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Research Article

Enantioselective Alcohol Oxidation Reactions Catalyzed by Unspecific Peroxygenases

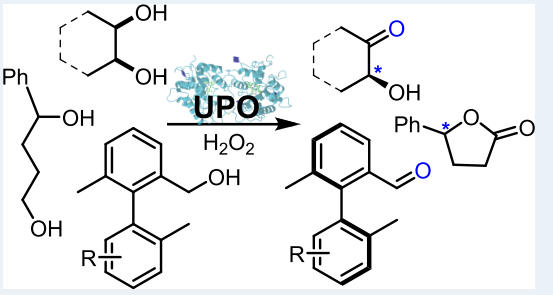
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ABSTRACT: The enantioselective oxidation of alcohols can be achieved via kinetic resolution of racemic alcohols or the desymmetrization of *meso*-diols. Biocatalytic alcohol oxidation approaches are known, using alcohol dehydrogenases and copper- and flavin-dependent oxidases, but the application of unspecific peroxygenases (UPOs) to enantioselective alcohol oxidation has received very little attention. As part of ongoing research into the catalytic promiscuity of UPOs, we present three distinct enantioselective biocatalytic alcohol oxidation approaches: the UPO-catalyzed oxidation of *meso*-diols via desymmetrization, the chemoselective oxidation of nonbenzylic sites in unsymmetrical diols, and the kinetic resolution of axially chiral biaryl alcohols. In each of the three reaction classes, the isolation of enantiomerically enriched oxidized products is demonstrated on a preparative scale.



KEYWORDS: *biocatalysis, unspecific peroxygenase, UPO, alcohol, diol, oxidation*

INTRODUCTION

Enantioselective alcohol oxidations are important transformations for the preparation of enantiomerically enriched oxygenated small molecules. Although the conversion of an alcohol into an aldehyde or ketone does not directly lead to the formation of a new point stereogenic center, this does not preclude alcohol oxidation strategies being used in asymmetric synthesis. For example, the enantioselective oxidation of racemic secondary alcohols (e.g., ***rac*-1**, Scheme 1A) can lead to the formation of enantiomerically enriched products (***ent*-1**) via kinetic resolution. Enantiomerically enriched products can also be formed via the oxidation of *meso*-diols via desymmetrization (e.g., **3** → **4**), with various abiotic catalytic methods known to promote both classes of oxidation (Scheme 1A).[1−25] Prominent recent examples include Lin and co-workers' method, in which a peptide catalyst bearing an aminoxyl radical enables the highly enantioselective oxidation of diols into lactones.[24] Phipps and co-workers have also shown that a cinchona alkaloid derived catalyst can enable the enantioselective synthesis of a wide range of linear and cyclic hydroxyketones from diol precursors.[25]

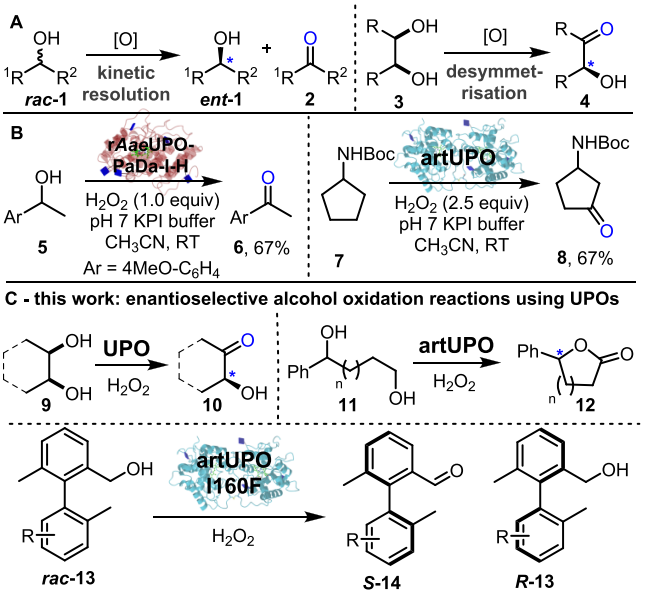
There has also been a significant amount of research on the asymmetric oxidation of diols using enzymes.[26,27] In the 1980s and 1990s, pioneering early work by Irwin and Jones[28] and Wong et al.[29] established that horse liver alcohol dehydrogenase (HLADH) can catalyze the enantioselective oxidation of diols leading to chiral lactone products. More recent work has continued to focus on heterologously expressed $NAD(P)^+$-dependent alcohol dehydrogenases (ADHs)[30−3132] but also on copper-dependent enzymes, such as laccase, in combination with TEMPO,[33] galactose oxidase,[34] and also flavin-dependent alcohol oxidases.[35]

