# Asymmetric oxidations at sulfur catalyzed by engineered strains that overexpress cyclohexanone monooxygenase†

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Recombinant strains of baker's yeast (*Saccharomyces cerevisiae*) and *Escherichia coli* expressing cyclohexanone monooxygenase from *Acinetobacter* sp. NCIB 9871 have been used as whole-cell biocatalysts for oxidations of several sulfides, dithianes and dithiolanes to the corresponding sulfoxides. The enantio- and diastereoselectivities of these reactions compare favorably with oxidations catalyzed by the purified monooxygenase or the parent microorganism (a class II pathogen). The facility of handling yeast reactions makes these biotransformations an attractive alternative route to optically pure sulfoxides.

Methods that exploit the ability of chiral sulfoxides to control the stereochemical outcome of reactions at nearby centers have become important additions to the repertoire of organic synthesis.[1–7] All of these strategies depend on access to homochiral sulfoxides and a variety of methods for their synthesis have been described.[8] Several useful sulfoxides are accessible in high optical purities by chemical oxidations followed by diastereomeric separation.[9] The direct asymmetric oxidations of prochiral thioethers provide a more direct route, but these reactions often display a variable degree of enantiomeric enrichment that is highly dependent on the nature of the substrate.[10–16] These limitations, coupled with a desire to develop ecologically more benign processes, have inspired a search for bioorganic oxidation methods. A large number of microorganisms[17–19] including baker's yeast[20] catalyze oxidations at sulfur. These biooxidations, however, have their own problems. Substrate acceptability is often limited and enantioselectivity can vary dramatically with substrate structure. Moreover, isolated redox enzymes require cofactors that must be regenerated for preparative-scale reactions and this adds to both the cost and complexity of these processes. Thus, there remains a need for simple, general oxidants that provide homochiral sulfoxides in high chemical and optical yields with minimal environmental impact.

Since the pioneering work by Walsh and co-workers,[21] *Acinetobacter* sp. NCIB 9871 cyclohexanone monooxygenase has been shown to accept a wide variety of thioethers and provide good yields of the corresponding sulfoxides.[22–31] Unfortunately, a variety of practical difficulties have limited the appeal of this enzyme to those with the expertise to handle the pathogenic *Acinetobacter* strain grown in the presence of organic inducers, purify the protein and set up a regeneration system for the essential NADPH cofactor.[32]

We have recently described a recombinant strain of baker's yeast (*Saccharomyces cerevisiae*) that overexpresses *Acinetobacter* sp. cyclohexanone monooxygenase.[33] These cells are non-pathogenic, simple to handle and monooxygenase production is induced by adding galactose to the growth medium. Whole cells of this "designer yeast" strain have been used in place of the purified enzyme to carry out Baeyer–Villiger oxidations and provide a variety of chiral δ- and ε-lactones, usually in optical purities >95%.[34–36] The growing yeast cells provide a constant supply of the enzyme and the NADPH cofactor, which dramatically simplifies the process.[37] Moreover, these oxidations do not require specialized equipment or training. In the case of Baeyer–Villiger oxidations where comparisons were possible, the yields and optical purities of the products from yeast-mediated reactions were virtually identical to those obtained from reactions utilizing the purified enzyme.[38] Given the success of our "designer yeast" in catalyzing asymmetric Baeyer–Villiger oxidations and the demonstrated ability of cyclohexanone monooxygenase to oxidize prochiral thioethers, we have applied the same yeast strain to synthesize chiral sulfoxides and these efforts are described here. Our results have shown that the engineered yeast strain can carry out asymmetric thioether oxidations; however, better results are sometimes obtained by using a strain of *Escherichia coli* that overexpresses the same enzyme. This latter reagent avoids side-reactions catalyzed by yeast enzymes and affords higher yields in some cases.

## Results and discussion

### Oxidations of acyclic thioethers by a baker's yeast strain expressing cyclohexanone monooxygenase

Phenyl methyl sulfide **1a** was oxidized by our engineered yeast strain to afford the optically pure sulfoxide **2a** in 95% yield with >99% ee (Scheme 1). This result is comparable to that obtained from the oxidation using purified cyclohexanone monooxygenase and an NADPH regeneration system[22] although no enzyme isolation or cofactor regeneration is required for reactions involving the engineered yeast strain. The optical purity of **2a** was determined by chiral-phase HPLC and its absolute configuration was assigned from its

† *Supplementary material available:* experimental details of the construction of an *E. coli* overexpression plasmid for cyclohexanone monooxygenase. For direct electronic access see http://www.rsc.org/suppdata/nj/1999/827/, otherwise available from BLDSC (No. SUP 57581, 5 pp.) or the RSC Library. See Instructions for Authors, 1999, Issue 1 (http://www.rsc.org/njc).

