CHARLES UNIVERSITY

Faculty of Science

Study Program: Organic Chemistry

Mgr. Vladimír Nosek

Biocatalytic Enantioselective Preparation of Chiral Sulfoxides

Biokatalytická enantioselektivní příprava chirálních sulfoxidů

Doctoral Thesis

Supervisor:

doc. RNDr. Jiří Míšek, Ph.D.

Prague, 2024

Declaration

I declare that I have completed this final thesis individually, under the supervision of doc. RNDr. Jiří Míšek, Ph.D., and that I have cited all the sources of information and literature used. This thesis, or any substantial part of it, has not been submitted for acquiring any other or the same academic degree.

Prohlášení

Prohlašuji, že jsem závěrečnou práci zpracoval samostatně, pod vedením školitele doc. RNDr. Jiřího Míška, Ph.D., a že jsem uvedl všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, dne 3.12.2024

Podpis:

Vladimír Nosek

Abstract

Chiral sulfoxides represent a class of compounds with significant biological activity, playing important role in various biochemical processes in numerous organisms and serve as active ingredients in prominent pharmaceuticals, such as Esomeprazole. Also, they have become valuable tools as chiral ligands, catalysts, and building blocks in asymmetric synthesis. Consequently, there is considerable interest in their enantioselective preparation. Despite the development of numerous methods for the asymmetric synthesis of chiral sulfoxides, general protocols that yield high enantiomeric excess (*ee* > 99%) for a variety of structurally different sulfoxides remain limited.

In this work, two innovative enzymatic protocols have been developed that are enantiocomplementary, enabling the synthesis of both (*R*)-sulfoxides and (*S*)-sulfoxides with high selectivity and enantiomeric excess through the process of kinetic resolution. The methods employ methionine sulfoxide reductase A (MsrA) for the preparation of (*R*)-sulfoxides and dimethylsulfoxide reductase (DmsABC) for (*S*)-sulfoxides. Both approaches demonstrated broad substrate scopes, allowing the synthesis of enantiopure sulfoxides that are otherwise challenging to obtain through conventional chemical or enzymatic routes. We also enhanced the enzymatic process by integrating a chemoenzymatic two-phase system using whole *E. coli* cells and an oxaziridine-based lipophilic oxidant, effectively extending the limits of enzymatic kinetic resolution with a robust deracemization protocol. Furthermore, employing a novel mutant of methionine sulfoxide reductase, we successfully expanded the substrate scope, addressing some of the limitations associated with natural enzyme.

Key words:

Biocatalysis, chiral sulfoxides, deracemization, kinetic resolution, sulfoxide reductases.

Abstrakt

Chirální sulfoxidy představují třídu sloučenin s významnou biologickou aktivitou, které hrají důležitou roli v různých biochemických procesech u mnoha organismů a slouží jako aktivní složky v prominentních farmaceutických přípravcích, například v Esomeprazolu. Rovněž se staly cennými nástroji ve využití jako chirální ligandy, katalyzátory a stavební bloky v asymetrické syntéze. Z tohoto důvodu je o jejich enantioselektivní přípravu značný zájem. Navzdory vývoji mnoha metod pro asymetrickou syntézu chirálních sulfoxidů zůstávají obecné protokoly, které by poskytovaly vysoký enantiomerní přebytek (*ee* > 99 %) pro širokou škálu strukturálně odlišných sulfoxidů, omezené.

V této práci byly vyvinuty dvě inovativní enzymatické metody, které jsou enantiokomplementární, což umožňuje syntézu jak (*R*)-sulfoxidů, tak i (*S*)-sulfoxidů s vysokou selektivitou a enantiomerním přebytkem prostřednictvím procesu kinetické rezoluce. Tyto metody využívají methioninsulfoxidreduktázu A (MsrA) pro přípravu (*R*)-sulfoxidů a dimethylsulfoxidreduktázu (DmsABC) pro přípravu (*S*)-sulfoxidů. Oba přístupy prokázaly široký rozsah substrátů, což umožňuje syntézu enantiomerně čistých sulfoxidů, které je obtížné získat běžnými chemickými nebo enzymatickými metodami. Enzymatický proces byl dále zdokonalen integrací chemoenzymatického dvoufázového systému využívajícího celé buňky *E. coli* a lipofilního oxaziridinového oxidantu, čímž byly účinně překonány fundamentální limity enzymatické kinetické rezoluce pomocí robustního protokolu deracemizace. Dále, použití nového mutantního enzymu methioninsulfoxidreduktázy nám dovolilo úspěšně rozšířit spektrum substrátů a překonat některá omezení spojená s přírodním enzymem.

Klíčová slova:

Biokatalýza, chirální sulfoxidy, kinetická rezoluce, deracemizace, sulfoxidreduktázy.

List of Publications

Publications based on this doctoral thesis:

1. Nosek V., Míšek J. Chemoenzymatic Deracemization of Chiral Sulfoxides. *Angew. Chem. Int. Ed.* **2018**, vol. 57, 9849-9852.

2. Nosek V., Míšek J. Enzymatic kinetic resolution of chiral sulfoxides – an enantiocomplementary approach. *Chem. Commun.* **2019**, vol. 55, 10480-10483.

3. Tarallo V., Kasireddy S., Nosek V., Míšek J. Development of a simple high-throughput assay for directed evolution of enantioselective sulfoxide reductases. *Chem. Commun.* **2020**, vol. 56, 5386-5388.

Additional publications not included in this doctoral thesis:

4. Makukhin, N.; Nosek, V.; Míšek, J. Development of a Ratiometric Fluorescent Probe with Two -Reactive Sulfoxides for Monitoring the Activity of Methionine Sulfoxide Reductase A. *Synthesis* **2018**, *50* (4), 772–777.

5. Tokarenko, A.; Nosek, V.; Míšek, J. Design, Synthesis, and Evaluation of Probes for Spatially Resolved Imaging of Enantioselective Sulfoxide Reductases. *J. Org. Chem.* **2022**, *87* (2), 1585–1588.

6. Jabczun, M.; Nosek, V.; Míšek, J. Complementary Strategies for Synthesis of Sulfinamides from Sulfur-Based Feedstock. *Org. Biomol. Chem.* **2023**, *21* (14), 2950–2954.

7. Nosek, V.; Míšek, J. Sulfinamide Crossover Reaction. *J. Org. Chem.* **2024**, *89* (11), 7927–7932.

Table of Contents

List of Abbreviation

A^-	anion
Ac	acetyl
aq	aqueous
AMP	ampicillin
Ar	aryl or aromate
API	active pharmaceutical ingredient
ATPase	adenosinetriphosphatase
BINOL	1,1'-bi-2-naphthol
Bn	benzyl
Bu	butyl
t-Bu	*tert*-butyl
BVMO	Baeyer-Villiger oxidase
BODIPY	4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene
C	conversion
CHMO	cyclohexanone monooxygenase
CHP	cumyl hydroperoxide
Cm	Chloramphenicol
COX-2	cyclooxygenase-2 (prostaglandin-endoperoxide synthase 2)
Cys	cysteine
δ	chemical shift in parts per million
d	doublet or days
D	dextrorotatory
DCM	dichloromethane
DET	diethyl tartrate

DIOP	2,3-*O*-isopropylidene-2,3-dihydroxy-1,4-bis(diphenylphosphino)butane
DMF	*N,N*-dimethylformamide
DmsABC	dimethylsulfoxide reductase
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
n-Dodec	dodecyl
dr	diastereomeric ratio
DRIFT	diffuse reflectance infrared Fourier transform
DTE	dithioerithritol
DTT	dithiothreitol
E^+	electrophile
EDG	electron donating group
ee	enantiomeric excess
e.g.	*exempli gratia*, for example
eq. or equiv.	equivalent
Et	ethyl
ESI	electrospray ionization
EWG	electron withdrawing group
FAD	flavin adenine dinucleotide
FG	functional group
Glu	glutamic acid
GST-tag	glutathione S-transferase tag
h	hour
HAMPO	hydroxyacetophenone monooxygenase
Hept	heptane
His-tag	polyhystidine tag, 6xhistidine

HOMO	highest occupied molecular orbital
HPLC	high performance liquid chromatography
HSAB	hard and soft acids and bases
HRMS	high resolution mass spectrometry
IPA	isopropylalcohol
IPTG	Isopropyl-ß-D-thiogalactopyranoside
IR	infrared spectroscopy
Kan	kanamycin
L	levorotatory
LA	Lewis acid
LB	Lysogeny broth or Luria-Bertani medium
LDA	lithium diisopropylamide
LEW buffer	Lysis-Equilibration-Wash buffer
LG	leaving group
*LG	chiral leaving group
LUMO	lowest unoccupied molecular orbital
q	quartet
rt	room temperature
m	multiplet
M	protein mass marker
MALDI	matrix-assisted laser desorption ionization
m-CPBA	*m*-chloroperbenzoic acid
Me	methyl
Met	methionine
MetSO	methionine sulfoxide
min	minute