In recent work, we have been studying the application of heme-dependent unspecific peroxygenases (UPOs) in organic synthesis. We have described a range of diverse chemical reactions, including regio- and enantioselective hydroxylation,[36,37] but also reactions that are reflective of the catalytic promiscuity of UPOs and other heme oxygenases, including halogenation,[38] cyclopropanation,[39] and rearrangement reactions following the UPO-catalyzed epoxidation of furans.[40,41] UPOs are an increasingly popular choice of biocatalyst for oxidation reactions as they are easy to prepare and apply at scale and, in contrast to some other redox enzymes, depend only upon the addition of hydrogen peroxide to effect oxidation reactions.[42,43] In the context of alcohol oxidation, it has previously been shown that UPOs catalyze the hydroxylation of the model compound ethylbenzene to 1-phenylethanol with high *ee* but also that overoxidation of the product to acetophenone can be observed.[44,45] This capacity for alcohol oxidation has been exploited for the

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Scheme 1. (A) Enantioselective Alcohol Oxidation Strategies; (B) Alcohol Oxidation with UPOs; and (C) This Work: Enantioselective Alcohol Oxidation Enabled by UPOs



oxidation of toluene derivatives or benzyl alcohol substrates to give aldehydes, ketones, and carboxylic acids.[46−52] Such oxidation can be performed directly using alcohol starting material (e.g., **5** → **6**, Scheme 1B)[49] and indirectly via an initial CH oxygenation and subsequent oxidation *in situ* (e.g., **7** → **8**, Scheme 1B).[50]

Despite the proven utility of UPOs to promote alcohol oxidation, their successful application in enantioselective alcohol oxidation is very limited. Zhang and co-workers showed that r*Aae*UPO can oxidize racemic secondary propargyl alcohols, substrates in which kinetic resolution might have been expected (i.e., a transformation of the type **5** → **6**), but only with "low enantioselectivity" (*E* value 3.6).[51] Similarly, Musa and coworkers showed that r*Aae*UPO oxidizes racemic secondary alcohols into ketones but conclusively showed that the enzyme efficiently oxidizes both enantiomers "without stereopreference".[52] Indeed, we know of only a single example of a transformation in which a UPO-catalyzed alcohol oxidation directly gives rise to an enantiomerically enriched product with a reported *ee*.[53,54] This case is found in a study by Zhang and co-workers, who showed that immobilized r*Aae*UPO catalyzes the conversion of hexane-1,5-diol into the methyl-δ-lactone in 55% *ee* on analytical scale.[53]

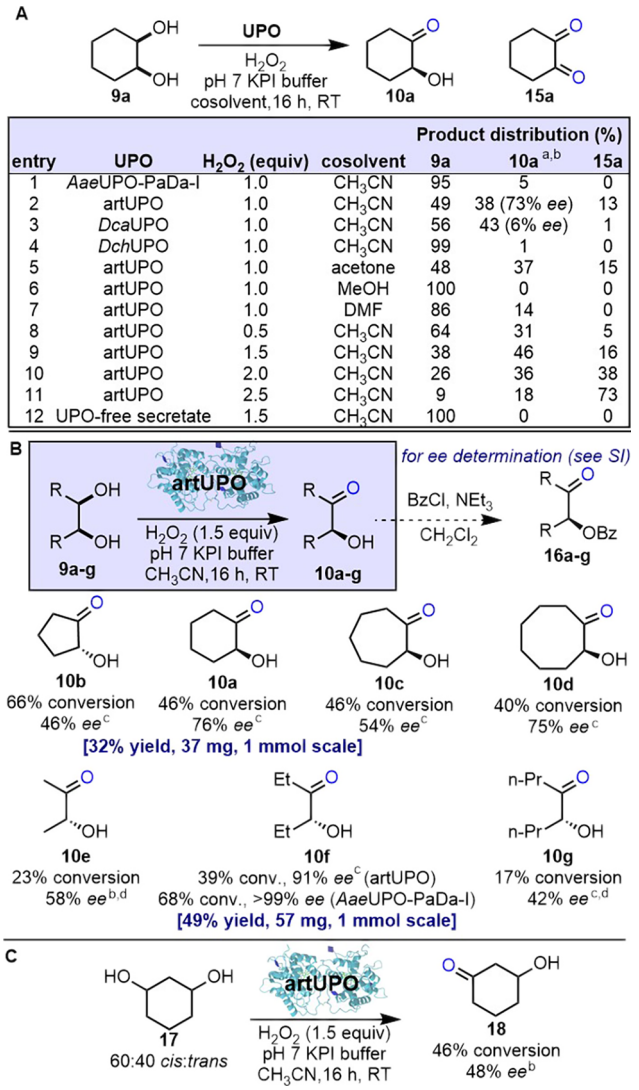
In this report, we detail the application of UPOs to three distinct classes of enantioselective alcohol oxidation reaction (Scheme 1C): the oxidation of *meso*-diols via desymmetrization (**9** → **10**), the chemoselective oxidation of nonbenzylic sites in unsymmetrical diols (**11** → **12**), and the kinetic resolution of axially chiral biaryl alcohols (*rac*-**13** → *S*-**14** and *R*-**13**). In each system, the isolation of enantiomerically enriched oxidized products on a preparative scale is demonstrated for the first time using UPOs.

