, L. Caggiano, R. F. W. Jackson, *Angew. Chem. Int. Ed.* **2005**, *44*, 7221–7223; c) H. Egami, T. Katsuki, *J. Am. Chem. Soc.* **2007**, *129*, 8940–8941; d) J. Sun, C. Zhu, Z. Dai, M. Yang, Y. Pan, H. Hu, *J. Org. Chem.* **2004**, *69*, 8500–8503; e) G. E. O'Mahony, A. Ford, A. R. Maguire, *J. Sulfur Chem.* **2013**, *34*, 301–341; f) J.-M. Brunel, P. Diter, M. Duetsch, H. B. Kagan, *J. Org. Chem.* **1995**, *60*, 8086–8088.
[5] a) S. Anselmi, N. Aggarwal, T. S. Moody, D. Castagnolo, *ChemBioChem* **2021**, *22*, 298–307; b) W. R. F. Goundry, B. Adams, H. Benson, J. Demeritt, S. McKown, K. Mulholland, A. Robertson, P. Siedlecki, P. Tomlin, K. Vare, *Org. Process Res. Dev.* **2017**, *21*, 107–113; c) Y. K. Bong, S. Song, J. Nazor, M. Vogel, M. Widegren, D. Smith, S. J. Collier, R. Wilson, S. M. Palanivel, K. Narayanaswamy, B. Mijts, M. D. Clay, R. Fong, J. Colbeck, A. Appaswami, S. Muley, J. Zhu, X. Zhang, J. Liang, D. Entwistle, *J. Org. Chem.* **2018**, *83*, 7453–7458; d) K. S. Koch R., Spelberg M., Tischler D., Schlomann M., Schlotissek A., Heine T., Bühler B., Schmid A., Willrodt C. International Patent, WO2018206823, PCT/EP2018/068978, **2018**..
[6] a) C. Willrodt, J. A. D. Gröning, P. Nerke, R. Koch, A. Scholtissek, T. Heine, A. Schmid, B. Bühler, D. Tischler, *ChemCatChem* **2020**, *12*, 4664–4671; b) G. de Gonzalo, A. Franconetti, *Enz. Microb. Technol.* **2018**, *113*, 24–28.
[7] S. Bordewick, A. Beier, K. Balke, U. T. Bornscheuer, *Enz. Microb. Technol.* **2018**, *109*, 31–42.
[8] a) F. Van Rantwijk, R. A. Sheldon, *Curr. Opin. Biotechnol.* **2000**, *11*, 554–564; b) M. Hobisch, D. Holtmann, P. G. De Santos, M. Alcalde, F. Hollmann, S. Kara, *Biotechnol. Adv.* **2021**, *51*, 107615; c) S. Lutz, K. Vuorilehto, A. Liese, *Biotechnol. Bioeng.* **2007**, *98*, 525–534; d) S. Lutz, E. Steckhan, A. Liese, *Electrochem. Commun.* **2004**, *6*, 583–587; e) T. Krieg, S. Huttmann, K.-M. Mangold, J. Schrader, D. Holtmann, *Green Chem.* **2011**, *13*, 2686–2689; f) I. Bassanini, E. E. Ferrandi, M. Vanoni, G. Ottolina, S. Riva, M. Crotti, E. Brenna, D. Monti, *Eur. J. Org. Chem.* **2017**, *2017*, 7186–7189; g) E. Churakova, I. W. C. E. Arends, F. Hollmann, *ChemCatChem* **2013**, *5*, 565–568; h) D. I. Perez, M. Mifsud Grau, I. W. C. E. Arends, F. Hollmann, *Chem. Commun.* **2009**, *44*, 6848–6850; i) W. X. Q. Robinson, T. Mielke, B. Melling, A. Cuetos, A. Parkin, W. P. Unsworth, J. Cartwright, G. Grogan, *ChemBioChem* **2023**, *24*, e202200558; j) X. Wei, C. Zhang, X. Gao, Y. Gao, Y. Yang, K. Guo, X. Du, L. Pu, Q. Wang, *ChemistryOpen* **2019**, *8*, 1076–1083.