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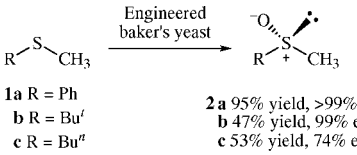



**Scheme 1**

optical rotation and comparison to literature values.[15] Likewise, *tert*-butyl methyl sulfide **1b** was oxidized to the corresponding (*R*)-sulfoxide **2b** in 99% ee as determined by chiral-phase GC and optical rotation values. The initially low isolated yield from this reaction was improved significantly (from 11% to 47%) by the use of a sealed flask to minimize substrate loss by evaporation. Using these conditions, *n*-butyl methyl sulfide **1c** was converted to **2c** in 53% isolated yield and 74% ee. In other cases, however, attempts to use our engineered yeast strain to effect thioether oxidations were less successful.[39] For this reason and because a recombinant *E. coli* strain expressing a bacterial dioxygenase has been used successfully in arene[40] and sulfide oxidations,[41] we elected to create an efficient *E. coli* expression system for cyclohexanone monooxygenase and evaluate its potential for sulfoxide synthesis.

### An efficient *E. coli* overexpression system for cyclohexanone monooxygenase

We constructed an *E. coli* overexpression plasmid for cyclohexanone monooxygenase using standard techniques (Fig. 1).[42] The polymerase chain reaction was first used to correct a three base pair deletion present in the 5′ region of our originally cloned gene.[33] Fortunately, this alteration in the *N*-terminal amino acid sequence did not appear to affect the catalytic activity or enantioselectivity of the protein expressed from the mutant gene.[38] A subsequent polymerase chain reaction incorporated a restriction site (*Nde*I) required to insert the gene encoding cyclohexanone monooxygenase into *E. coli* expression plasmid pET 22b(+). In this final construct, designated pMM4, protein expression is driven by the strong T7 promoter that can be controlled by adding isopropylthio-β-D-galactoside to the culture medium. This plasmid was used to transform *E. coli* BL21(DE3) to create the final strain [BL21(DE3)(pMM4)] that overexpresses cyclohexanone monooxygenase at a level of *ca.* 20% of total protein. This quantity is approximately five-fold higher than the previous *E. coli* overexpression strain[43] and ten- to twenty-fold higher than typical expression levels in *S. cerevisiae*.[44] The recombinant *E. coli* strain is a very efficient catalyst for asymmetric Baeyer–Villiger oxidations and these results will be described in detail elsewhere.

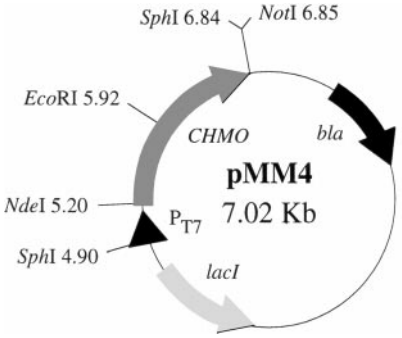
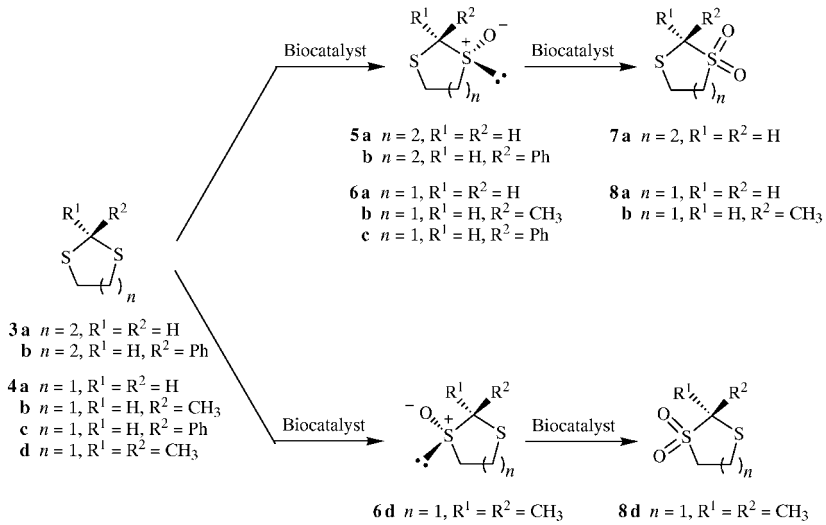



**Fig. 1** Partial restriction map of pMM4. This plasmid directs the efficient synthesis of *Acinetobacter* sp. cyclohexanone monooxygenase when present in *E. coli* BL21(DE3). The CHMO gene is transcribed by T7 RNA polymerase and the protein accumulates in soluble form in the cytoplasm. The *bla* gene encodes β-lactamase to confer resistance to ampicillin and the *lacI* gene product is involved in regulating monooxygenase expression.