MS	mass spectrometry or molecular sieves
MsrA	methionine sulfoxide reductase A
MsrB	methionine sulfoxide reductase B
MW	microwave
MWCO	molecular weight cut-off
NADH	nicotinamid adenine dinucleotid
NADPH	nicotinamide adenine dinucleotide phosphate
Naph	naphtyl
NCS	*N*-chlorsuccinimide
n.d.	not determined
NMP	*N*-methylpyrrolidone
NMR	nuclear magnetic resonance
NSAIDs	non-steroidal anti-inflammatory drugs
Nu	nucleofile
P	product
n-Pent	pentyl
Ph	Phenyl
Phe	Phenylalanine
ppm	parts per million
Pr	propyl
i-Pr	isopropyl
R	“Rest of the molecule”, “residue” = substituent
R	*rectus*
Rf	*retention factor*
rac	racemic
ROS	reactive oxygen species

rt	room temperature
S	*sinister*
s	singlet
salan	reduced salen ligand
salen	N,N-bis(salicylidene)ethylenediamine
SDS-PAGE	sodium dodecyl sulfate - polyacrylamide gel electrophoresis
Sec	selenocysteine
S_N2	bimolecular nucleophilic substitution
t	triplet
t or temp	temperature
TBHP	tert-butyl hydroperoxide
TCEP	tris(2-carboxyethyl)phosphine
TET	tetracycline
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	tetramethylsilan or trimethylsilyl
TOF	time of flight
Tol	tolyl
tr	time of retention
Tris	tris(hydroxymethyl)aminomethane
Trp	tryptophan
Trx	thioredoxin
TrxR	thioredoxin reductase
Ts	*p*-toluensulfonyl - "tosyl"
Tyr	tyrosine

UV	ultraviolet
VIS	visible
v/v	volume per unit volume

"*Improvise, adapt, overcome.*"

Bear Grylls, adventurer and wilderness survival expert

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1. Introduction

1.1. Chiral Sulfur Compounds

Stereogenic centers in organic compounds are often located on the carbon atom with four different substituents. However, different atoms can serve as stereogenic centers in organic compounds. Most commonly sulfur and phosphorus can provide configurationally stable stereogenic centers. Chiral organic sulfur compounds represent a diverse class that carries chirality on the sulfur atom in various oxidation states (+II, +IV; +VI) (Figure 1).[1–4] These compounds have experienced renewed interest in organic chemistry in recent years. One of the most important functional groups of these compounds is sulfoxides which contain chiral sulfur in +IV oxidation state. One reason for their growing appeal is their presence in many successful medications, where they serve as active components. Also, their widespread use as chiral auxiliary groups, ligands, and intermediates makes them attractive targets for applications in asymmetric synthesis.

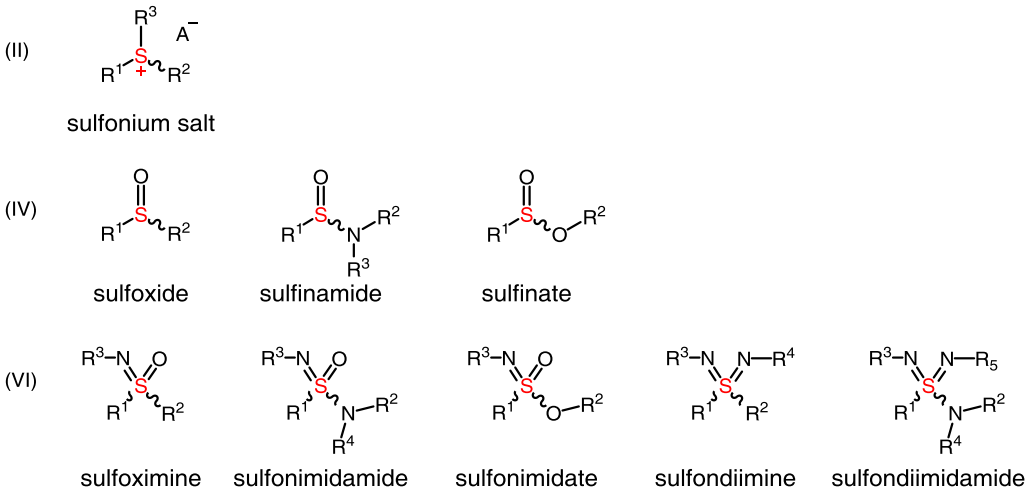


Figure 1: Several examples of chiral compounds containing sulfur stereogenic centers at different oxidation states.

Sulfoxides adopt tetrahedral topology around the central sulfur atom (Figure 2). Provided the two C-substituents are not identical, the sulfoxides are configurationally stable chiral compounds. Typical energy barrier for racemization is in the range of 35-43 kcal mol^{-1}. Thus, racemization of sulfoxides can occur thermally only at high temperatures, usually exceeding 200°C.[5,6]

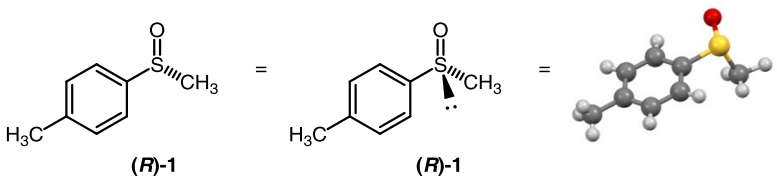
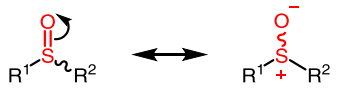


Figure 2. The structural formula of (R)-methyl-p-tolyl sulfoxide and the corresponding X-ray crystal structure.[7]

The nature of the sulfinyl group, sulfur-oxygen double bond (S=O) in sulfoxides, is not formed by a typical π bond as in the case of carbon-oxygen bond (C=O), because oxygen contributes electrons from a non-bonding electron pair to the d-orbital of sulfur, an overlap occurs, and the result is a d_{π}-p_{π} bond. The sulfoxide bond should be rather understood more accurately as a partial double bond, which is represented by two resonance structures (Scheme 1).[5] For simplicity, only one of these representations is chosen, which may vary in notation in different publications. One of the characteristics of the S=O bond is that it is highly polarized with a large dipole moment, giving it a dipolar aprotic feature.[8,9] This is reflected in the much higher polarity of sulfoxide compounds in comparison with the corresponding sulfones or sulfides.



Scheme 1. Different resonance structures of sulfoxides.

The electronic structure of sulfoxides is responsible for their Lewis basicity.[10,11] This is related to the energy level of the HOMO and LUMO molecular orbitals (Figure 3). The HOMO is predominantly located on the sulfur and oxygen atoms, acting as a nucleophilic site.[12] Conversely, the LUMO is situated on the sulfur atoms and adjacent carbon atoms, giving it an electrophilic character that makes it susceptible to nucleophilic attack.[12]

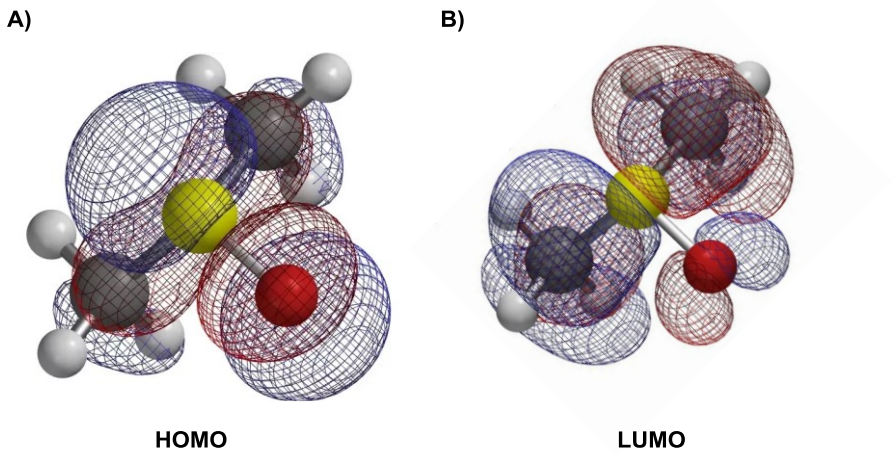


Figure 3. Molecular orbitals of DMSO. A) highest occupied molecular orbital (HOMO) B) lowest unoccupied molecular orbital (LUMO).[12]

1.2. Natural Chiral Sulfoxides with Biological Activity

Sulfoxides have been found in a number of natural products, and many of them exhibit significant biological activity. An interesting example is the highly poisonous α-amanitin **2**, which is an active ingredient of a death cap mushroom poison (Figure 4).[13] α-Amanitin is a bicyclic peptide that contains a chiral sulfoxide moiety in the bridging region. α-Amanitin inhibits RNA polymerase II by non-covalent binding under ‘‘bridge helix’’ between pol II subunits, Rpb1 and Rpb2.[14] Interestingly, the natural (*R*)-sulfoxide stereoisomer is approximately 20 times more toxic than its (*S*)-analog that is believed to be due to the difference in a 3D structure.[15] Thanks to its high toxicity to eukaryotic cells, it is a promising agent for antibody-drug conjugates.[16] Therefore, effective total synthesis was also developed.[17,18]

