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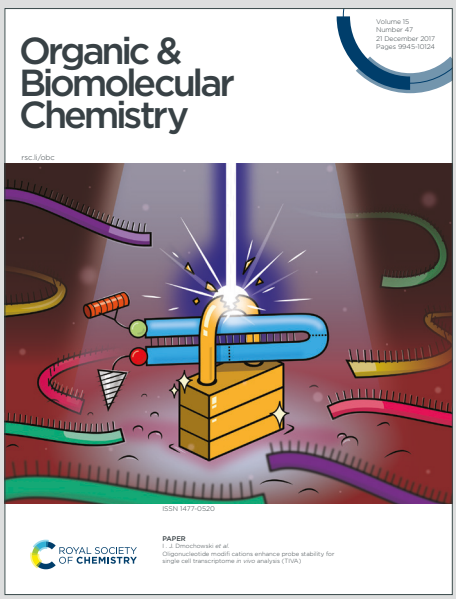

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ARTICLE

Biocatalytic synthesis of heterobiaryl sulfoxides: A comparative study between Baeyer-Villiger monooxygenases and unspecific peroxygenases

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The biocatalytic sulfoxidation of heterobiaryl indole- and pyrrole-based sulfides was investigated using unspecific peroxygenases (UPOs) and Baeyer–Villiger monooxygenases (BVMOs) as complementary oxidative biocatalysts. Among the UPOs tested, only the UPO from *Marasmius rotula* showed outstanding catalytic efficiency, reaching up to 99% conversion at substrate concentrations as high as 60 mM, with excellent chemoselectivity toward sulfoxides (>90%), albeit with moderate enantioselectivities (17–64% *ee*). In contrast, screening of a panel of BVMOs revealed superior stereochemical control: TmCHMO enabled the sulfoxidation of indole-based sulfides with enantioselectivities up to 94% ee, while OTEMO proved particularly effective for pyrrole-based substrates, affording sulfoxides in up to 90% ee. Reaction parameters such as temperature, pH, cosolvent and substrate loading have been optimized, allowing reaction rates of up to 22.4 mmol·L^{-1}·h^{-1} at 50 mM substrate concentration without enantioselectivity loss. Overall, pyrrole-based sulfides displayed higher optical purities than indole analogues under BVMO catalysis, whereas UPOs excelled in terms of productivity and operational simplicity. Selected BVMO- and UPO-catalyzed reactions were successfully scaled up, demonstrating the practical applicability of these biocatalytic systems. These results highlight the complementary strengths of UPOs and BVMOs for the efficient and selective synthesis of chiral heterobiaryl sulfoxides.

Introduction

Heterobiaryl sulfoxides constitute an important class of sulfur-containing organic compounds characterized by a sulfoxide moiety linking two aromatic rings, at least one of which is heteroaromatic. These compounds, as well as heterobiaryl systems in general, have attracted considerable attention in organic synthesis, medicinal chemistry, and materials science owing to their distinctive stereoelectronic properties, chiral versatility, and ability to serve as key intermediates in asymmetric catalysis and the design of bioactive molecules.[1] The presence of a heteroaromatic ring confers additional reactivity and binding capabilities, enabling these sulfoxides to participate in asymmetric transformations and molecular recognition processes. Among the various approaches for the synthesis of these valuable molecules in enantiopure form,[2] the biocatalyzed asymmetric oxidation of prochiral sulfides offers a wide set of advantages, including mild and environmentally friendly conditions and excellent selectivities.[3] In this context, two classes of oxidative enzymes — Baeyer-Villiger monooxygenases (BVMOs)[4] and unspecific peroxygenases (UPOs)[5]— have emerged as promising biocatalysts for enabling these transformations. BVMOs are flavin-dependent monooxygenases renowned for their ability to catalyze the Baeyer-Villiger oxidation.[6] Beyond this classical transformation, BVMOs have demonstrated remarkable proficiency in the oxidation of heteroatoms, including nitrogen, phosphorus, boron, and sulfur, enabling the asymmetric sulfoxidation of prochiral sulfides. This capability has been widely exploited to access a broad range of chiral sulfoxides with high enantioselectivity. UPOs, a class of fungal-derived peroxygenases, are heme-thiolate enzymes that catalyze the direct transfer of an oxygen atom from hydrogen peroxide (H_2O_2) to a variety of substrates, including sulfides.[7] In contrast to BVMOs, UPOs do not require nicotinamide adenine dinucleotide phosphate (NADPH) as a cofactor, relying exclusively on H_2O_2 as the cosubstrate. This feature simplifies their practical implementation and aligns well with green chemistry principles by employing a readily available and environmentally benign oxidant. Moreover, the robustness of UPOs under these reaction conditions often surpasses that of many existing methodologies, highlighting their potential for