■ RESULTS AND DISCUSSION

Our previous work has focused on the application of representative members of the two major types of UPOs: artUPO from Family I[55,56] and r*Aae*UPO-PaDa-I-H[57−59] from Family II. These families are distinguished by shorter and longer sequences, respectively,[60,61] and also by active site topologies[56,62] that confer differences in catalytic properties including regio- and stereoselectivity.[36−51,56,63] Accordingly, we began our studies on alcohol oxidations again using these enzymes, in the expectation of revealing complementary activities of the two enzyme families toward these substrates. In all cases, the UPOs used were expressed in *Pichia pastoris* (*Komagataella phaffii*) and used as crude secretates. Details of cloning, expression, and enzyme production can be found in our previous publications.[39,56,59,63,64]

First, the UPO-mediated oxidative desymmetrization of *meso*-diols was explored, starting with the conversion of cyclohexane-1,2-diol **9a** into hydroxy ketone **10a** (Scheme 2A). Diol **9a** was used to challenge a range of both

Scheme 2. (A) Optimization of the UPO Oxidation of Diol 9a; (B) artUPO Oxidation of *meso* 1,2-diols; and (C) Oxidation of 1,3 Diol 17



				Product distribution (%)		
entry	UPO	H_2O_2 (equiv)	cosolvent	9a	10a [a,b]	15a
1	*Aae*UPO-PaDa-I	1.0	CH_3CN	95	5	0
2	artUPO	1.0	CH_3CN	49	38 (73% *ee*)	13
3	*Dca*UPO	1.0	CH_3CN	56	43 (6% *ee*)	1
4	*Dch*UPO	1.0	CH_3CN	99	1	0
5	artUPO	1.0	acetone	48	37	15
6	artUPO	1.0	MeOH	100	0	0
7	artUPO	1.0	DMF	86	14	0
8	artUPO	0.5	CH_3CN	64	31	5
9	artUPO	1.5	CH_3CN	38	46	16
10	artUPO	2.0	CH_3CN	26	36	38
11	artUPO	2.5	CH_3CN	9	18	73
12	UPO-free secretate	1.5	CH_3CN	100	0	0

[a]Substrate distribution measured by GC (BGB-175 IG column or HP-5 column; see the Supporting Information for details). [b]*ee* directly measured by GC with a chiral stationary phase (BGB-175 column; see the Supporting Information for details). [c]*ee* of the benzoate ester derivative **16** measured by HPLC (Chiralpak IG column; see the Supporting Information for details). [d]The assignment of absolute stereochemistry is made in analogy to the assignment of **10f**.

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Family I and Family II UPOs in pH 7.0 aq. buffer and an organic solvent at RT, with H_2O_2, used as a stoichiometric oxidant, added slowly via a syringe pump. The relative distribution of unreacted diol **9a**, product **10a**, and overoxidized 1,2-diketone product **15a** was measured using GC. In selected cases, a separate GC analysis was also performed using a chiral column to measure the *ee* of **10a**. Of the UPOs tested, artUPO offered the best combination of product formation and enantioselectivity (38% conversion into **10a** with 73% *ee*, entry 2). Acetonitrile proved to be the best organic co-solvent among those tested (entries 2 and 5–7). Focusing on artUPO, the amount of H_2O_2 was varied (entries 2 and 8–11), and the transformations proceeded broadly as expected; the consumption of **9a** increased as more H_2O_2 was used, with a concomitant increase in overoxidation into diketone **15a**. The use of 1.5 equiv H_2O_2 (entry 9) offered the best compromise of formation of the desired product **10a** while minimizing overoxidation (see Supporting Information Section S2 for further details). In addition, a "UPO-free" secretate, derived from the fermentation of *P. pastoris* cells that had not had a UPO gene integrated into their chromosomal DNA, was tested in a control reaction, which confirmed that there was no oxidation reaction by the secretate and H_2O_2 in the absence of UPO (entry 16).[65]

Examples of the selective oxidation of **9a** using isolated enzymes appear to be scarce, but Adlercreutz showed that whole cells of *Gluconobacter oxydans* catalyzed the oxidation of **9a** into the (*S*)-ketol with 100% conversion and 97% *ee*.[66] Allen and co-workers also showed that a whole-cell system of *Pseudomonas putida* strain ML2 expressing the *cis*-diol dehydrogenase BCDD converted **9a** into the (*S*)-ketol product with 11% yield and >98% *ee*.[67] More recently, Yu and co-workers reported the evolution of an ADH from *Bacillus subtilis* (*Bs*BDH) to give an enzyme also capable of oxidizing **9a** to the (*S*)-ketol with 73% yield and an *ee* of >99%.[68]