### Oxidations of dithianes using engineered baker's yeast and *E. coli* strains

Colonna and co-workers have used purified cyclohexanone monooxygenase along with an NADPH regeneration system to oxidize dithiane **3a** to (*R*)-sulfoxide **5a** in 81% yield and >98% ee along with a 19% yield of sulfone **7a** (Scheme 2; Table 1).[25] The high optical purity of **5a** was due to both the enzyme's preference in the oxidation of **3a** and subsequent enantioselective oxidation of (*S*)-**5a**, also catalyzed by cyclohexanone monooxygenase.[45] Similar results were obtained when Furstoss and co-workers used whole *Acinetobacter* sp. NCIB 9871 cells in place of purified cyclohexanone monooxygenase (Table 1).[28] On the other hand, when our engineered yeast cells expressing cyclohexanone monooxygenase were grown in the presence of **3a**, sulfoxide **5a** was isolated in only 18% yield (Table 1). A control experiment in which the unmodified yeast cells were grown in the presence of **3a** revealed that native yeast enzymes were also capable of forming both sulfoxide and sulfone products. To avoid this competition, the *E. coli* strain that overexpresses cyclohexanone monooxygenase was used to oxidize **3a** to the (*R*)-sulfoxide in 73% isolated yield. Loss of the volatile substrate and/or product by evaporation, either during the oxidation or during workup, likely accounts for the slightly depressed yield of **5a** relative to the amount expected from GC analysis. This oxidation was complete within 20 h and only a small amount of starting material and sulfone were present at the end of the reaction (Fig. 2). The absolute configuration of **5a** was assigned by comparing the sign of its optical rotation to literature values. Given the results of Colonna *et al.*,[25] it is likely




**Scheme 2**

**Table 1** Biocatalytic oxidations of dithianes

| Substrate | Catalyst | **3** : **5** : **7**[a] | Sulfoxide **5** | | | Sulfone **7** | | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | *trans* : *cis* | Yield[b] (%) | % ee | Yield[b] (%) | % ee | |
| **3a** | Isolated enzyme | 0 : 81 : 19 | —[c] | 81 | >98 | 19 | — | 25 |
| | *Acinetobacter* | N.R.[d] | — | 76 | 98 | 20 | — | 28 |
| | *S. cerevisiae* 15C | 82 : 1 : 17 | — | N.D.[e] | N.D. | N.D. | — | This paper |
| | 15C(pKR001) | 1 : 42 : 57 | — | 18 | 90 | 19 | — | This paper |
| | *E. coli* BL21(DE3) | 100 : 0 : 0 | — | — | — | — | — | This paper |
| | BL21(DE3)(pMM4) | 3 : 91 : 6 | — | 73 | 84[f] | N.D. | — | This paper |
| **3b** | Isolated enzyme | 0 : 100 : 0 | 50 : 1 | N.R. | 28 | — | — | 29 |
| | *S. cerevisiae* 15C | 92 : 8 : 0 | N.D. | N.D. | N.D. | — | — | This paper |
| | 15C(pKR001) | 66 : 34 : 0 | 9 : 1 | 30 | 30[f,g] | — | — | This paper |
| | *E. coli* BL21(DE3) | 100 : 0 : 0 | — | — | — | — | — | This paper |
| | BL21(DE3)(pMM4) | 34 : 66 : 0 | 19 : 1 | N.D. | 12[f,g] | — | — | This paper |

[a] Determined by gas chromatography. [b] Isolated yield after chromatographic purification. [c] Not applicable. [d] Not reported. [e] Not determined. [f] Optical purities were determined by chiral-phase GC. [g] Optical purity of the major diastereomer.

that extending the reaction time in the presence of the engineered *E. coli* cells would result in an even greater optical purity of the sulfoxide by preferential oxidation of (*S*)-**5a** to the sulfone. A control experiment established that native *E. coli* enzymes are not capable of oxidizing **3a** (Table 1). Moreover, incubating racemic sulfoxide **5a** with the unmodified *E. coli* host strain for extended times failed to yield any sulfone product (Scheme 3). The engineered *E. coli* strain thus acts as the whole-cell equivalent of purified cyclohexanone monooxygenase that does not require enzyme purification or cofactor regeneration.