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practical applications. When comparing BVMOs and UPOs in the context of asymmetric sulfoxidation of prochiral sulfides, the following features emerge:

- Cofactor Requirements: BVMOs are flavoenzymes that typically require NADPH as a reducing cofactor, necessitating in situ cofactor regeneration system or the use of whole cells biocatalysts.[8] In contrast, UPOs directly utilize H_2O_2 as the cosubstrate, eliminating the need for additional cofactors and simplifying reaction design.
- Substrate Scope and Selectivity: BVMOs have demonstrated high enantioselectivity toward a wide range of sulfide substrates, including bulky and pharmaceutically relevant compounds. Furthermore, protein engineering strategies have significantly expanded their substrate scope and enhanced selectivity. UPOs also display broad substrate tolerance and can achieve high product concentrations with satisfactory enantioselectivity. However, enantiomeric outcomes are more strongly dependent on the specific enzyme and reaction conditions employed. In general, BVMO-catalyzed reactions exhibit superior chemoselectivity, whereas UPO-catalysed processes may suffer from competing side reactions.
- Operational Stability and Reaction Conditions: Immobilized UPOs have shown exceptional stability under non-aqueous conditions,[9] enabling transformations facilitating at high substrate concentrations with reduced solvent usage. This behaviour is well aligned with sustainable chemistry principles by minimizing waste and improving process efficiency. Although highly effective, BVMOs typically require more tightly controlled aqueous environments and the presence of cofactors, which may complicate large-scale implementations.
- Practical Applications and Scalability: The robustness of UPOs under various conditions, including neat reactions, make them attractive candidates for industrial processes where high product titers and operational simplicity are essential. BVMOs, on the other hand, offer outstanding selectivity and the possibility of enzyme engineering to finely tune their catalytic properties, rendering them particularly suitable for applications in which precise enantiocontrol is required. Notably, several examples of larger scale BVMO-catalyzed sulfoxidations have been reported, employing engineered enzymes to access valuable APIs with high yields and optical purities.[10]

To enable a direct comparison between BVMOs and UPOs, a representative series of indole- and pyrrole-based heterobiaryl sulfides was selected as model substrates for biocatalytic sulfoxidations. Apart from their importance as pharmaceutical and synthetic scaffolds and their application as chiral auxiliaries, the presence of a heteroaromatic ring (indole or pyrrole) introduces distinct electronic and steric environments that can strongly influence enzyme–substrate recognition, oxygen-transfer efficiency, and stereochemical outcome. This allows the evaluation of how each enzyme family responds to electronically differentiated aromatic systems. In addition, the heterobiaryl architecture provides sufficient steric demand around the sulfur atom to challenge the active sites of the studied BVMOs and UPOs, making these substrates appropriate probes for assessing catalytic scope and enantiodiscrimination. This approach allowed the evaluation of both enzyme classes in terms of activity and selectivity, as well as a direct comparison of their catalytic performance in achieving the desired asymmetric sulfoxidation.

Results and discussion

Sulfoxidation of indole-based sulfides

A set of indole-based heterobiaryl sulfides (**1a-i**) containing the indole ring has been prepared following the literature procedure.[11] Among them, compound 1a was selected as a model substrate to be tested in sulfoxidation reactions catalyzed by a set of BVMOs, including 2-oxo-Δ3-4,5,5-trimethylcyclopentenylacetyl-CoA 1,2-monooxygenase (OTEMO) from *Pseudomonas putida* ATCC 17453,[12] cyclohexanone monooxygenase from *Thermocrispum municipale* (*Tm*CHMO),[13] polycyclic ketone monooxygenase (PockeMO) from *Thermothelomyces thermophila*,[14] phenylacetone monooxygenase from *Thermofibida fusca* (PAMO),[15] cyclopentadecanone monooxygenase (CPDMO) from *Pseudomonas sp*. HI-70,[16] and the recently described ancient BVMO (AncBVMO2.2).[17] All enzymes have been expressed as fusion proteins, fused to an improved variant of phosphite dehydrogenase from *Pseudomonas stutzeri*.[18] The reactions were carried out at 30ºC for 48 hours using phosphite as cosubstrate for the NADPH cofactor recycling (see Supporting Information). Although BVMOs have previously been shown to catalyse certain nitrogen oxidations,[19] complete chemoselectivity for the sulfoxide formation was observed for all substrates tested, with no detectable sufone overoxidation product neither N-oxidation products.