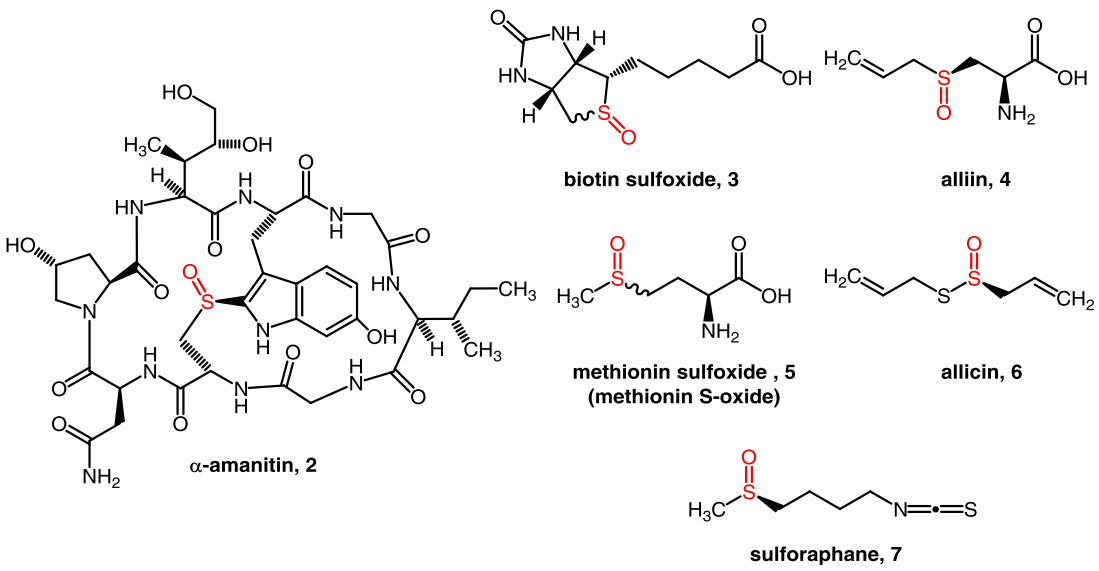


Figure 4. Examples of biologically active compounds bearing chiral sulfoxide moiety commonly present in nature.

A number of chiral sulfur compounds participate in many processes of general metabolism and might be involved in the maintenance of homeostasis.[5,19–21] This includes examples of oxidized derivatives which originated from biotin (biotin sulfoxide **3**), cysteine (allin **4**), or methionine (methionine sulfoxide **5**), etc. (Figure 4). Methionine sulfoxide (MetSO) **5** can be found both in a free form (in plasma) and bound in a number of proteins, where it can perform important functions. Allicin **6** is a substance with antimicrobial and antifungal effects, which imparts a characteristic odor of garlic and originates from its chiral sulfoxide precursor allin **4.** Alliin is enzymatically cleaved by the enzyme alliin lyase (4.4.1.4) as self-defense of plants, common for garlic or onion by squeezing or slicing.[22,23] The other example includes sulforaphane **7**, which has an antibacterial effect and is also being tested for its anti-cancer activity (Figure 4).[24]

1.3. Chiral Sulfoxides as Active Pharmaceutical Ingredients

Chiral sulfoxides have been used with great success in the pharmaceutical industry, where a number of these biologically active sulfoxides were among the best-selling drugs.[25] A prime example is Esomeprazole **8,** which was the second best-selling drug in 2014 (over $6.1 billion in sales) (Figure 5).[26] Esomeprazole is a proton pump inhibitor (H^+/K^+ ATPase blocker) and is used to treat stomach and intestinal ulcers.[27–29] The active substance of Esomeprazole is enantiomerically pure (*S*)-sulfoxide, which in clinical studies showed significantly higher efficacy and bioavailability

than the racemic analog.[30,31] This example demonstrates that the absolute configuration of chiral sulfoxides in biologically active substances can play a major role.

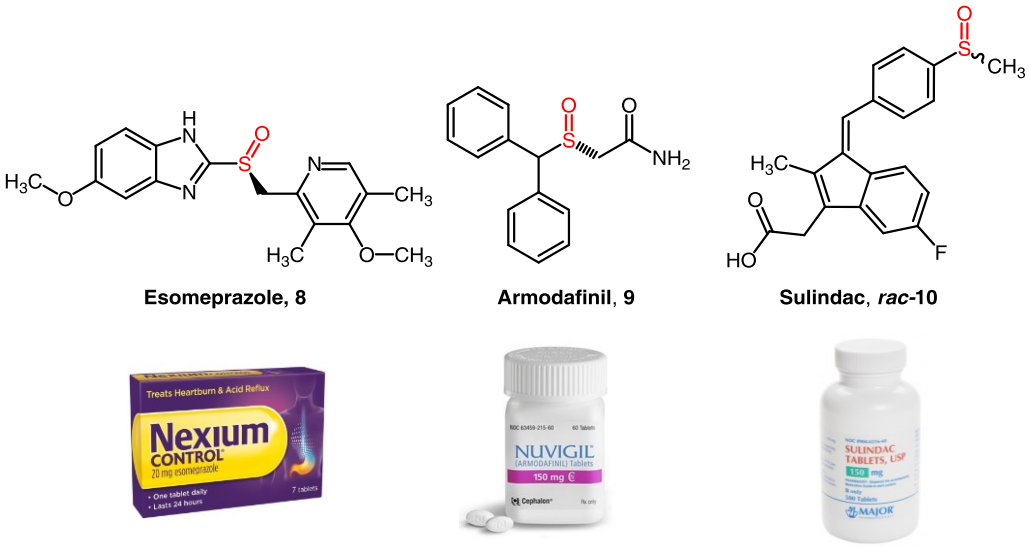


Figure 5. Examples of marketed drugs with a chiral sulfoxide moiety.

Another example of a successful drug containing chiral sulfoxide is Armodafinil **9**, which is used as a stimulant drug against sleep disorders like narcolepsy (Figure 5).[32,33] It has been shown that the (*R*)-enantiomer has a significantly longer biological half-life and therefore can operate much longer in the body than the complementary (*S*)-enantiomer, which is rapidly metabolized.[34] Interestingly, the first large-scale resolution of the racemic drug was achieved by HPLC chromatography with a chiral stationary phase that helped accelerate the drug registration process.[35] Sulindac **10** is a non-steroidal anti-inflammatory drug (NSAID) inhibiting prostaglandin-endoperoxide synthase 2 (COX-2) (Figure 5).[36,37] It is marketed in a racemic form. The active form of the drug is sulfide, which is obtained by enzymatic reduction of the sulfoxide prodrug with complex pharmacokinetics.[38] The NSAID activity itself is unaffected by the sulfoxide absolute configuration, however, it has been demonstrated that the (*S*)-enantiomer has a stronger ability to induce the P450 enzyme system as compared to the (*R*)-enantiomer.[39] Therefore, the (*R*)-enantiomer may offer a better safety profile due to these differences in metabolism.[39] It also showed a significant anticancer activity when used alongside other therapeutics.[40,41] Interestingly, ingestion in combination with DMSO decreases the plasma level of the Sulindac sulfide and causes peripheral neuropathy, suggesting these are competitive substrates for enzymatic reduction.[42–44] A further illustration of the increasing importance of sulfoxides in the pharmaceutical industry is demonstrated by other drugs and drug candidates (Figure 6).