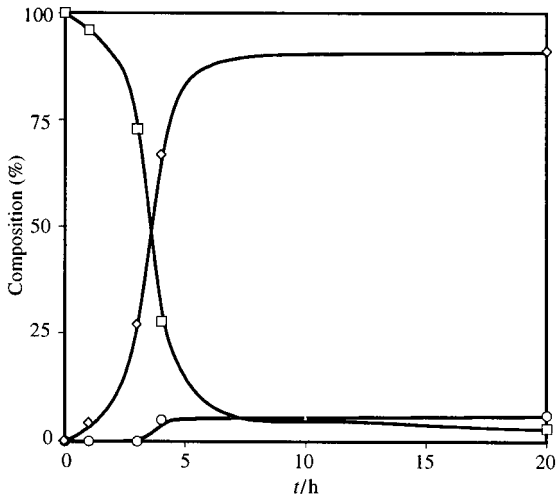
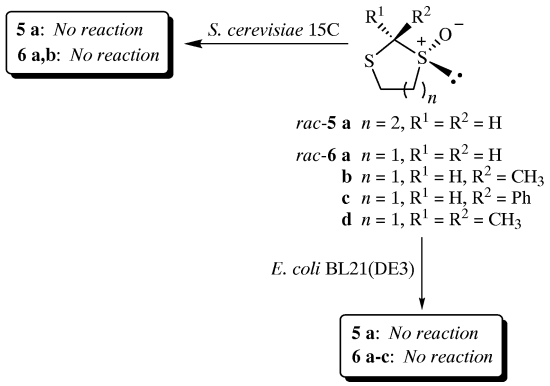



**Fig. 2** Oxidation of 1,3-dithiane **3a** by *E. coli* BL21(DE3)(pMM4). This oxidation was performed with 8.3 mM substrate and monitored periodically by chiral-phase GC. Symbols: (□) dithiane **3a**; (◇)(*R*)-sulfoxide **5a**; (○) sulfone **7a**.




**Scheme 3**

Similar results were observed for 2-phenyl-substituted compound **3b** (Table 1). While the engineered yeast strain afforded the expected (*R*)-sulfoxide, its efficiency was compromised by competing oxidations carried out by native yeast enzymes. This competition was avoided by substituting the engineered *E. coli* strain. Unfortunately, cyclohexanone monooxygenase itself displays relatively low enantioselectivity for **3b**, which limits the utility of all routes based on this catalyst.

### Oxidations of dithiolanes using engineered baker's yeast and *E. coli* strains

Chiral sulfoxides derived from 1,3-dithiolanes are also valuable synthetic intermediates and several groups have investigated the use of cyclohexanone monooxygenase as a chiral oxidant for their synthesis. Purified cyclohexanone monooxygenase converts unsubstituted **4a** to the (*R*)-sulfoxide in 94% yield and >98% ee, although this is accompanied by 6% of sulfone **8a** (Scheme 2; Table 2).[25] Unfortunately, in this case, both the yeast and *E. coli* strains engineered to overexpress cyclohexanone monooxygenase afforded mainly the sulfone product. Control reactions in which the unmodified yeast and *E. coli* strains were grown in the presence of dithiolane **4a** or racemic sulfoxide **6a** showed no reaction (Table 2 and Scheme 3, respectively), demonstrating that the overoxidation observed was due to cyclohexanone monooxygenase. Since purified cyclohexanone monooxygenase oxidizes **6a** to sulfone **8a** more slowly than it converts **4a** to **6a**, we suspect that the rate of diffusion of sulfoxide **6a** across the cell membrane is relatively slow, which would allow **6a** to accumulate in the presence of the monooxygenase. A similar situation was found for methyl-substituted dithiolane **4b** (Table 2).

By contrast, both the engineered yeast and *E. coli* strains oxidized phenyl-substituted **4c** to the corresponding (*R*)-sulfoxide with high chemo- and diastereoselectivity (Table 2). Because native yeast enzymes also oxidized **4c** (Table 2), the engineered *E. coli* strain was preferred for this reaction. Native *E. coli* enzymes did not oxidize sulfoxide **6c** (Scheme 3). Here, however, as with phenyl-substituted dithiane **3b**, cyclohexanone monooxygenase displayed relatively low enantioselectivity for this substrate.