At 30 °C, low conversions (8%, see Table S1) were obtained with *Tm*CHMO and PockeMO, affording sulfoxide **2a** as the sole product and with no evidence of sulfone overoxidation. In view of the fact that these results and the known thermostability of these enzymes, both enzymes were also tested at 45ºC (Table 1, entries 1 and 2), affording (+)-**2a** in 44% conversion and 35% ee when *Tm*CHMO was employed, whereas for PockeMO both lower conversions and optical purities were measured (entry 2). With the aim of increasing the system activity, sulfoxidations were carried out at 60ºC in presence of *Tm*CHMO and PockeMO (entries 3 and 4). Higher conversions were achieved, but (+)-**2a** was obtained with a very low enantiomeric excess (9% *ee*) using *Tm*CHMO (entry 8), whereas no modification was observed for PockeMO (11% *ee*, entry 9). Previous studies on both *Tm*CHMO and PockeMO have shown that this biocatalyst showed an improved performance in presence of low percentages (10% v/v) of hydrophilic solvents as MeOH or 1,4-dioxane.[20] By this reason, sulfoxidation of 1a was conducted in presence of these two cosolvents. The use of 1,4-dioxane (entry 5) led to a slightly higher conversion, but with an important drop in sulfoxide optical purity. On the other hand, the reaction in 10% v/v methanol afforded the final compound with low conversion (18%), being observed an increase in the process selectivity (71% ee, entry 6).

Sulfoxidation of substrate **1a** was also tested in the presence of different UPOs, from *Marasmius rotula* (*Mro*UPO),[21]

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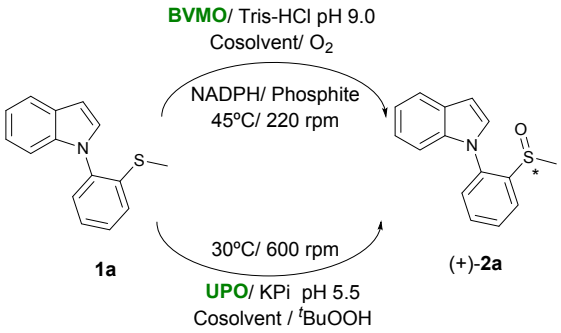
Chaetomium globosum (*Cgl*UPO),[22] *Coprinopsis cinerea* (*rCci*UPO)[23] and *Humicola insolens* (*rHin*UPO).[24] As initial approach, the reactions were conducted using acetone as cosolvent and H_2O_2 as cosubstrate. However, control experiments showed a non-enzymatic sulfoxidation yielding 10-15% conversion after 2 hours, a process that was not observed in the BVMO-catalyzed sulfoxidations. To suppress the undesired chemical oxidation, *tert*-butyl hydroperoxide (^{t}BuOOH) and H_2O_2 were subsequently compared as cosubstrates in reactions performed with acetonitrile (MeCN) or acetone (two water-miscible solvents commonly used in UPOs-catalysed reactions due to their low impact on enzyme activity). These experiments demonstrated that non-enzymatic sulfoxidation occurred only in the presence of H_2O_2 (Figure SX). Consequently, ^{t}BuOOH was selected as the cosubstrate for reactions. Furthermore, discarding peroxide is necessary for future scale-up experiments due to the risk of explosion from certain combinations of acetone and peroxide.[25] The reactions of substrate **1a** with several UPOs only showed good results with *Mro*UPO,[21] whereas for *Cgl*UPO, *rCci*UPO, and *rHin*UPO no oxidation was observed. Several reactions were conducted using acetone and MeCN in 10-30% concentration (Tables 1 and S5) observing a positive effect at higher cosolvent concentrations. The use of 30% cosolvent delivered excellent conversions (99%) using 0.1 mol% of UPO and 2 mM of peroxide. As a result, 98% of (+)-**2a** was obtained under the best conditions (entry 10) presenting a 62% enantiomeric excess. Remarkably, the enantiomeric excess was retained during the optimization process and the cosolvent, its concentration and the conversion rate did not impact in contrast to BVMOs.

Table 1. Biocatalyzed sulfoxidation of prochiral heterobiaryl sulfide **1a** employing BVMOs and *Mro*UPO



Entry	Biocatalyst	Solvent	T (ºC)	t (h)	Conv. (%)[a]	*ee* (%)[b]
1	*Tm*CHMO	None	45	48	44	35
2	PockeMO	None	45	48	21	12
3	*Tm*CHMO	None	60	48	60	9
4	PockeMO	None	60	48	33	11
5	*Tm*CHMO	10% dioxane	45	48	48	13
6	*Tm*CHMO	10% MeOH	45	48	15	71
7	*Mro*UPO	20% acetone	30	24	83 (98)	64
8	*Mro*UPO	20% MeCN	30	24	72 (93)	62
9	*Mro*UPO	30% acetone	30	24	99 (97)	60
10	*Mro*UPO	30% MeCN	30	24	99 (98)	62

[a] Determined by GC/MS. [b] Measured by HPLC; n.d. not determined. In parenthesis, the percentage of sulfoxide obtained.