Our attention then turned to testing the optimized conditions on the transformation of other *meso*-diols to hydroxyketones (**9a–g** → **10a–g**, Scheme 2B). Reliably measuring the *ee* of products **10a–g** directly via GC was challenging. Therefore, in this series, after measuring conversion into **10a–g**, the product was derivatized into the corresponding benzoate ester (**15**) to enable the *ee* to be measured by HPLC (see Supporting Information Section S3 for full preparative and analytical details). Cyclic *meso*-diols **9a–d** were tested, and in all cases, artUPO enabled the formation of the expected five- to eight-membered ring hydroxyketone products **10a–10d** (40–66% conversion, 46–76% *ee*). The absolute stereochemistry of **10a** was assigned by direct comparison to the literature data (HPLC elution times for the published benzoate ester **16a** and optical rotation data for **10a**).[25] The assignments of homologues **10b–d** were made by comparing their optical rotation data of the corresponding benzoate ester **16b–d** to the published data (see the Supporting Information). Notably, this analysis suggested that the five-membered ring product **10b** was formed in the opposite enantiomeric series to the six- to eight-membered ring homologues. We attempted to rationalize the observations on cyclic diol oxidation using modeling with Autodock VINA[69] and the structure of artUPO previously determined by our group.[56] Models of the small substrate **9b** suggested that this might adopt various poses within the active site of similar energies, resulting in moderate *ee*. In addition, the hydrophobic residues above and around the heme (V69, I160, and I91) permit the substrate to lie in plane with the heme (Figure S43A). However, for larger substrates, such as **9a** (Figure S43B) and especially *cis*-cyclooctane-1,2-diol **9d** (Figure S43C), these residues restrict the binding of the larger rings to fewer conformations, with the ring more perpendicular to the plane of the heme, perhaps partially explaining the higher selectivity with these substrates.

Measuring accurate isolated yields for the transformations of **9a–9d** was hampered by the high volatility of the products, but in a representative case, 37 mg of the six-membered ring product **10a** was isolated in acceptable yield following a 1 mmol scale artUPO biotransformation, corresponding to an approximate total turnover number (TTN, expressed as μmol product/μmol enzyme) of 2.76×10^3. Allen and co-workers also showed that BCDD in *P. putida* can oxidize **9b** into the (*S*)-ketol product in 63% yield with >98% *ee*,[67] but biotransformations of larger *cis*-diols **9c** and **9d** have not to our knowledge been reported.

Moving onto linear *meso*-diols, butane-2,3-diol **9e** was also oxidized by artUPO to form the volatile hydroxy ketone **10e** in 23% conversion and 58% *ee*. The interconversion of **9e** and **10e** has been the focus of a number of studies on ADHs including work by Shen and co-workers, in which an ADH from *Serratia marcescens* was shown to catalyze the transformation of **9e** into (*R*)-**10e**.[70] However, the oxidative biotransformation of longer linear *cis*-diols appears to have received limited attention. Among the longer compounds, hexane-3,4-diol **9f** was the most successful of those tested in this series, with artUPO promoting its conversion into hydroxy ketone **10f** with 39% conversion and 91% *ee*. Furthermore, switching to a Family II UPO, r*Aae*UPO-PaDa-I-H, allowed the same transformation to be performed with improved conversion and even higher *ee* (68%, >99% *ee*), with this biotransformation also performed on 1 mmol scale (57 mg of enantiomerically pure **10f** was isolated, corresponding to a yield of 49% and a TTN of 3.68×10^2). Homolog **9g** revealed the limits of this transformation, with only 17% conversion into product **10g** observed in this case, likely due to the increased steric demands on this larger diol substrate. The absolute stereochemistry of **10f** was assigned by comparison to the literature data (HPLC elution times for the published enantiomer of benzoate ester **16f** and optical rotation data for the enantiomer of **10f**).[25] The assignments of homologs **10e** and **10g** were made by analogy.

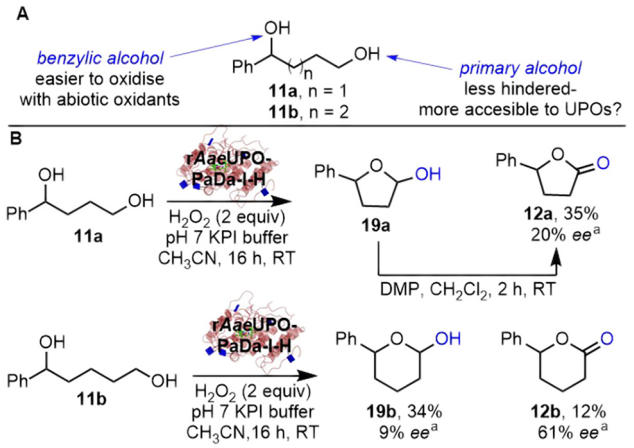
A cyclic 1,3-diol was also successfully transformed by artUPO (Scheme 2C). Thus, diol **17** (used as 60:40 *cis*/*trans* mixture) was converted into hydroxyketone **18** in 46% conversion and 48% *ee*. The proclivity of product **18** to undergo $E1_{CB}$ elimination to form 2-cyclohexen-1-one discouraged further investigations in this case, but nonetheless, the successful application of artUPO in this alternative diol class is encouraging.