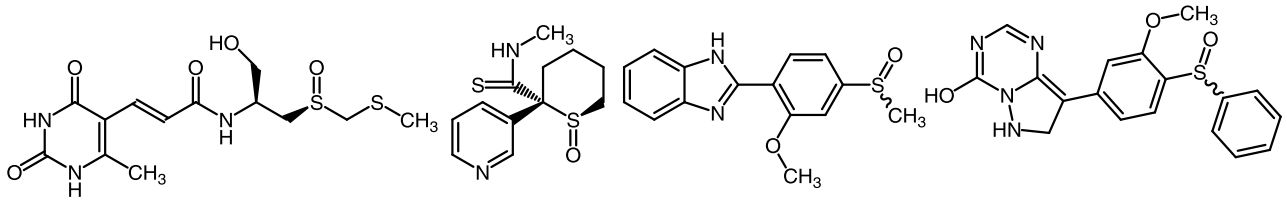
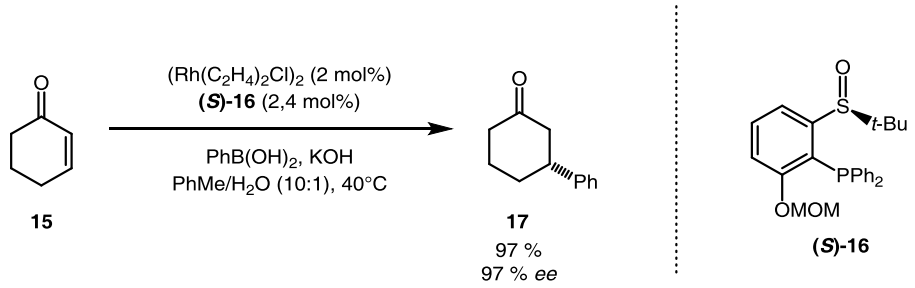


Figure 6. Other examples of bioactive sulfoxides. Aprikalim[45], Sparsomycin[46], Sulmazole[47], BOF-4272.[48]

1.4. Chiral Sulfoxides in Organic Synthesis

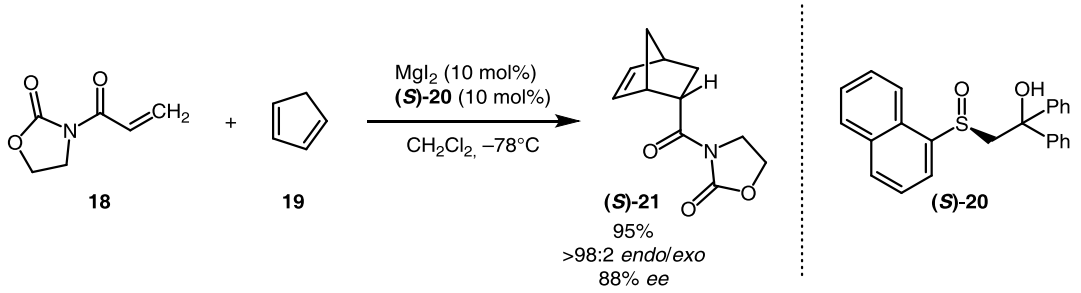
1.4.1. Sulfoxides as Chiral Ligands

Sulfoxide moiety can be coordinated with metals either through oxygen or sulfur atom. This interaction can be rationalized according to Pearson's HSAB theory where hard oxygen coordinates to hard metals and soft sulfur to soft metals.[49–52] This may result in different metal complex characteristics and functionalities. An example of the successful application of sulfoxides as a ligand is a rhodium-catalyzed conjugate addition to unsaturated ketones (Scheme 2). The authors utilized a relatively simple ligand **16**, where the only carrier of chiral information is the sulfinyl group. Good to excellent yields and enantioselectivities were achieved.



*Scheme 2. Asymmetric conjugate addition utilizing chiral sulfoxide **16** as a ligand.*

Another example of sulfoxides acting as ligands is their application in asymmetric catalysis for Diels-Alder reactions. Hydroxysulfoxide **(*S*)-20** forms a Mg(II) complex that catalyzes reactions with substrates **18** and **19** to yield major product ***endo*-21** with high enantioselectivity (Scheme 3).[53]



Scheme 3. Lewis acid-catalyzed Diels-Alder reaction with a sulfoxide ligand.

Ligands bearing one or more sulfoxide groups have recently become a successful and popular design motif in a wide range of reactions (Figure 7).[11,54–56]

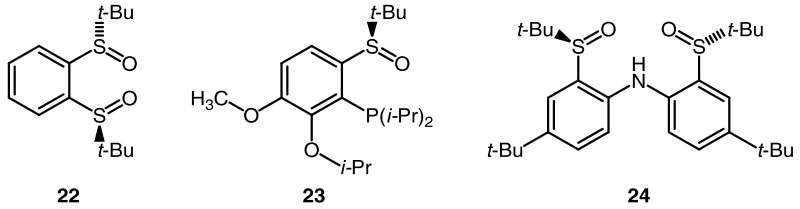
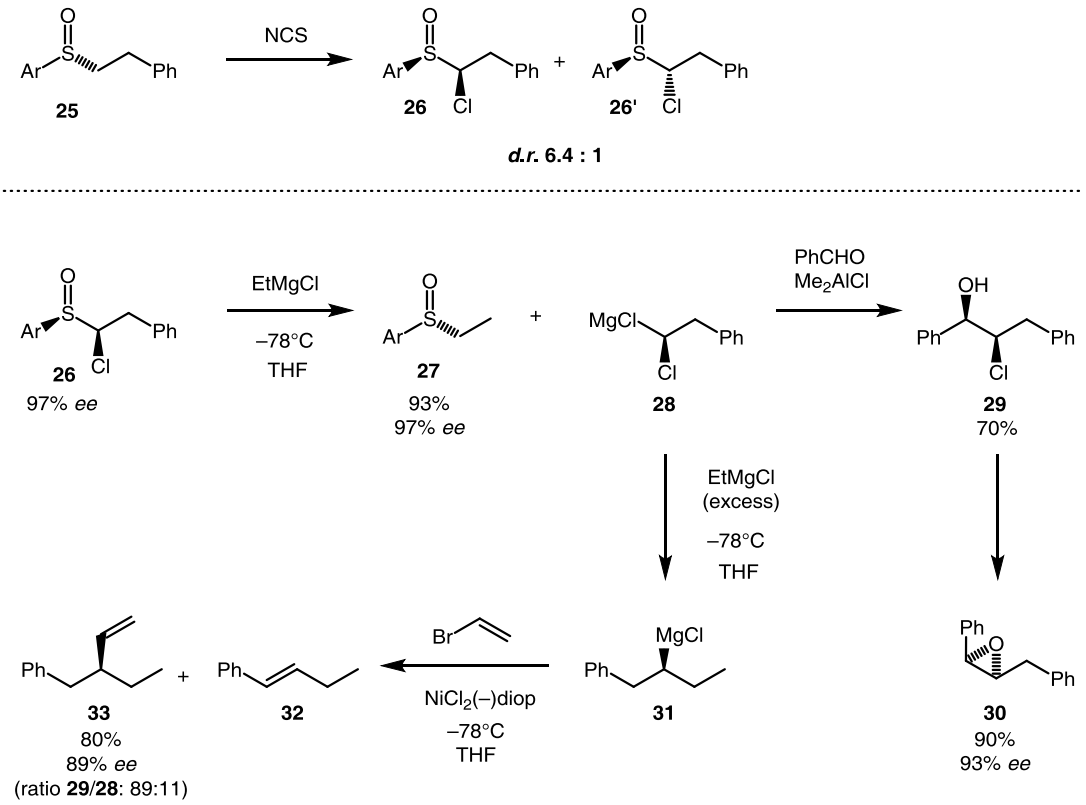


Figure 7. Examples of other sulfoxide ligands used in asymmetric catalysis.

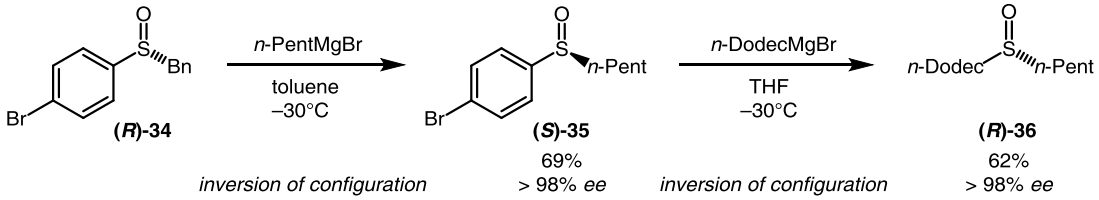
1.4.2. Sulfoxides As Chiral Auxiliaries and Chiral Synthetic Precursors

Chiral sulfoxides are also widely used as chiral auxiliary groups in a large variety of reactions due to their efficient chirality transfer. An interesting application of chiral sulfoxides is in the unusual preparation of enantioenriched Grignard reagents (Scheme 4).[57] The enantiopure sulfoxide **25** can be chlorinated in the α-position to form a diastereomeric mixture, where the main diastereoisomer **26** can be separated by recrystallization.[58] The reaction of an intermediate **26** with EtMgCl proceeds as a magnesium/sulfinyl exchange and the formation of a new chiral Grignard reagent **28** and enantioenriched sulfoxide **27** is observed.[57–60] The chiral Grignard reagent **28** can be utilized for stereoselective Kumada–Corriu cross-coupling reaction to get alkenes **32** and **33**,[60] or directly quenched by addition to aldehyde leading to asymmetric formation of chlorohydrin **29**, which is subsequently transformed to an epoxide **30**.[57,58] Chirality transfer is achieved with high selectivity in all cases.