After establishing the sulfoxidation of **1a** using the BVMOs PockeMO and *Tm*CHMO, as well as *Mro*UPO, the biocatalytic sulfoxidation was extended to other prochiral sulfides bearing different substituents on the indole ring (Table S2). Best results obtained for each enzyme are shown in Scheme 1. Thus, when fluorinated analogues substituted at the 5-, 6- and 7- positions (substrates **1b-d**) were employed, the corresponding sulfoxides (+)-**2b-d** were obtained with good enantioselectivities using *Tm*CHMO, particularly in the case of (+)-**2d**, which was isolated with 94% *ee* when the reaction was performed in 10% v/v MeOH. Conversions for these substrates were low to moderate, reaching a maximum of 51% for **2d** in presence of this cosolvent, highlighting the beneficial effect of MeOH for this substrate. In contrast, PockeMO catalyzed reactions of the fluorinated substrates afforded significantly lower enantioselectivities, the best result being the formation of (+)-**2c** with 34% conversion and 32% *ee*. A different trend was observed for the sulfoxidation of the 5-chloro derivative **1e**, for which PockeMO outperformed *Tm*CHMO, delivering (+)-**2e** with 17% conversion and 66% *ee*. In comparison, **1e** proved to be a poor substrate for *Tm*CHMO, resulting in both low conversion and low optical purity. The presence of electron-donating substituents was also tolerated by PockeMO. Sulfoxidation of the 5-methoxy derivative **1f** afforded (+)-**2f** with 37% conversion and good enantiomeric excess (77% *ee*). By contrast, this substrate was poorly accepted by TmCHMO, yielding only 13% of (+)-**2f** with 17% *ee* after 48 h at 45ºC. For the methyl-substituted derivatives **1g** (5-methyl) and **1h** (6-methyl), low conversions were observed for both BVMOs. Nevertheless, (+)-**2g** was obtained with 39% *ee* in the PockeMO-catalysed reaction, whereas (+)-**2h** was formed with only 13% ee in presence of *Tm*CHMO, representing the highest value for this substrate. Finally, the influence of the alkyl chain at the sulfur atom was examined by replacing the methyl group with an ethyl substituent (**1i**). Low conversions were observed for both BVMOs, affording (+)-**2i** with 21% *ee* when catalysed by PockeMO, while *Tm*CHMO provided even lower optical purity.

The reactions catalyzed by *Mro*UPO were carried out using 30% MeCN and 0.1 mol% of enzyme generally obtaining very high of sulfoxides (up to 96%) with the most of sulfides **1b-h** after 24 hours (no sulfoxide was observed with the ethyl derivative 1i). In general, the chemoselectivity to sulfoxides attained for this biocatalyst was very high (>93%), except for the 5-methyl derivative **1g** where some reaction byproducts were observed (also but in lower proportion was achieved for the 6-methyl derivative **1h**). For this substrate, the benzylic positions turned out to be particularly active delivering a broad number of products such as alcohols, aldehydes and carboxylic acids, greatly reducing the selectivity of the sulfoxide, thus achieving (+)-**2g** in 57% of total products. In addition, it is important to remark that although the oxidative cleavage of ethers is a typical UPO-reaction, not only was this not the case with **1f** but also the sulfoxide (+)-**2f** was recovered as the exclusive product. In general, low to moderate optical purities were achieved for the (+)-sulfoxides **2b-i**, with enantiomeric excesses ranging from 21 to 64%, being observed the best results for those indoles bearing functional groups at the 5-position, as shown for **1b** (5-

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fluoro), **1e** (4-chloro), and **1g** (5-methyl), recovering the corresponding sulfoxides with optical purities around to 60%.