Next, attention turned to exploring the oxidation of unsymmetrical diols **11a** and **11b**, the oxidation of which leads to lactone products. The generation of lactones from symmetrical diols using ADHs and other enzymes has been demonstrated many times[71] from early examples by Irwin and Jones[28] with horse liver alcohol dehydrogenase (HLADH) through to more recent examples by Hollmann et al.[72] and Gotor-Fernández et al.[73] Unsymmetrical diols such as **11a** and **11b** pose an interesting chemoselectivity question (Scheme 3A). Using abiotic oxidants, oxidation of the activated benzylic alcohol is typically favored; for example, using mild oxidants like MnO_2, highly selective benzylic oxidation can be achieved.[74] In contrast, it was reasoned that the steric demands of the UPO active site may favor selective oxidation of the primary, nonbenzylic alcohol. This idea was tested by reacting racemic 1-phenylbutane-1,4-diol **11a** with r*Aae*UPO-PaDa-I-H and 2 equiv of H_2O_2 in pH 7.0 aq. buffer and 20% (v/v) acetonitrile at RT. The

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Scheme 3. UPO Oxidation of Unsymmetrical Diols: (A) Chemoselectivity Challenge and (B) r*Aae*UPO-PaDa-I-H Oxidation of Unsymmetrical Diols 11a and 11b[a]



[a]*ee* of 12a and 12b measured by HPLC (Chiralpak IG column; see the Supporting Information for details).

expected chemoselectivity was indeed seen, with a 10:3 mixture of lactol **19a** and lactone **12a** obtained, with each product arising via an initial oxidation of the primary alcohol. No products were observed arising from the oxidation of the benzylic alcohol. We were unable to separate products **19a** and **12a**, but reacting the mixture with Dess–Martin periodinane enabled lactol **19a** to be further oxidized into lactone **12a**, which was subsequently isolated in 35% overall yield and 20% *ee*.

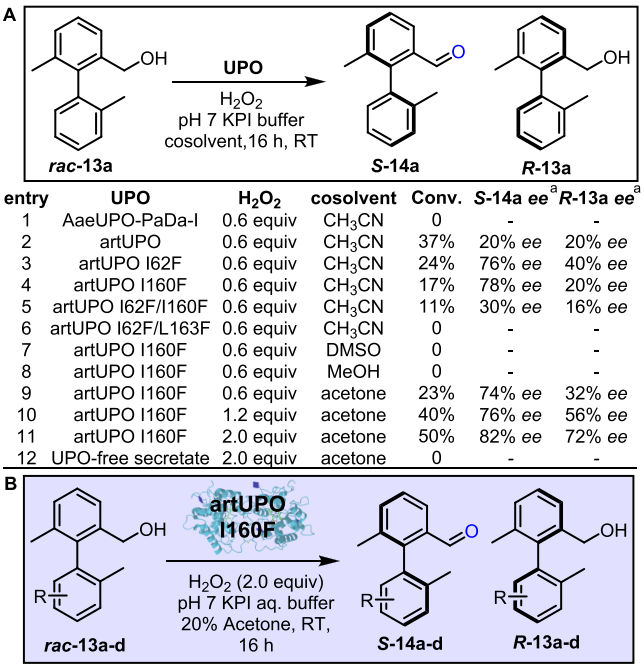
Homologous diol **11b** reacted similarly, affording lactol **19b** and lactone **12b** under the same conditions. In this case, these products were separable, allowing an isolated yield of each (34% and 12%, respectively) to be obtained. The *ee*'s of **19b** and **12b** were also each measured, with the *ee* of lactone **12b** being notably higher than that of lactol **19b**.

Finally, the kinetic resolution of axially chiral biaryl alcohols was investigated. The biotransformation of atropoisomeric compounds is of significant contemporary interest.[75] We were confident that racemic biaryl alcohols of the form ***rac*-13**[76] would have a sufficiently high rotational barrier for UPOs to enable their kinetic resolution via the selective oxidation of one of the two atropisomers.[76−80] Relevant methods based on abiotic alcohol oxidation have been reported,[81−84] and enzyme-mediated oxidation has been used successfully in a similar context, for example, by Clayden and co-workers using galactose oxidase variants to give hydroxyaldehydes with up to 99% *ee*.[85,86] More recently, Collins et al. have harnessed asymmetric alcohol oxidation using alcohol dehydrogenases (ADHs)— alongside achiral borane reductants—to effect redox-powered autonomous directional C–C bond rotation.[87]