Using either the engineered yeast or *E. coli* strains, dimethyl-substituted dithiolane **4d** afforded the (*S*)-sulfoxide, in agreement with the results obtained by Pasta and coworkers using purified enzyme (Scheme 2; Table 2).[29] Reversals of stereoselectivity that accompany changes in substrate structure are well-precedented for cyclohexanone monooxygenase.[27] The enantioselectivities of oxidations using

**Table 2** Biocatalytic oxidations of dithiolanes

| | | | Sulfoxide **6** | | | Sulfone **8** | | |
|---|---|---|---|---|---|---|---|---|
| Substrate | Catalyst | **4 : 6 : 8**[a] | *trans* : *cis* | Yield[b] (%) | % ee | Yield[b] (%) | % ee | Reference |
| **4a** | Isolated enzyme | 0 : 94 : 6 | —[c] | 94 | >98 | 6 | — | 25 |
| | *Acinetobacter* | N.R.[d] | — | 71 | 95 | 16 | — | 30 |
| | *S. cerevisiae* 15C | 100 : 0 : 0 | — | — | — | — | — | This paper |
| | 15(pKR001) | 5 : 25 : 70 | — | 20 | 75[e] | 45 | — | This paper |
| | *E coli* BL21(DE3) | 100 : 0 : 0 | — | — | — | — | — | This paper |
| | BL21(DE3)(pMM4) | 0 : 15 : 85 | — | 13 | 86 | 47 | — | This paper |
| **4b** | Isolated enzyme | 0 : 94 : 6 | ⩾50 : 10 | N.R. | 50[f] | N.R. | N.R. | 29 |
| | *S. cerevisiae* 15C | 100 : 0 : 0 | — | — | — | — | — | This paper |
| | 15C(pKR001) | 1 : 22 : 77 | 10 : 1 | 16 | 20[e,f] | 15 | 76[e] | This paper |
| | *E. coli* BL21 (DE3) | 100 : 0 : 0 | — | — | — | — | — | This paper |
| | BL21(DE3)(pMM4) | 34 : 31 : 35 | 5 : 1 | 35 | 40[e,f] | 16 | 73[e] | This paper |
| **4c** | *S. cerevisiae* 15C | 81 : 19 : 0 | 1 : 2 | N.D.[g] | 70[f] | — | — | This paper |
| | 15C(pKR001) | 6 : 94 : 0 | 32 : 1 | 74 | 20[e,f] | — | — | This paper |
| | *E. coli* BL21(DE3) | 100 : 0 : 0 | — | — | — | — | — | This paper |
| BL21(DE3)(pMM4) | 13 : 87 : 0 | 40 : 1 | 60 | 20[e,f] | — | — | — | |
| **4d** | Isolated enzyme | 0 : 100 : 0 | —[c] | N.R. | 65 | — | — | 29 |
| | *S. cerevisiae* 15C | 100 : 0 : 0 | — | — | — | — | — | This paper |
| | 15(pKR001) | 0 : 86 : 14 | — | 84 | 48[e] | 10 | — | This paper |
| | *E. coli* (BL21(DE3) | 100 : 0 : 0 | — | — | — | — | — | This paper |
| | BL21(DE3)pMM4) | 9 : 67 : 24 | — | 46 | 69[e] | 6 | — | This paper |

[a] Determined by gas chromatography. [b] Isolated yield after chromatographic purification. [c] Not applicable. [d] Not reported. [e] Optical purities were determined by chiral-phase GC. [f] Optical puritiy of the major diastereomer. [g] Not determined

either the engineered yeast or *E. coli* strains were virtually identical to that observed for the purified monooxygenase. Over-oxidation to sulfone **6d** was a relatively minor side-reaction when the engineered yeast strain was employed.

## Conclusion

In summary, our results demonstrate that microbial strains genetically engineered to express cyclohexanone monooxygenase can be useful substitutes for the purified enzyme in thioether oxidations. These reactions are simple to perform and provide chiral sulfoxides with chemical and optical yields comparable to those obtained by traditional methods. The major difficulty associated with this approach is the interference of native enzymes that compete for the thioether substrate. However, by judicious choice of host cell (*S. cerevisiae* or *E. coli*), such problems can be minimized.