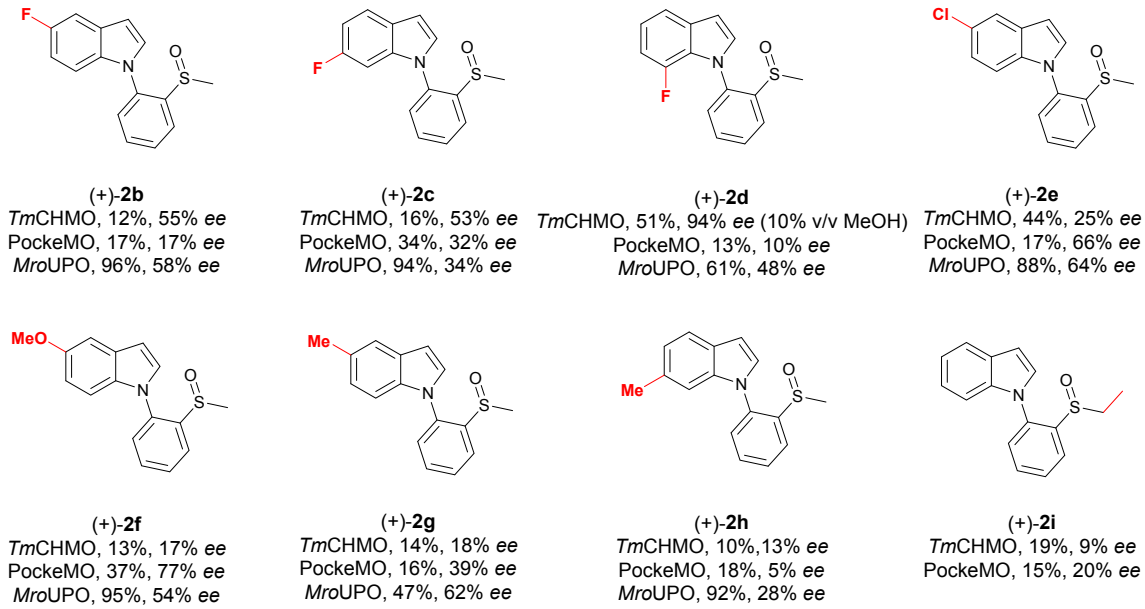


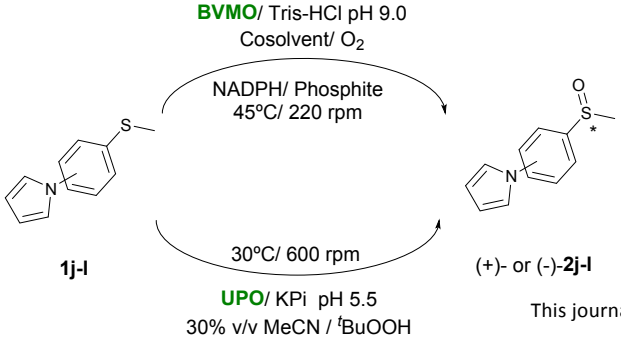
Figure 1. Sulfoxidation of heterobiaryl sulfides **1b**-i catalyzed by PockeMO, *Tm*CHMO and *Mro*UPO.

Enzymatic sulfoxidation of pyrrole-based sulfides

In view that the heterobiaryl sulfides lead to sulfoxidation procedures with generally low activity and moderate enantioselectivity, a set of pyrrole-based sulfides (**1j-n**) were tested as substrates in the biocatalysed oxidations. These compounds were prepared following a previously described procedure based on a Paal–Knorr reaction, between the corresponding (methylthio)anilines and 2,5-dimethoxytetrahydrofuran in glacial acetic acid,[26] affording 1j-n in 47% to 58% yields. For those pyrrole-based sulfurs containing ethyl and isopropyl alkyl substituents (1m and 1n, respectively), the S-alkylation was performed first, as previously described, following by the Paal-Knorr reaction.[27]

Enzymatic sulfoxidation of compounds **1j-n** were carried out in presence of BVMOs (see Table S3). For the best biocatalyst, the effect of the temperature (45ºC), as well as the presence of 1,4-dioxane or methanol as cosolvents (10% v/v), were analyzed in these oxidative procedures (Table 2). Depending on the substrate structure and the enzyme employed, it was possible to get the optically active sulfoxides in high optical purities. Among the BVMOs evaluated for the sulfoxidation of substrate **1j**, *Tm*CHMO displayed the best performance in buffer pH 9.0 at 30ºC, being recovered (+)-**2j** with moderate conversion but a high optical purity (*ee*=88%, entry 1). When the temperature was increased up to 45ºC, there was no improvement in the system activity but a high decrease in selectivity was observed (entry 2). The presence of organic cosolvents in 10% v/v has a positive effect when employing 1,4-dioxane, as shown in entry 4, as the conversion is doubled in the same reaction time (63%), whereas the high optical purity is maintained (90% *ee*). For the 3-pyrrole derivative (**1k**), the best results were obtained in the reactions catalysed by PockeMO and OTEMO, being achieved a higher conversion with this last catalyst (entry 6). When the reaction was carried out at 30ºC in 10% v/v methanol as cosolvent, it was possible to recover (-)-**2k** with 85% conversion and 87% *ee*, as shown in entry 8. The use of 1,4-dioxane or 45ºC led to worse results for OTEMO on this substrate. Again, the best results in the biooxidation of 1-(4-(methylthio)phenyl)-1H-pyrrole (**1l**) were achieved with both PockeMO and OTEMO, being observed a different enantiopreference depending on the BVMO. PockeMO led to (+)-**2l** in 17% conversion and 72% ee (entry 10), whereas OTEMO afforded (-)-**2l** in 16% conversion and 79% *ee* (entry 11). Further optimization of this last catalyst regarding the temperature and the presence of cosolvents did not improve the results achieved in buffer at 30ºC

Table 2. Biocatalyzed sulfoxidation of compounds **1j-n** in presence of BVMOs and MroUPO.