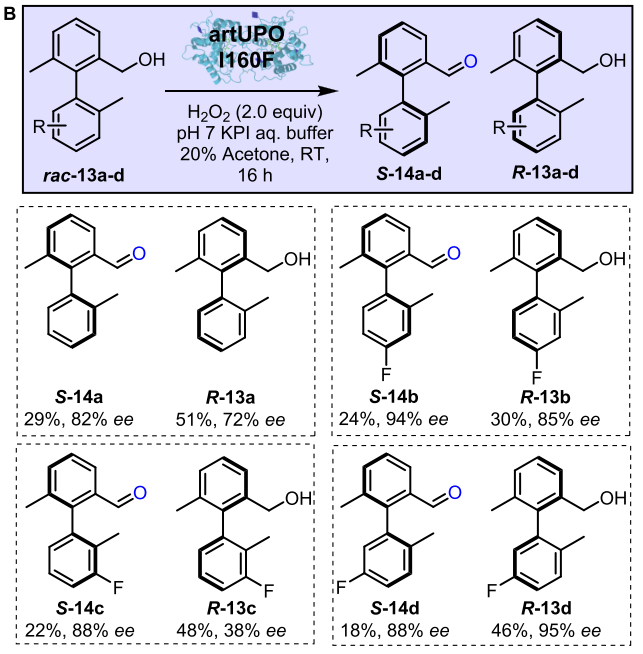
To the best of our knowledge, the only example of a UPO being used in the enantioselective synthesis of an axially chiral biaryl system is an oxidative biaryl coupling reaction, discovered by Müller and co-workers, during studies in the biosynthesis of phlegmacin natural products.[88] UPO-mediated alcohol oxidation reactions have not been applied previously to the asymmetric oxidation of axially chiral molecules, either via desymmetrization or via kinetic resolution. We therefore synthesized racemic biaryl alcohol ***rac*-13a** and explored its oxidation using different UPOs and H_2O_2, with 0.6 equiv of oxidant used initially, targeting the ≈50% conversion needed for efficient kinetic resolution (Scheme 4).

Using r*Aae*UPO-PaDa-I-H, no conversion of ***rac*-13a** was observed (Scheme 4A, entry 1). This result was not wholly surprising given previous observations that this UPO does not usually accept such sterically demanding substrates.[37] We therefore turned to artUPO, which is generally better at converting bulkier substrates, and 37% conversion into aldehyde ***S*-14a** was observed (Scheme 4A, entry 2). Both the oxidized product (***S*-14a**) and unreacted starting material (now ***R*-13a**) were isolated, each with a small but measurable enantiomeric enrichment (both 20% *ee*). The (*R*)-enantiomer of **14a** is a published compound, and this enabled the absolute stereochemical assignment of ***R*-13a** to be made via simple MnO_2 oxidation into ***R*-14a** and comparing their optical rotation data.[89] The absolute stereochemistry of ***S*-14a** could also be confidently assigned, as its NMR data were identical to those of ***R*-14a** but its optical rotation data had the opposite sign.

Scheme 4. Atroposelective Oxidation of Biaryl Alcohols[b]



entry	UPO	H_2O_2	cosolvent	Conv.	*S*-14a *ee*[a]	*R*-13a *ee*[a]
1	AaeUPO-PaDa-I	0.6 equiv	CH_3CN	0	-	-
2	artUPO	0.6 equiv	CH_3CN	37%	20% *ee*	20% *ee*
3	artUPO I62F	0.6 equiv	CH_3CN	24%	76% *ee*	40% *ee*
4	artUPO I160F	0.6 equiv	CH_3CN	17%	78% *ee*	20% *ee*
5	artUPO I62F/I160F	0.6 equiv	CH_3CN	11%	30% *ee*	16% *ee*
6	artUPO I62F/L163F	0.6 equiv	CH_3CN	0	-	-
7	artUPO I160F	0.6 equiv	DMSO	0	-	-
8	artUPO I160F	0.6 equiv	MeOH	0	-	-
9	artUPO I160F	0.6 equiv	acetone	23%	74% *ee*	32% *ee*
10	artUPO I160F	1.2 equiv	acetone	40%	76% *ee*	56% *ee*
11	artUPO I160F	2.0 equiv	acetone	50%	82% *ee*	72% *ee*
12	UPO-free secretate	2.0 equiv	acetone	0	-	-



[a]*ee* measurements done by HPLC (IBN5 column; see the Supporting Information). [b](A) optimization of the UPO oxidation of **rac-13a** and (B) artUPO I160F oxidation of **rac-13a-d**.

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To improve the stereoselectivity, mutation of smaller hydrophobic residues in the tunnel approaching the active site to variants with larger phenylalanines was explored, as this approach was successful in improving the *ee* during a previous study focused on artUPO-mediated cyclopropanation.[39] A selection of single and double artUPO mutants, produced using methods described in that study,[39] was tested (Scheme 4A, entries 3–6), and the mutant artUPO I160F (Scheme 4A, entry 3) proved to be the most effective in terms of the *ee* of the aldehyde product ***S*-14a** (78% *ee*).