## Experimental

NMR spectra were obtained on Varian Unity 400, Bruker AMX 400 or Varian XL-200 instruments. All spectra were recorded in $CDCl_3$ solutions unless otherwise indicated and referenced to solvent ($\delta = 7.24$ for $^1H$ and 77.0 for $^{13}C$ spectra) or TMS. IR spectra were recorded from thin films on a Nicolet 520 FT-IR spectrophotometer. Optical rotations were measured on a Perkin Elmer 241 polarimeter operating at ambient temperature. All measurements were performed in chloroform solutions unless otherwise indicated. Packed column gas chromatography was performed on a Shimadzu GC-9A instrument equipped with a flame ionization detector and a custom-packed column (1/8″ × 1 m, 5% OV-101 on 100/120 Supelcoport, Supelco, Inc.) with helium as carrier gas. Capillary gas chromatography was performed on a Hewlett-Packard 5890 chromatograph employing a 0.54 mm × 15 m DB-1301 column (J&W, Inc.) or a Shimadzu GC-9A employing a 0.32 mm × 30 m ß-Dex 225 column (Supelco). The injector and detector temperatures were maintained at 225 °C. HPLC analyses were performed on a Beckman System Gold personal chromatograph using an Econosphere silica column (4.6 × 250 mm, Alltech) coupled with a Chiralcel OD-H column (4.6 × 150 mm, Daicel Chemical Industries, Ltd.) Mixtures of hexanes and 2-propanol were used as the mobile phase. Distillations were carried out using a Kugelrohr apparatus. Thin layer chromatography was performed on pre-coated silica gel 60 plates (Whatman). Flash chromatography was performed on silica gel (200–425 mesh, Fisher). Potassium carbonate was oven-dried at 80 °C and cooled to room temperature prior to use. Methylene chloride was dried over anhydrous $K_2CO_3$, distilled and stored over 3 Å molecular sieves. Other solvents were purified by fractional distillation. All other reagents were obtained from commercial suppliers and used as received.

### General procedure for synthesizing dithiolanes and dithianes

The aldehyde or ketone (20 mmol) and 1,2-ethanedithiol (20 mmol, 1.7 mL) was dissolved in 40 mL of $CH_2Cl_2$. The solution was cooled in an ice-water bath and $BF_3$–$OEt_2$ (7.9 mmol, 1.0 mL) was added dropwise. The cold bath was then removed and the reaction mixture was vigorously stirred for 1 h at room temperature. The mixture was poured into saturated $NaHCO_3$ and ice. The organic layer was washed thoroughly with saturated $NaHCO_3$ and the combined aqueous phases were extracted with $CH_2Cl_2$. The combined organic phases were dried over anhydrous $MgSO_4$ and concentrated by rotary evaporation. The residue was purified when necessary by vacuum distillation or flash chromatography on silica gel using a hexane–ethyl acetate mixture. Dithanes were synthesized in a similar manner except that 1,3-propanedithiol was used in place of 1,2-ethanedithiol.

### General procedure for yeast-mediated oxidations

To a 250-mL baffled Erlenmeyer flask was added 100 mL of YP-Gal medium (1% bacto-yeast extract, 2% bacto-peptone, 2% galactose), 100 μL of substrate (0.51–0.94 mmol, depending on the substrate), 3 μL of cyclohexanone and 0.2 g of frozen yeast cells.[37] If β-cyclodextrin was required, it was also added at this time.[46] The reaction mixture was shaken at 30 °C, 250 rpm until GC or HPLC analysis showed complete consumption of the starting material or an unchanged ratio of starting material and product over a 4 h time period. At this

time, the reaction mixture was centrifuged to remove yeast cells (5,000 × *g*, 10 min). The supernatant was saturated with NaCl and extracted with EtOAc (5 × 50 mL). The cell pellet was resuspended in *ca.* 20 mL of water and extracted twice with 50 mL of EtOAc. The combined organic extracts were washed once with brine, dried over anhydrous $Na_2SO_4$ and concentrated by rotary evaporator. The residue was purified by flash chromatography on silica gel using 3 : 1 hexanes–acetone as the eluant.

### General procedure for *E. coli*-mediated oxidations

Recombinant *E. coli* strain BL21(DE3)(pMM4) from a frozen stock was streaked on LB plates (1% bacto-tryptone, 0.5% bacto-yeast extract, 1% NaCl, 1.5% bacto-agar) containing 200 μg $mL^{-1}$ ampicillin, then the plate was incubated at 30 °C until colonies were 1–2 mm in size. A single colony from this plate was used to inoculate 10 mL of liquid LB medium (1% bacto-tryptone, 0.5% bacto-yeast extract, 1% NaCl) containing 200 μg $mL^{-1}$ ampicillin in a sterile 50-mL Erlenmeyer flask. After shaking at 37 °C, 250 rpm overnight, 1 mL of this saturated culture was used to inoculate 100 mL of liquid LB medium containing 200 μg $mL^{-1}$ ampicillin supplemented with 10 mL of 20% glucose in a 250-mL baffled Erlenmeyer flask. The culture was shaken at 37 °C, 250 rpm until the $OD_{600}$ was between 0.8 and 1.0. At this point, 10 μL of isopropyl-thio-β-D-galactoside (IPTG) stock solution (200 mg $mL^{-1}$) was added followed by 100 μL of substrate (0.51–0.94 mmol, depending on the substrate). If β-cyclodextrin was needed for the reaction, it was added at this point. The final reaction mixture was shaken at room temperature, 250 rpm until GC showed complete consumption of the starting material or when the ratio of product and starting material remained unchanged over a 4 h time period. At this time, the reaction mixture was saturated with NaCl and extracted with ethyl acetate (5 × 50 mL). The combined extracts were washed once with brine, dried over anhydrous $Na_2SO_4$ and concentrated by rotary evaporator. The residue was purified by flash chromatography on silica gel using 3 : 1 hexanes–acetone as the eluant.