*Scheme 4. The preparation of an enantioenriched Grignard reagent **28** and **31** from a chiral sulfoxide **26**.*

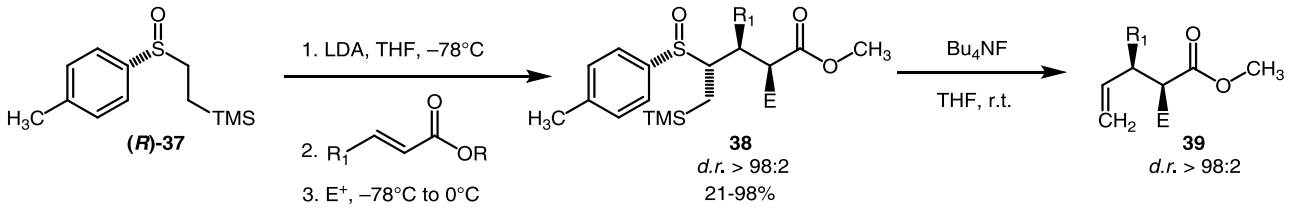
Interestingly, certain chiral sulfoxides can be utilized for the enantioselective preparation of new sulfoxide compounds with different C-substituents.[61] This strategy employs an unusual carbanion as the leaving group. In a sequential two-step reaction, sulfoxide **34** was firstly reacted with *n*-pentylmagnesium bromide to substitute the benzyl group, producing intermediate **35**. Subsequent addition of *n*-dodecylmagnesium bromide to this intermediate, substituting 4-bromophenyl group, resulted in sulfoxide **36** (Scheme 5).[62] The opposite enantiomer was obtained by simply changing the order of Grignard reagent addition. Despite achieving high *ee* values, the method is limited to aliphatic Grignard reagents. Success depends on pK_a differences between the nucleophile and leaving groups, influencing regioselectivity and *ee*.[58,61,63,64]



*Scheme 5. Stereoselective sytnthesis of new sulfoxides by nucleophilic substitution on a precursor sulfoxide **34**.*

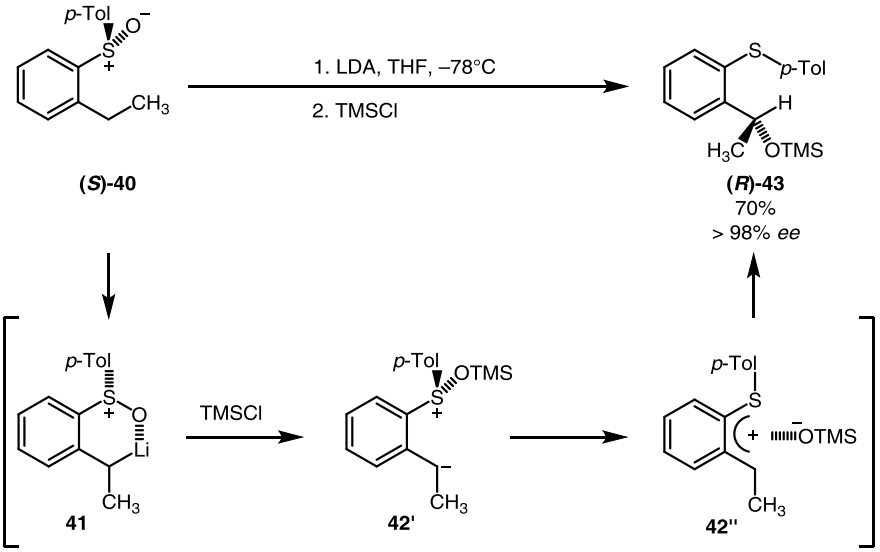
Because sulfoxide is an electron-accepting group, it can stabilize the negative charge on the α-carbon. Sulfoxides significantly increase the acidity of the adjacent hydrogens (pK_a ~ 33).[65,66] This

characteristic led to the development of a whole range of synthetic applications with optically active α-sulfinyl carbanions.[65,66] They found use in asymmetric substitutions and additions to activated double bonds or aldehydes. Sterically bulky substituents on the sulfinyl group are usually required to maintain high stereoselectivities. An example of a successful application reaching high stereoselectivity is depicted in Scheme 6.[67] The deprotonated α-position of sulfoxide **37** nucleophilically attacks an α,β-unsaturated ester, followed by the reaction of the resulting enolate with an electrophile. This process forms a new molecule **38** with three new stereogenic centers, exhibiting complete stereoselectivity. Desulfenylation/elimination with Bu_4NF yielded product **39** quantitatively and verified the stereochemistry of the prior addition reaction.[67]



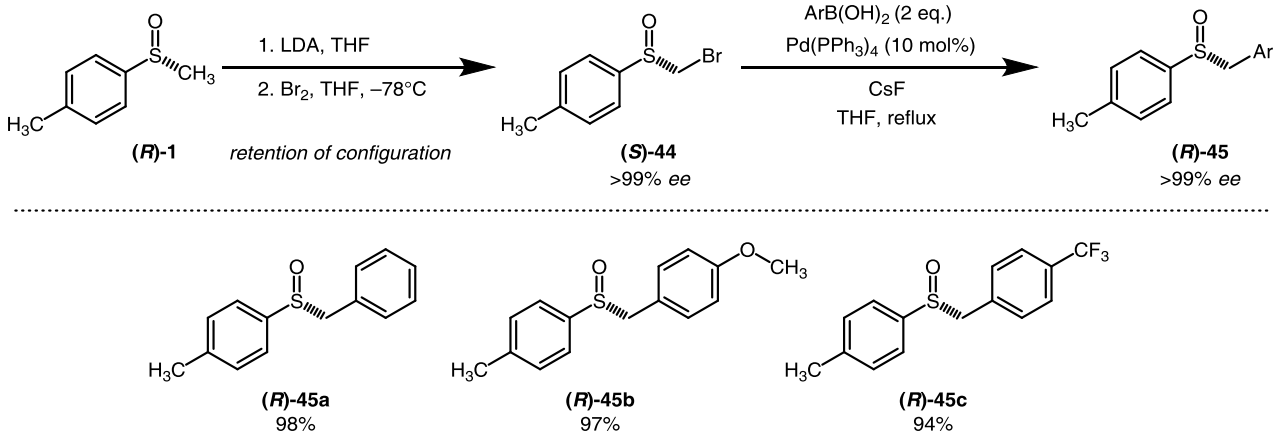
Scheme 6. An example of Michael addition where sulfoxide acts as chiral nucleophile.

Chiral sulfoxides may be used as precursors in various types of rearrangement reactions or (hetero)Diels-Alder reaction.[68] There are some examples where chirality has been transferred from sulfur to carbon enantiospecifically with high *ee* values.[69–71] Illustrating sulfur-to-carbon chirality transfer, Scheme 7 shows a vinylogous Pummerer rearrangement that performs a selective 1,4-oxygen migration when ortho-sulfinyl benzyl carbanion **41** encountered trimethylsilyl halide, the reaction produced trimethylsilylether **42** with high enantiomeric excess, which is easily deprotected to benzyl alcohol with NH_4Cl.[70]



Scheme 7. Enantiomerically pure chiral sulfoxide in rearrangement reaction with the transmission of chiral information to the product.

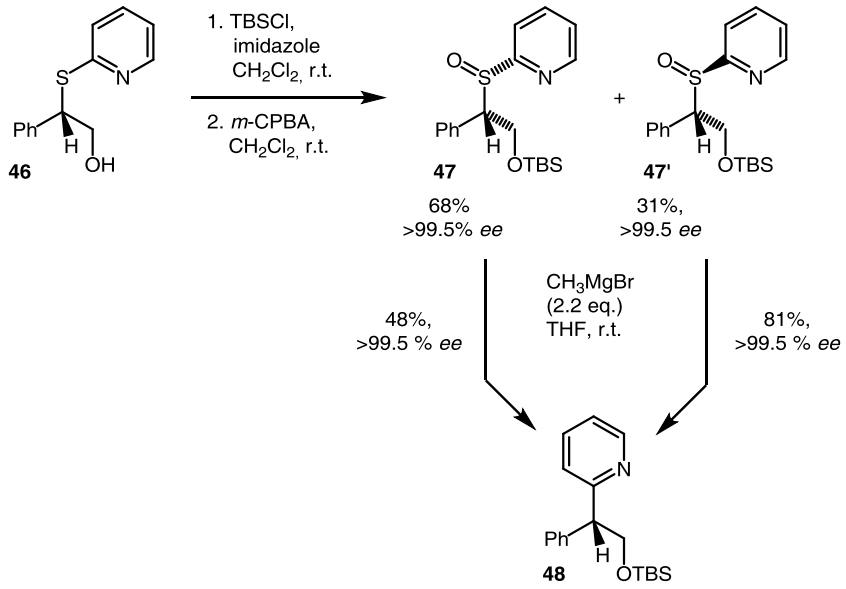
Chiral sulfoxides play an important role as starting materials in many synthetic applications. Enantiopure α-halosulfoxides can react in traditional palladium-catalyzed couplings (Scheme 8).[72] Benzylic sulfoxides **45** were obtained with preservation of chiral information with *ee* > 99%.