Conversion dropped to 17% using the artUPO I160F mutant, but following additional optimization to the organic co-solvent (Scheme 4A, entries 7–9) and increasing the number of equivalents of H_2O_2 (Scheme 4A, entries 9–11) a protocol able to effect 50% conversion was established, with both the product ***S*-13a** and unreacted alcohol ***R*-14a** formed with improved enantiomeric ratios (82% and 72% *ee*, respectively).[90]

With an improved protocol established, the optimized artUPO I160F method was then applied to preparative scale biotransformation of biaryl alcohols ***rac*-13a–d** on 0.2 mmol scale (Scheme 4B). In each case, efficient kinetic resolution was achieved, with enantiomerically enriched aldehydes (***S*-14a–d**, 82–94% *ee*) and alcohols (***S*-13a–d**, 38–95% *ee*) isolated in each case.[91] In the case of **14a**, the yield of 29% corresponded to an approximate TTN of 1.70×10^2. Full details on substrate syntheses, analysis, and *ee* measurements are included in the Supporting Information. The absolute stereochemistries of all products are assumed to mirror those validated for ***S*-14a** and ***R*-13a**; this assumption is supported by the consistent elution order during HPLC analysis.

The binding of enantiomers of **14d** in the active site of artUPO was again examined with modeling using Autodock VINA.[69] The lowest energy poses for each enantiomer (Figure 1) presenting the benzylic carbon of the alcohol to compound I are distinguished by the positioning of their respective pendant aromatic ring substituents. For the (*R*)-enantiomer (the disfavored enantiomer), its methyl group is situated near the side chains of V69 and L65. Mutation of I160 to phenylalanine in the selective mutant I160F would be expected to push the (*R*)-enantiomer further toward these V69 and L65 side chains, thus further disfavoring binding. In contrast, the equivalent methyl group of the favored (*S*)-enantiomer does not experience the same steric restriction and hence can bind and be oxidized more readily.

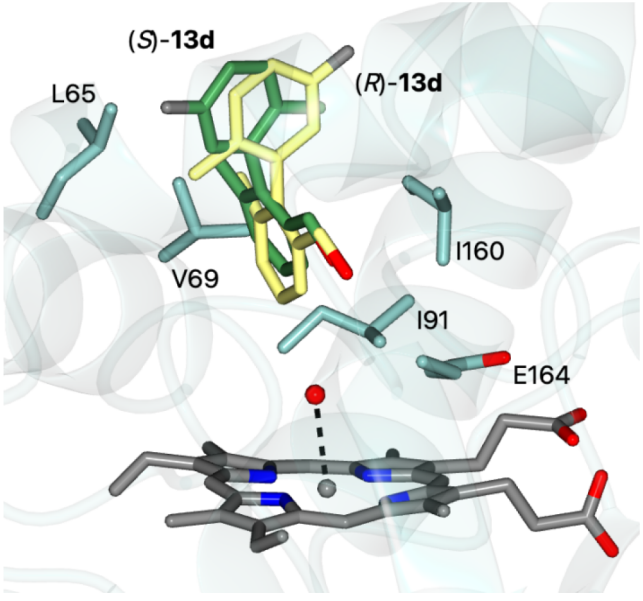


Figure 1. Structure of wild-type artUPO (PDB 7ZNM) modeled with compound I and with the (*S*)- and (*R*)-enantiomers of **14d** in green and yellow, respectively, modeled into the active site.

It appears that substrates ***rac*-13a–d** lie on the edge of what artUPO I160F can tolerate sterically; a 4-chlorinated substrate (**rac-13e**; see the Supporting Information) was also tested, but only 8% conversion into the analogous aldehyde ***S*-14e** (66% ee) was observed using this larger substrate, perhaps because of a steric clash of the chloro substituent with the side chain of L65 (Figure 1). Additional enzyme engineering, for example, to reduce bulk around the UPO active site, would likely be needed to improve the conversion of larger substrates.[92]

■ MATERIALS AND METHODS

Enzyme Production

The cloning and expression of the UPOs used in this study and their preparation from fermentations of *P. pastoris* have been described previously.[39,56,59,63,64] Enzymes were added to reactions in the form of the crude secretate from the *Pichia* fermentations.

Experimental procedures and compound characterization data for all transformations featured in this manuscript are included in the Supporting Information. A representative, preparative scale biotransformation for each of the three major reaction classes featured in the manuscript (Schemes 2–4) is included below:

Representative Procedure for Symmetrical Diol Oxidation: Oxidation of 9f by r*Aae*UPO-PaDa-I-H

A flask containing a stirred solution of liquid r*Aae*UPO-PaDa-I-H secretate (5 mL, 37 U mL^{-1}),[59] pH 7 KPi buffer (50 mL, 100 mM in H_2O), H_2O (15 mL), MeCN (20 mL), and *meso*-hexane-3,4-diol **9f** (118 mg, 1.00 mmol, 1.0 equiv) was gradually charged with H_2O_2 (2.00 mmol, 2.0 equiv in 10 mL H_2O) over a period of 10 h using a syringe pump (Scheme 2 results). After full addition, the solution was stirred for a further 6 h before the solution was extracted with diethyl ether (50 mL × 5). The combined organic layers were washed with brine, dried ($MgSO_4$), filtered, and carefully concentrated to approx. 5 mL volume. The solution was then directly loaded onto a column where the product was then purified using flash column chromatography (silica, 0% → 20% Et_2O in DCM), giving the product **10f** as a colorless oil (56.8 mg, 49%, >99% *ee*).