### General procedures for monitoring biotransformations

For GC analysis, 100 μL aliquots of the reaction mixture were removed periodically and mixed with 100 μL of EtOAc, vortexed vigorously for 30 s and centrifuged at 7,500 rpm in a microcentrifuge for 1 min. The EtOAc also contained 100 ppm methyl benzoate as an internal standard. The organic phase was removed and analyzed by GC. For HPLC analysis, 2 mL aliquots of the reaction mixture were removed and extracted with an equal volume of EtOAc. The organic layer was then concentrated to one half to one third of its original volume by a stream of nitrogen, then an aliquot of the residue was analyzed by HPLC.

### General procedure for chemical oxidations

The sulfur compound (10 mmol) was dissolved in 50 mL of methanol and cooled to approximately 15 °C. To this was added $NaIO_4$ (11 mmol, 2.35 g) dissolved in 10 mL of warm water at a rate that kept the reaction temperature below 20 °C. After the addition was complete, the reaction was stirred for 30–90 min at room temperature until TLC analysis showed complete consumption of the starting material. The mixture was evaporated to near dryness, then the residue was diluted with distilled water and extracted with EtOAc or $CH_2Cl_2$. The combined organics were dried over $Na_2SO_4$ and concentrated by rotary evaporator. Pure products were obtained by flash chromatography on silica gel using 2 : 1 petroleum ether–acetone as the eluant.

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## References

1 G. Solladié, *Synthesis*, 1981, 185.
2 M. Mikolajczyk and J. Drabowicz, *Top. Stereochem.*, 1982, **13**, 333.
3 M. R. Barbachyn and C. R. Johnson, in *Asymmetric Synthesis*, ed. J. D. Morrison and J. W. Scott, Academic Press, New York, 1984. pp. 227–261.
4 G. H. Posner, *Acc. Chem. Res.*, 1987, **20**, 72.
5 H. L. Holland, *Chem. Rev.*, 1988, **88**, 473.
6 J. Drabowicz, P. Kielbasinski, and M. Mikolajczyk, in *The Chemistry of Sulfones and Sulfoxides*, ed. S. Patai, Z. Rappoport and C. J. M. Stirling, John Wiley and Sons, New York, 1988. pp. 233–278.
7 A. J. Walker, *Tetrahedron: Asymmetry*, 1992, **3**, 961.
8 E. G. Mata, *Phosphorus, Sulfur*, 1996, **117**, 231.
9 R. F. Bryan, F. A. Carey, O. D. Dailey, R. J. Maher and R. W. Miller, *J. Org. Chem.*, 1978, **43**, 90.
10 P. Pitchen, P. P. E. Duñach, M. N. Deshmukh and H. B. Kagan, *J. Am Chem. Soc.*, 1984, **106**, 8188.
11 H. B. Kagan, E. Duñach, C. Memecek, D. Pitcher, O. Samuel and S.-H. Zhao, *Pure Appl. Chem.*, 1985, **57**, 1911.
12 S.-H. Zhao, O. Samuel and H. B. Kagan, *Tetrahedron*, 1987, **43**, 5135.
13 F. A. Davis, R. ThimmaReddy and M. C. Weismiller, *J. Am. Chem. Soc.*, 1989, **111**, 5964.
14 V. K. Aggarwal, G. Evans, E. Moya and J. Dowden, *J. Org. Chem.*, 1992, **57**, 6390.
15 J.-M. Brunel, P. Diter, M. Duetsch and H. B. Kagan, *J. Org. Chem.*, 1995, **60**, 8086.
16 P. B. C. Page, R. D. Wilkes, E. S. Namiwindwa and M. J. Witty, *Tetrahedron*, 1996, **52**, 2125.
17 B. J. Auret, D. R. Boyd and H. B. Henbest, *J. Chem. Soc. C*, 1968, 2371.
18 H. L. Holland, H. Popperl and R. W. Ninniss, *Can. J. Chem.*, 1985, **63**, 1118.
19 H. Ohta, Y. Okamoto and G. Tsuchihashi, *Agric. Biol. Chem.*, 1985, **49**, 671.
20 J. Beecher, P. Richardson, S. Roberts and A. Willets, *Biotech. Lett.*, 1995, **17**, 1069.
21 D. R. Light, D. J. Waxman and C. Walsh, *Biochemistry*, 1982, **21**, 2490.
22 G. Carrea, B. Redigolo, S. Riva, S. Colonna, N. Gaggero, E. Battistel and D. Bianchi, *Tetrahedron: Asymmetry*, 1992, **3**, 1063.
23 F. Secundo, G. Carrea, S. Dallavalle and G. Franzosi, *Tetrahedron: Asymmetry*, 1993, **4**, 1981.
24 G. Ottolina, P. Pasta, G. Carrea, S. Colonna, S. Dallavalle and H. L. Holland, *Tetrahedron: Asymmetry*, 1995, **6**, 1375.
25 S. Colonna, N. Gaggero, A. Bertinotti, G. Carrea, P. Pasta and A. Bernardi, *J. Chem. Soc. Chem. Commun.*, 1995, 1123.
26 G. Ottolina, P. Pasta, D. Varley and H. L. Holland, *Tetrahedron: Asymmetry*, 1996, **7**, 3427.
27 S. Colonna, N. Gaggero, P. Pasta and G. Ottolina, *Chem. Commun.*, 1996, 2303.
28 V. Alphand, N. Gaggero, S. Colonna and R. Furstoss, *Tetrahedron Lett.*, 1996, 6117.
29 S. Colonna, N. Gaggero, G. Carrea and P. Pasta, *Tetrahedron: Asymmetry*, 1996, **7**, 565.
30 V Alphand, N. Gaggero, S. Colonna P. Pasta, and R. Furstoss, *Tetrahedron*, 1997, **53**, 9695.
31 S. Colonna, N. Gaggero, G. Carrea and P. Pasta, *Chem. Commun.*, 1997, 439.
32 For recent advances in NADPH regeneration technology applied to cyclohexanone monooxygenase, see F. Secundo, G. Carrea, S. Riva, E. Battistel and D. Bianchi, *Biotechnol. Lett.*, 1993, **15**, 865 and S. Rissom, U. Schwarlinek, M. Vogel, V. I. Tishkov and U. Kragl, *Tetrahedron: Asymmetry*, 1997, **8**, 2523.
33 J. D. Stewart, K. W. Reed and M. M. Kayser, *J. Chem. Soc., Perkin Trans. 1*, 1996, 755.