Scheme 8. Palladium-catalyzed Suzuki coupling of enantiopure α-halosulfoxides.

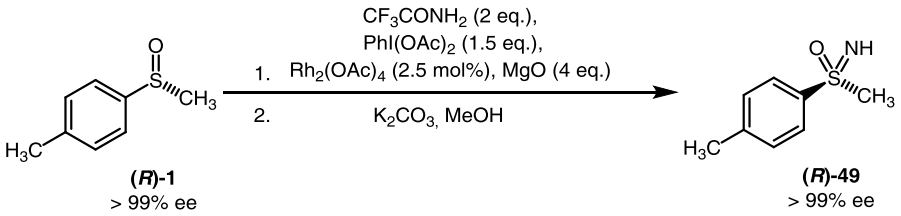
Heterocyclic aromatic sulfoxides can be utilized for C-C bond-forming reactions without any transition metal catalyst. It is hypothesized that it may proceed via a stabilized hypervalent sulfur intermediate.[73–75] Recently, a broad range of valuable heterocyclic building blocks was obtained using a procedure that starts with sulfinyl chloride and involves two consecutive Grignard additions, followed by the formal extrusion of SO, leading to the formation of a new C-C

bond on the sp^2 carbons.[73] Similarly, heteroaryl sulfoxides **47** or **47'** were utilized in stereoretentive coupling reaction for the preparation of (hetero)diaryl alkanes **48** with high *ee* up to 99.5% (Scheme 9).[76]



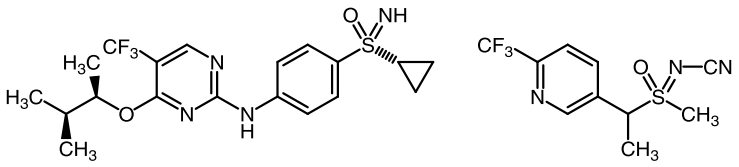
Scheme 9. Grignard reaction on heteroaromatic sulfoxides with formal SO extrusion leading to the formation of the new C(sp^2)-C(sp^3) bond.

Moreover, chiral sulfoxides can be transformed into higher oxidation state sulfur compounds, such as sulfoximines.[77] Typically, this transformation uses nitrene (metalonitrene) chemistry. Nitrene is usually generated *in situ* from hypervalent iodo-compounds in the presence of a rhodium or copper catalyst (Scheme 10).[78,79] Alternatively, chloramines and azides can also be used for this type of transformation.[77,80]



Scheme 10. Chiral sulfoximines are synthesized via rhodium-catalyzed nitrene transfer to sulfoxides.

These chiral compounds offer a unique 3D tetrahedral topology enabled by another substituent (=NR). The new acidobasic characteristics and spatial arrangement can be used to further refine the physiological and biological characteristics of active pharmaceutical ingredients (APIs) such as Roniciclib **50** or crop protection agents such as Sulfoxaflor **51** (Scheme 11).[77,81,82]



50
Roniciclib
(pan-CDK Inhibitor candidate)

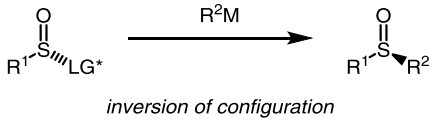
51
Sulfoxaflor
(insecticide)

Scheme 11. Biologically active sulfoximines derived from chiral sulfoxides, Roniciclib and Sulfoxaflor.

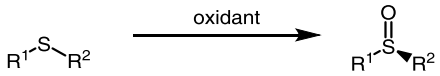
1.5. Enantioselective Preparation of Chiral Sulfoxides

Chiral sulfoxides are typically prepared asymmetrically through three main methods (a-c), and other methods (d) (Scheme 12):

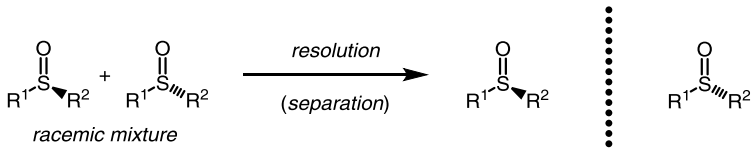
a) nucleophilic substitution of diastereomerically pure sulfinates



b) enantioselective oxidation of prochiral sulfides



c) kinetic resolution of racemic sulfoxides

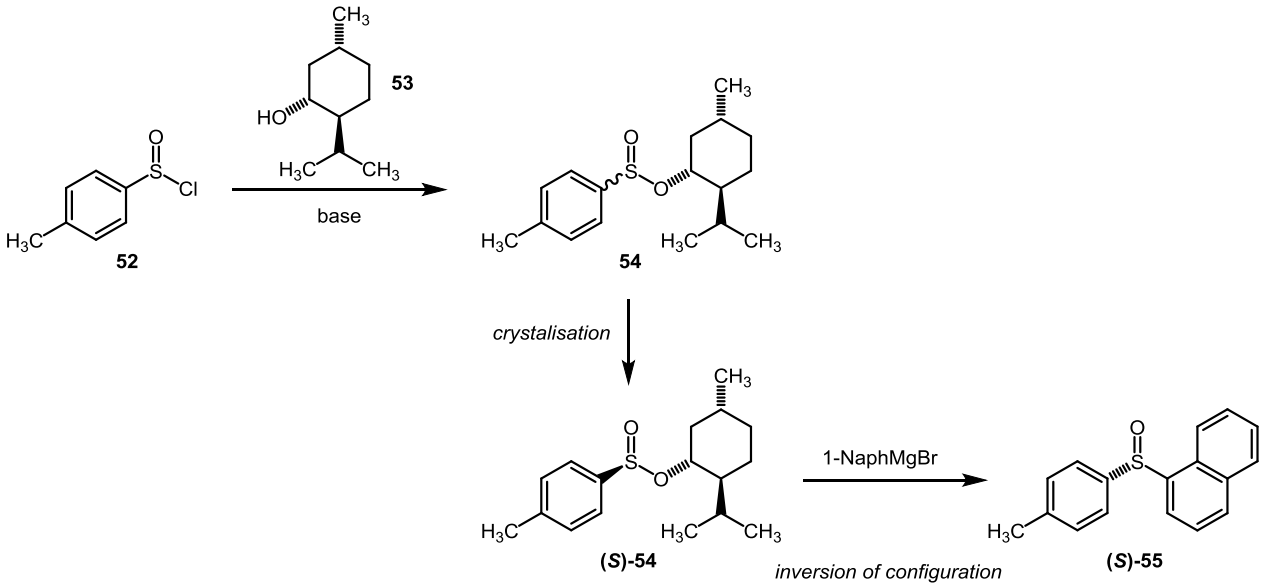


d) other methods

Scheme 12. Current key approaches in asymmetrical preparation of chiral sulfoxides.

1.5.1. Nucleophilic Substitution of Diastereomerically Pure Sulfinates

One of the first methods for asymmetric preparation of chiral sulfoxides was a procedure developed by Andersen.[83,84] This approach is based on nucleophilic substitution of diastereoisomerically pure sulfinates with organometallic reagents (Scheme 13). Sulfinyl chloride **52** reacts with a chiral alcohol **53** to form a chiral sulfinate **54**. The resulting diastereomers of **54** are separated and undergo nucleophilic substitution with an organometallic reagent to form sulfoxide **55** stereoselectively.



Scheme 13. Preparation of chiral sulfoxides by nucleophilic substitution method.

Ever since the inception of this method, numerous modifications have been developed.[85–94] Figure 8 highlights some of the most popular chiral precursors (**56**, **57**, and **58**) used in the synthesis of enantioenriched sulfoxides. While this approach can deliver chiral sulfoxides with good to excellent enantiomeric excess (*ee*), it has several limitations. The process relies on optically pure starting materials, requiring complex optimization and often challenging purification steps, such as multiple crystallizations or chromatography. Additionally, the preparation of alkyl alkyl sulfoxides can be difficult or impractical due to the challenge of separating optically pure sulfinates from oily mixtures. A further common issue is the inability to obtain the minor diastereomer in sufficient quantity or quality to prepare the complementary sulfoxide enantiomer. Further methodological improvements, such as the introduction of a chiral sulfite **59** or sulfimidate precursors **60** and **61** addressed some of these issues.