Representative Procedure for Unsymmetrical Diol Oxidation: Oxidation of 11b by r*Aae*UPO-PaDa-I-H

A flask containing a stirred solution of liquid r*Aae*UPO-PaDa-I-H secretate[59] (1 mL, 37 U mL^{-1}), pH 7 KPi buffer (10 mL, 100 mM in H_2O), MeCN (4 mL), H_2O (3 mL), and 1-phenylpentane-1,5-diol **11b** (36.1 mg, 0.2 mmol, 1.0 equiv) was gradually charged with H_2O_2 (0.4 mmol, 2.0 equiv in 2 mL H_2O) over a period of 10 h using a syringe pump (Scheme 3 results). After full addition, the solution was stirred for a further 6 h before the solution was extracted with 3× EtOAc. The combined organic layers were washed with brine, dried ($MgSO_4$), filtered, and concentrated to afford the crude product as a yellow oil. This was then purified via flash column chromatography (silica, 30% EtOAc in hexane) to afford lactol **19b** (12.1 mg, 34%, 9% *ee*) and lactone **12b** (4.2 mg, 12%, 61% *ee*), each as colorless oils.

Representative Procedure for the Atroposelective Oxidation of Biaryl Alcohols: Kinetic Resolution of 13a by artUPO I160F

To a round-bottom flask containing a magnetic stirring bar were added liquid artUPO I160F secretate (1.0 mL, 9.5 U mL^{-1})[39] and KPi buffer

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(100 mM, pH = 7, 10 mL). The solution was diluted by the addition of deionized water (3 mL) followed by addition of the appropriate alcohol **rac-13a** (0.2 mmol, 1.0 equiv, final concentration 10 mM) in acetone (4 mL) (Scheme 4 results). Next, 5 mL of an 80 mM H_2O_2 solution (2 equiv, prepared from 40 μL 30% H_2O_2 in 5 mL deionized water) was added over a 10 h period using a syringe pump. After the H_2O_2 addition was complete, the reaction was then stirred at room temperature for a further 6 h. The reaction mixture was then extracted with ethyl acetate (3 × 20 mL). The combined organic phases were then washed with brine (20 mL), dried over anhydrous $MgSO_4$, and concentrated *in vacuo* to give the crude product mixture, which was purified by flash column chromatography on silica gel (eluent: hexane/EtOAc = 20:1–10:1) to provide the corresponding enantiomerically enriched products (*S*)-**14a** (12.0 mg, 29%, 82% *ee*) and (*R*)-**13a** (17.0 mg, 51%, 72% *ee*).

CONCLUSIONS

The development of enzyme-catalyzed processes for redox transformations will benefit from the application of easy-to-use biocatalysts and the demonstration of their use in preparative scale reactions. Here we have demonstrated that UPOs, in addition to established roles in preparative hydroxylation reactions and others, can also be applied to the selective oxidations of alcohols to generate new series of enantio-enriched products. Three distinct classes of UPO-mediated alcohol oxidations have been demonstrated, including the desymmetrization of *meso*-diols and the chemoselective oxidation of nonbenzylic sites in unsymmetrical diols. It is interesting to note that these alcohol-to-ketone conversions tend to be less enantioselective than C–H oxygenation reactions (to form chiral alcohols) catalyzed by UPOs,[45] although an explanation of this phenomenon awaits more detailed analysis using structure and modeling calculations.

The enantioselectivity of the reported processes is in some cases inferior at this stage compared to the best whole-cell[66] or NAD(P)H-dependent oxidoreductase-catalyzed examples,[70] for which enantioenriched products such as **10a** have higher *ee*'s. However, as with those enzymes, the UPO selectivity might reasonably be improved using mutation. In that case, the advantages of UPOs might be more fully realized, as earlier methods require either the handling of whole-cell organisms or the need for cofactor recycling. The kinetic resolution of axially chiral biaryl alcohols is also promising as, following the demonstration of the suitability of UPOs for these transformations and also the improvements achievable using mutants, further applications to broader ranges of substrates may be envisaged.

ASSOCIATED CONTENT

Supporting Information

This material is available free of charge via the Internet at https://pubs.acs.org/doi/10.1021/acscatal.6c05786.

Experimental procedures, additional reaction optimization, compound characterization data, NMR spectra, and enzyme production (PDF)

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ABBREVIATIONS

UPO	unspecific peroxygenase
ADH	alcohol dehydrogenase
TEMPO	2,2,6,6-tetramethylpiperidin-1-oxyl

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