[9] a) J. S. Dordick, M. A. Marletta, A. M. Klibanov, *Biotechnol. Bioeng.* **1987**, *30*, 31–36; b) J. S. Dordick, M. A. Marletta, A. M. Klibanov, *Proc. Natl. Acad. Sci. USA* **1986**, *83*, 6255–6257.
[10] a) C. Kohlmann, L. Greiner, W. Leitner, C. Wandrey, S. Lütz, *Chem. Eur. J.* **2009**, *15*, 11692–11700; b) S. K. Karmee, C. Roosen, C. Kohlmann, S. Lütz, L. Greiner, W. Leitner, *Green Chem.* **2009**, *11*, 1052–1055; c) Y. R. Li, Y. J. Ma, P. L. Li, X. Z. Zhang, D. Ribitsch, M. Alcalde, F. Hollmann, Y. H. Wang, *ChemPlusChem* **2020**, *85*, 254–257; d) G. de Gonzalo, *Sus. Chem.* **2020**, *1*, 290–297.
[11] R. A. Sheldon, *Curr. Opin. Green Sustain. Chem.* **2019**, *18*, 13–19.
[12] a) P. Molina-Espeja, S. Ma, D. M. Mate, R. Ludwig, M. Alcalde, *Enzyme* **2015**, *73–74*, 29–33; b) P. Molina-Espeja, E. Garcia-Ruiz, D. Gonzalez-Perez, R. Ullrich, M. Hofrichter, M. Alcalde, *Appl. Environ. Microbiol.* **2014**, *80*, 3496–3507.
[13] R. Ullrich, J. Nüske, K. Scheibner, J. Spantzel, M. Hofrichter, *Appl. Environ. Microbiol.* **2004**, *70*, 4575–4581.
[14] a) F. Tonin, F. Tieves, S. Willot, A. Van Troost, R. Van Oosten, S. Breestraat, S. Van Pelt, M. Alcalde, F. Hollmann, *Org. Process Res. Dev.* **2021**, *25*, 1414–1418; b) F. Tieves, F. Tonin, E. Fernández-Fueyo, J. M. Robbins, B. Bommarius, A. S. Bommarius, M. Alcalde, F. Hollmann, *Tetrahedron* **2019**, *75*, 1311–1314.
[15] P. De Santis, N. Petrovai, L.-E. Meyer, M. Hobisch, S. Kara, *Front. Chem.* **2022**, *10*, 985997.
[16] J. B. Wang, A. Ilie, M. T. Reetz, *Adv. Synth. Catal.* **2017**, *359*, 2056–2060.
[17] P. Wang, X. Han, X. Liu, R. Lin, Y. Chen, Z. Sun, W. Zhang, *Chem. Eur. J.* **2022**, *28*, e202201997.
[18] P. Gomez de Santos, I. Mateljak, M. D. Hoang, S. J. Fleishman, F. Hollmann, M. Alcalde, *J. Am. Chem. Soc.* **2023**, *145*, 3443–3453.
[19] a) Y. Wang, N. Teetz, D. Holtmann, M. Alcalde, J. M. A. van Hengst, X. Liu, M. Wang, W. Qi, W. Zhang, F. Hollmann, *ChemCatChem* **2023**, *15*, e202300645; b) T. Peng, J. Tian, Y. Zhao, X. Jiang, X. Cheng, G. Deng, Q. Zhang, Z. Wang, J. Yang, Y. Chen, *Angew. Chem. Int. Ed.* **2022**, *61*, e202209272; c) Y. Zhao, X. Jiang, S. Zhou, J. Tian, P. Yang, Y. Chen, Q. Zhang, X. Xu, Y. Chen, J. Yang, *Org. Biomol. Chem.* **2023**, *21*, 3417–3422.

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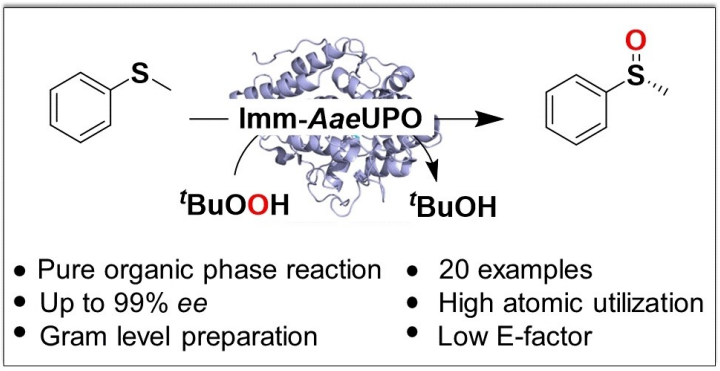
RESEARCH ARTICLE

Asymmetric Sulfoxidations in Non-Aqueous Media



Immobilised peroxygenase showed superb stability under neat reaction conditions, allowing the asymmetric sulfoxidation reaction in very high product titre with a decent E-factor.

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