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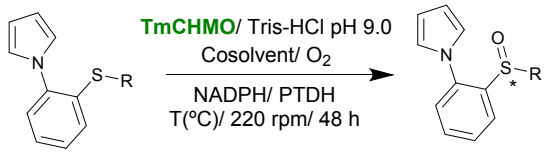
Entry	Pyrrole	BVMO	Cosolvent	T (ºC)	t (h)	Conv. (%)[a]	*ee* (%)[b]	*Config*
1	2-Pyrrole-	*Tm*CHMO	None	30	48	40	88	(+)
2	2-Pyrrole-	*Tm*CHMO	None	45	48	32	55	(+)
3	2-Pyrrole-	*Tm*CHMO	10% MeOH	30	48	90	60	(+)
4	2-Pyrrole-	*Tm*CHMO	10% dioxane	30	48	63	90	(+)
5	3-Pyrrole-	PockeMO	None	30	48	38	68	(-)
6	3-Pyrrole-	OTEMO	None	30	48	42	70	(-)
7	3-Pyrrole-	OTEMO	None	45	48	41	60	(-)
8	3-Pyrrole-	OTEMO	10% MeOH	30	48	85	87	(-)
9	3-Pyrrole-	OTEMO	10% dioxane	30	48	53	70	(-)
10	4-Pyrrole-	PockeMO	None	30	48	17	72	(+)
11	4-Pyrrole-	OTEMO	None	30	48	16	79	(-)
12	4-Pyrrole-	OTEMO	None	45	48	16	46	(-)
13	4-Pyrrole-	OTEMO	10% MeOH	30	48	29	70	(-)
14	4-Pyrrole-	OTEMO	10% dioxane	30	48	17	55	(-)
15	2-Pyrrole-	*Mro*UPO	30% MeCN	30	5	99 (95)	31	(+)
16	3-Pyrrole-	*Mro*UPO	30% MeCN	30	5	93 (94)	21	(-)
17	4-Pyrrole-	*Mro*UPO	30% MeCN	30	5	81 (92)	17	(+)

[a] Determined by GC/MS. [b] Measured by HPLC; n.d. not determined. In parenthesis, the percentage of sulfoxide obtained.

Sulfides **1j-l** were also tested with *Mro*UPO under similar conditions to the indole derivatives. Nevertheless, the pyrrole-derivatives showed better affinity to the UPO resulting in a sulfone production that forced us to use a stoichiometric amount of peroxide. After 5 hours, high conversions (81-99%) were achieved with good selectivity to the sulfoxides 2j-l (entries 15-17) with minor amounts of hydroxy, hydroxy-sulfinyl and/or sulfone compounds. Regrettably, sulfoxides were recovered with low enantiomeric excesses, from 17% for (+)-**2l** to 31% for (+)-**2j**.

In analogy to the indole-based substrates, the influence of the alkyl substituent at the sulfur atom was also examined for the pyrrole derivatives. However, it should be noted that, for the indole substrate **1i**, PockeMO afforded higher enantioselectivity (21% *ee*) than *Tm*CHMO (9% *ee*), indicating that the optimal BVMO depends on the specific substrate structure. As observed in Table 3, increasing the size of the alkyl group from methyl to ethyl of isopropyl resulted in a marked decrease in both activity and selectivity compared to the methyl analogue. Sulfoxidation of the ethyl-substituted sulfide **1m** afforded (+)-**2m** with only 18% conversion and 69% *ee* in the reaction catalysed (entry 1). Increasing the reaction temperature to 45 ºC improved conversion to 25%, but the enantiomeric excess dropped to 60% (entry 2). For the isopropyl-substituted sulfide **1n**, *Tm*CHMO was the only BVMO able to catalyse the sulfoxidation reaction, leading to (+)-**2n** in 10% conversion and 51% *ee* (entry 5). Raising the temperature led to higher conversion, accompanied by a slight decrease in the enantiomeric excess (entry 6). The addition of cosolvents (10% v/v methanol or 1,4-dioxane) had comparable effects for **1m** and **1n**. Methanol enhanced conversion but caused a dramatic loss of enantioselectivity (entries 3 and 7), whereas 1,4-dioxane afforded similar results to those obtained in absence of cosolvents (entries 4 and 8). Finally, *Mro*UPO was also evaluated for the oxidation of sulfides **1m** and **1n**. However, no sulfoxide formation was detected, and only hydroxylated products were observed (Figure S3).