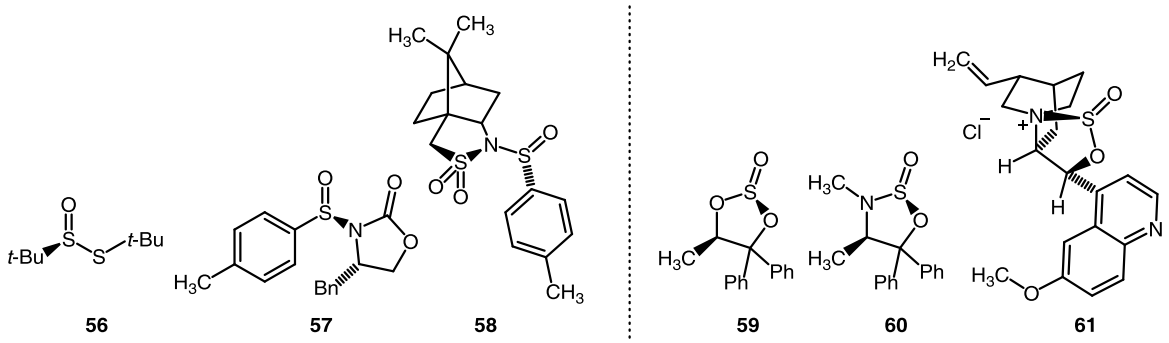
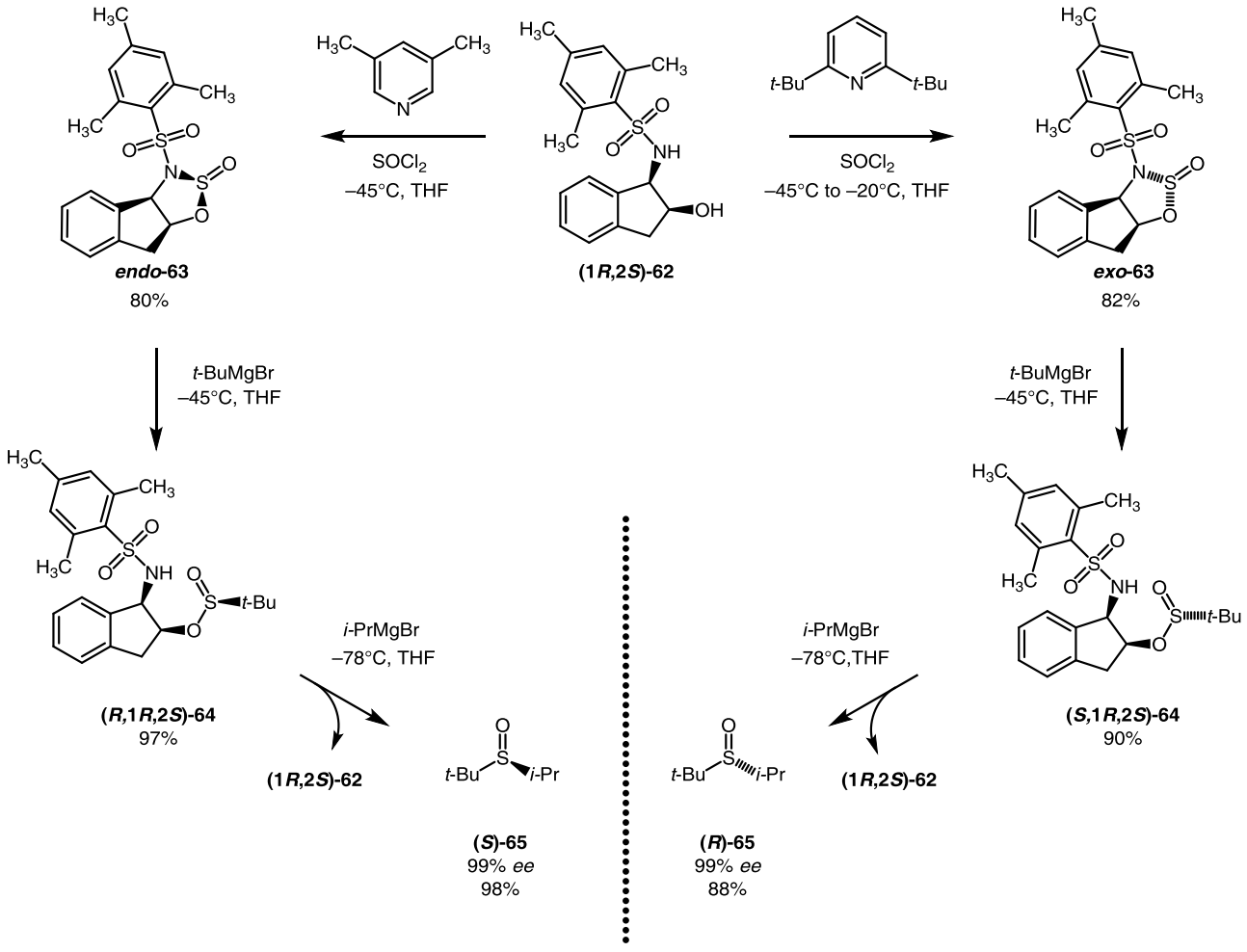


Figure 8. Examples of chiral synthons used for the preparation of sulfoxides by nucleophilic substitution.

Probably, the most universal protocol was developed by the group at Sepracor (Scheme 14).[89] This approach effectively employs diastereomerically pure oxathiazolidine-S-oxides derivatives ***endo*-63** and ***exo*-63**, which are derived from the starting material **(*1R,2S*)-62**. During the reaction with thionyl chloride, the choice of base determines the reaction pathway, guiding it towards either the *endo* or *exo* product. A subsequent nucleophilic substitution with a Grignard reagent causes the sulfonamide group to leave, and an inversion of configuration occurs at the sulfur center, consistent with an S_N2 reaction, though some literature also suggests a possible addition-elimination mechanism.[91] The addition of the second Grignard reagent provides the resulting chiral sulfoxide. This method has been shown to produce various chiral sulfoxides with high enantiomeric excess values and relatively high yields.[89] The main advantage of the method is that it produces both enantiomers **65** from a single chiral synthon **(*1R,2S*)-62**.



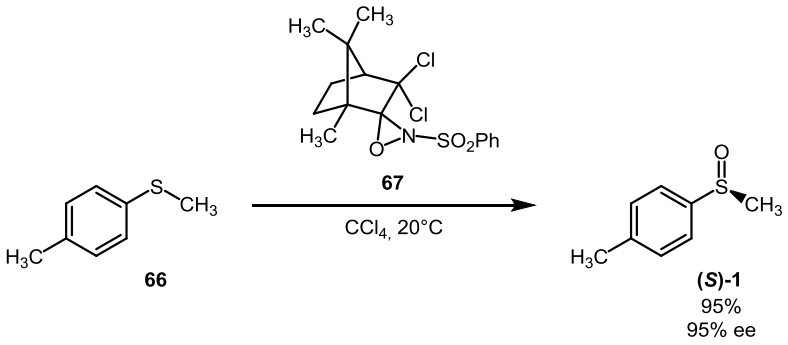
Scheme 14. Complementary asymmetric pathways to both sulfoxide enantiomers.

1.5.2. Asymmetric Oxidation of Prochiral Sulfides

The asymmetric oxidation of prochiral sulfides is currently one of the most utilized methods for the preparation of chiral sulfoxides. The pros and cons of both stoichiometric and catalytic methods will be discussed in the following section.

1.5.2.1. Stochiometric oxidation

The first efforts to asymmetrically oxidize sulfides to sulfoxides were undertaken with chiral peroxyacids as the oxidant. However, rather low stereoselectivity was observed.[95] Davis introduced chiral oxaziridines that proved more effective due to their electrophilicity and steric hindrance[96–100]. Later, it was reported the application of sulfamyloxaziridines, which are easier to prepare, provide chiral sulfoxides with *ee* up to 68%.[101] Subsequent studies introduced oxaziridine **67**, which has proven effective for enantioselective oxidation of sulfides. (Scheme 15).[102]



*Scheme 15. Enantioselective oxidation of sulfides with oxaziridine **67**.*

Another improvement of the method was achieved through the *in situ* generation of chiral oxaziridines from chiral imines (Figure 9).[103–105]

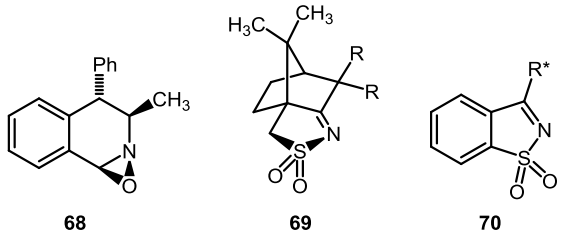
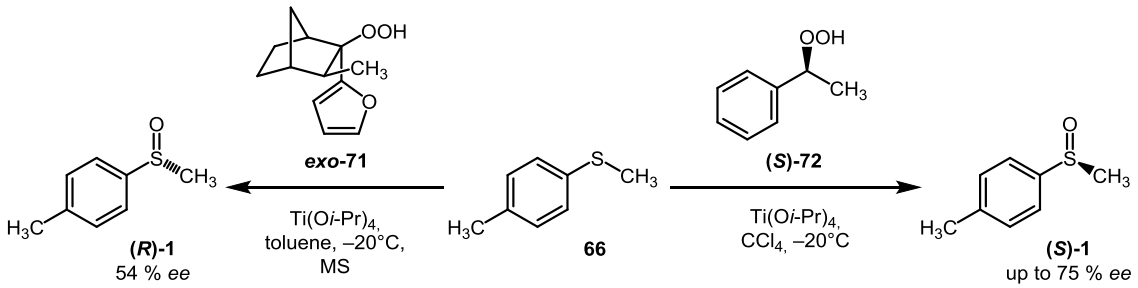