34 J. D. Stewart, K. W. Reed, J. Zhu, G. Chen and M. M. Kayser, *J. Org. Chem.*, 1996, **61**, 7652.
35 J. D. Stewart, K. W. Reed, C. A. Martinez, J. Zhu, G. Chen and M. M. Kayser, *J. Am. Chem. Soc.*, 1998, **120**, 3541.
36 M. M. Kayser, G. Chen and J. D. Stewart, *J. Org. Chem.*, 1998, **63**, 7103.
37 M. M. Kayser, G. Chen and J. D. Stewart, *Synlett*, 1999, 153.
38 J. D. Stewart, *Curr. Org. Chem.*, 1998, **2**, 211.
39 In the case of phenyl *n*-propyl sulfide, extended reactions in the presence of the engineered yeast strain gave only a low yield of the corresponding (*R*)-sulfoxide in 25% ee. Neither ethyl thiophenylacetate nor methyl β-(thiophenyl)ethyl ether afforded any sulfoxide products, even after extended reaction times. Moreover, when the racemic sulfoxides derived from these thioethers were incubated with the yeast strain, all traces of the sulfoxides disappeared within 78 h. We did not investigate the fates of these materials further.
40 G. J. Zylstra and D. T. Gibson, *Genetic Eng.*, 1991, **13**, 183.
41 C. C. R. Allen, D. R. Boyd, H. Dalton, N. D. Sharma, S. A. Haughey, R. A. S. McMordie, B. T. McMurray, G. N. Sheldrake and K. Sproule, *J. Chem. Soc., Chem. Commun.*, 1995, 119.
42 Details of this construction are provided as Supplementary Material.
43 Y.-C. J. Chen, O. P. Peoples and C. T. Walsh, *J. Bacteriol.*, 1988, **170**, 781.
44 S. D. Emr, *Methods Enzymol.*, 1990, **185**, 231.
45 Using racemic sulfoxide **5a** and purified cyclohexanone monooxygenase, Colonna and co-workers estimated an enantioselectivity value of 12 for the oxidation of **5a** to **7a**.
46 R. Bar, *Trends Biotechnol.*, 1989, **7**, 2.

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