Table 3. *Tm*CHMO biocatalyzed sulfoxidation of prochiral sulfides **1m,n**



1m,n (+)-**2m,n**

1m R: Et
1n R: ^{i}Pr.

Entry	R	Solvent	T (ºC)	Conv. (%)[a]	*ee* (%)[b]
1	Et	None	30	18	69
2	Et	None	45	25	60
3	Et	10% MeOH	30	33	40
4	Et	10% dioxane	30	20	65
5	^{i}Pr	None	30	10	51
6	^{i}Pr	None	45	23	44
7	^{i}Pr	10% MeOH	30	28	15
8	^{i}Pr	10% dioxane	30	12	49

[a] Determined by GC/MS. [b] Measured by HPLC; n.d. not determined.

BVMO-catalyzed sulfoxidation on the indole- and pyrrole-based sulfides have been performed at multimilligram scale, as shon in the Supporting Information (section S6). Although BVMO-catalyzed reactions afforded the corresponding sulfoxides with high enantiomeric excesses (up to 94% *ee*), the isolated yields

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at this scale were moderate, typically ranging from 15 to 41%. These values reflect the incomplete conversions observed for several substrates under conditions optimized for enantioselectivity, as well as the substrate-dependent activity of the enzymes. In some cases, prolonged reaction times or higher temperatures led to improved conversion but were accompanied by loss of enantiopurity. Thus, a compromise between activity and stereocontrol was necessary. Despite the moderate isolated yields, the excellent enantioselectivities, high chemoselectivity (no overoxidation to sulfones), and the use of mild aqueous conditions highlight the synthetic potential of these BVMOs for the preparation of optically active heterobiaryl sulfoxides.

Study of sulfoxidation parameters

The effect of pH on the biocatalysed sulfoxidation of compounds **1j** and **1l** was examined by studying the oxidation of **1j** catalysed by *Tm*CHMO and the sulfoxidation of **1l** catalysed by OTEMO under cosolvent-free conditions. For both substrates, increased conversions were observed at pH values above 7.0, with comparable activities maintained throughout the pH range of 8.0–9.5 (Table S4). Notably, pH variations within this range had no significant impact on stereoselectivity, as the corresponding sulfoxides were consistently obtained with similar enantiomeric excesses in both *Tm*CHMO- and OTEMO-catalysed reactions. Thus, aside from the enhanced catalytic activity at mildly basic pH, no pronounced pH-dependent effects on enantioselectivity were detected for either BVMO.
In contrast, evaluation of the unspecific peroxygenase MroUPO using substrate **1j** confirmed its previously reported acidic pH optimum. Maximum conversion (91%) was achieved at pH 5.5, whereas a sharp decline in activity was observed at higher pH values, dropping to only 5% conversion at pH 8.5.[21]
The effect of substrate concentration on the most relevant BVMO-catalysed sulfoxidations was subsequently examined. To enable a meaningful comparison of conversions achieved at different reaction times, the reaction rate was defined as the mmoles of sulfide oxidized by the enzyme per hour and per liter (mmol L^{-1} h^{-1}). This parameter was evaluated for the sulfoxidation of indole-based sulfide **1d** catalyzed by *Tm*CHMO in 10% v/v MeOH at 45 ºC, for the sulfoxidation of pyrrole derivative **1j** catalyzed by the same enzyme in 10% v/v 1,4-dioxane at 30 ºC, and for the oxidation of pyrrole-based compound **1k** catalyzed by OTEMO in the presence of 10% v/v methanol at 30 ºC. For all three sulfides, the reaction rate increased as the substrate concentration was raised above 5.0 mM, reaching a maximum at 50 mM, as shown in Figure 1. Under these conditions, reaction rates of 9.7 mmol L^{-1} h^{-1} for (+)-**2d**, 22.4 mmol L^{-1} h^{-1} for (+)-**2j**, and 15.6 mmol L^{-1} h^{-1} for (-)-**2k** were achieved. At higher substrate concentrations a decrease in reaction rate was observed. However, the rates remained superior to those obtained at 5.0 mM. Importantly, the enantiomeric excesses of the resulting sulfoxides were unaffected by substrate concentration across the entire range studied, indicating that process selectivity is maintained even at elevated substrate loadings.

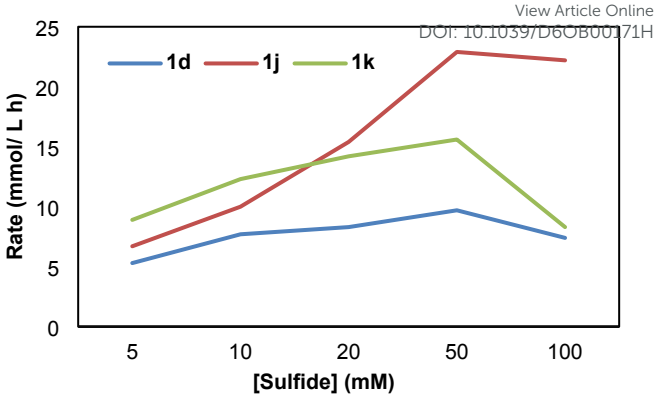

Figure 2. Effect of the sulfides concentration in the reaction rate of the *Tm*CHMO-biocatalyzed sulfoxidations of **1d** (blue line) and **1j** (red line), and in the sulfoxidation of **1k** catalyzed by OTEMO (green line).

The biooxidations catalyzed by *Mro*UPO on substrates **1e** and **1j**, which have led to the best performance for those indole- and pyrrole-based sulfides, respectively, have been scaled up to a 60 mM substrate concentration. To ensure proper sulfide solubilization, acetonitrile percentage was increased up to 60% v/v. Both conversion and selectivity were comparable to those achieved at lower substrate concentration. Thus, the biooxidaton of 1e occurred with a 95% conversion, with an 88% of sulfoxide (+)-**2e** and 12% of sulfone overoxidation product. Regarding the pyrrole-based substrate, 84% of the starting material was converted, leading to an 88% of sulfoxide (+)-**2j**, and a 12% of hydroxy-sulfinyl compound. Enantiomeric excesses were maintained at these concentrations.

Conclusions

This comparative study between Baeyer–Villiger monooxygenases (BVMOs) and unspecific peroxygenases (UPOs) in the asymmetric sulfoxidation of heterobiaryl sulfides has shown the distinct yet complementary catalytic features of both enzyme families. UPO from *Marasmius rotula* exhibited high catalytic efficiency, achieving conversions close to quantitative values under mild, cofactor-independent conditions. The enzyme demonstrated broad substrate tolerance and high chemoselectivity towards sulfoxides, although the enantiomeric excesses obtained were moderate (17–64% *ee*) and only slightly affected by changes in cosolvent or peroxide systems. These results underline the operational simplicity and robustness of UPOs, which make them particularly attractive for sustainable oxidation processes at preparative scales. In contrast, BVMOs such as *Tm*CHMO, PockeMO, and OTEMO displayed lower to moderate activity but remarkably higher stereochemical control, reaching enantiomeric excesses up to 94% for selected substrates. Their activity and selectivity were found to be strongly dependent on reaction parameters such as temperature, solvent composition, and substrate electronic properties. The presence of small amounts of methanol or 1,4-dioxane often enhanced both conversion and selectivity, demonstrating that fine-tuning of reaction media can significantly influence BVMO-catalyzed

sulfoxidations. Regarding substrate scope, indole-based sulfides afforded moderate optical purities for both enzymatic systems, whereas pyrrole-based derivatives led to improved enantioselectivities, particularly in BVMO-catalyzed oxidations. In these cases, *Tm*CHMO and OTEMO proved especially effective, yielding optically active sulfoxides in high ee values and moderate conversions.

Overall, UPOs emerged as highly productive and robust catalysts suitable for straightforward, green oxidation procedures, while BVMOs provided superior asymmetric induction under optimized cofactor-dependent conditions. The complementary behaviour of both enzyme classes offers a sustainable platform for the synthesis of several chiral heterobiaryl sulfoxides, allowing the selection of the most appropriate biocatalyst depending on whether conversion efficiency or enantiomeric purity is prioritized.

Author contributions

Conceptualization: A.G.B, A.R and G.d.G; Resources; M.F, N.L, R.F, K.S. and A.G; Investigation: P.V.D, J.C.G., D.G.R. and A.G.B. Supervision: A.G.B, A.R. and G.d.G. Methdology: A.R and G.d.G. Funding acquisition: R.F. and A.G.; Wrting-original draft: A.R. and G.d.G; Writing- reviewing and editing: N.L., M.F., R.F., A.G. A.G.B., A.R and G.d.G.

Conflicts of interest

There are no conflicts to declare

Data availability

The data supporting this article have been included as part of the Supplementary Information. Supplementary information: synthesis and characterization of products, NMR and HPLC spectra and full screening of biooxidation procedures.

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Notes and references

‡ Footnotes relating to the main text should appear here. These might include comments relevant not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

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The data supporting this article have been included as part of the Supplementary Information. Supplementary information: synthesis and characterization of products, NMR and HPLC spectra and full screening of biooxidation procedures.