Figure 9. Examples of chiral oxaziridine oxidants and imine precursors used for in situ generation of oxidants.[104–106]

Chiral peroxides **71** and **72** were used as chiral selectors for asymmetric oxidation mediated by transition metals (Scheme 16).[107–111] Part of the process involved kinetic resolution through the selective overoxidation of one enantiomer to sulfone, enhancing the *ee*.[108–111]

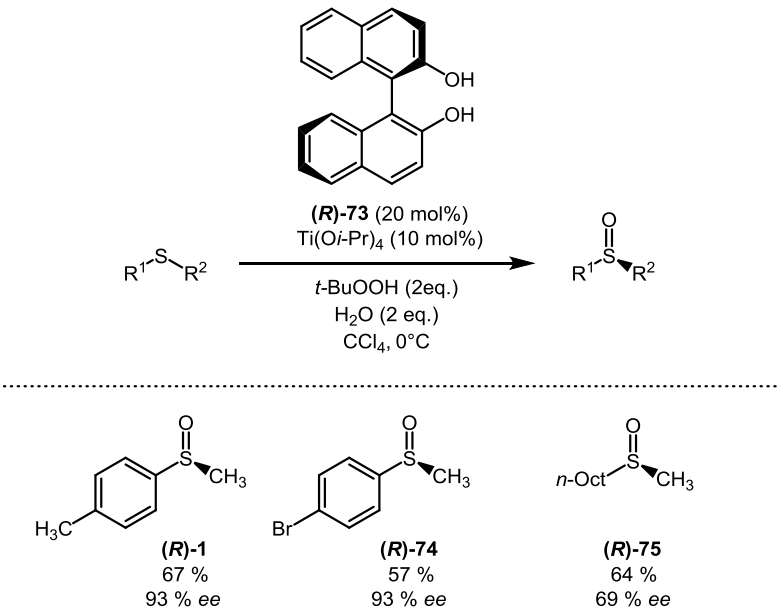


Scheme 16: Chiral peroxides as a chiral source for asymmetric oxidation with titanium catalyst.

1.5.2.2. Chemocatalytic oxidation

The most well-studied method for asymmetric catalytic oxidation of prochiral sulfides relies on catalysis by transition metal complexes.[112] There were two original stochiometric protocols that were developed independently by Kagan and Modena.[113–115] Both authors utilized the principle of Sharpless's catalysis for asymmetric epoxidation. In the beginning, both methods used a stoichiometric amount of a titanium catalyst ($Ti(OR)_4$, where R = Et, *i*-Pr), diethyl tartrate (DET), serving as a chiral auxiliary group, and an oxidizing agent (*tert*-butyl hydroperoxide (TBHP) or cumene hydroperoxide (CHP)).

The major progress was the discovery that it is sufficient to use only a substoichiometric amount of a transition metal as a catalyst.[54,116] This significantly shifted the efficiency of synthesis and thus interest in this class of compounds. Extensive research has enhanced these approaches, focusing on the use of a wide range of chiral ligands, like chiral diols or triols (Figure 10).[54,116–121] Notable examples are BINOL from Uemura's modification (Scheme 17).[122,123] Various oxidants, including peroxides and peroxyacids, were also employed.[54,112,116]



Scheme 17. Modification of the original method of preparing chiral sulfoxides, using a catalytic amount of Ti-catalyst.

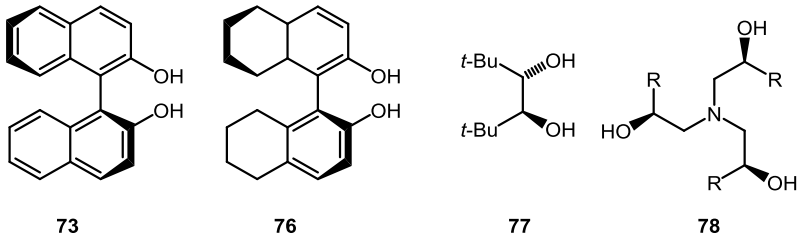
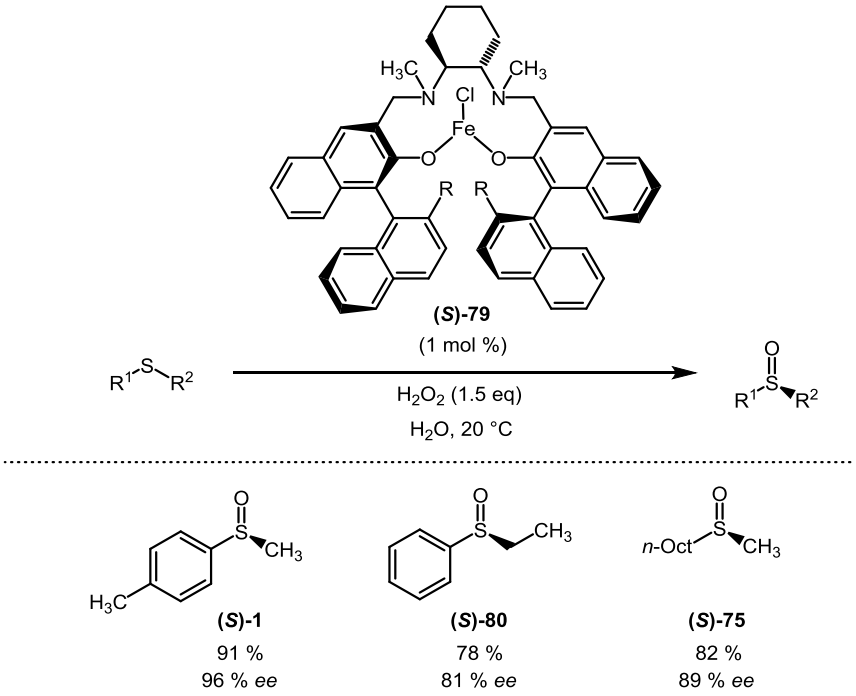


Figure 10. A few examples of chiral ligands for Ti-catalyzed asymmetric oxidation of sulfides.

Overoxidation to sulfone is a common phenomenon in these reactions. Interestingly, some protocols have leveraged this tendency to improve *ee* values by designing kinetic resolution conditions that exploit selective overoxidation.[124–126] In such cases, one enantiomer is preferentially oxidized to the sulfone, leaving the other intact, which leads to a reduction in overall yield but enhances enantiopurity.[54,109,125–127] However, this also necessitates the separation of the sulfone byproduct. Consequently, the overall outcome of oxidation reactions catalyzed by transition metals often combines both asymmetric oxidation and kinetic resolution strategies.

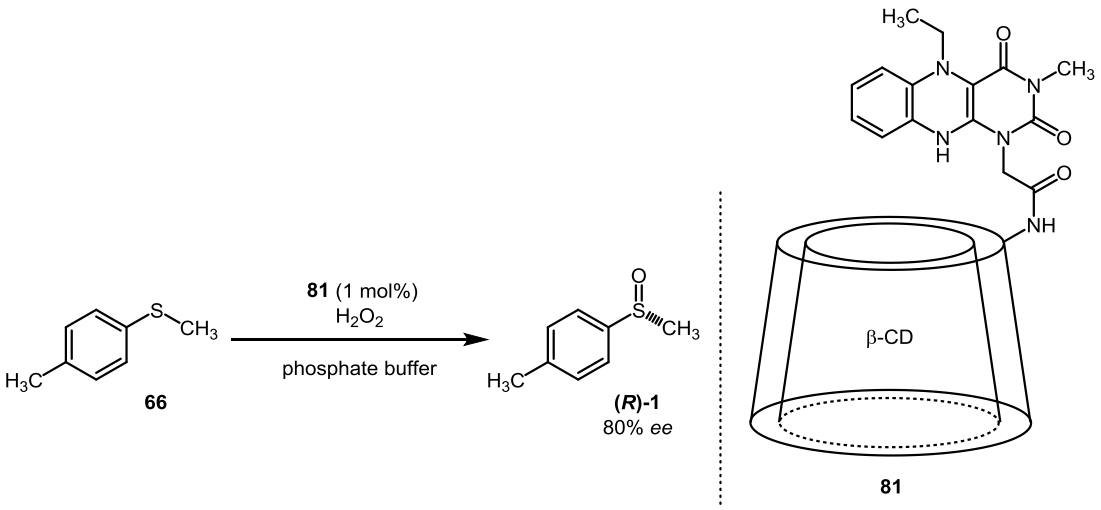
Modern versions of the oxidation reaction use complexes based on Schiff bases,[128–130] and salen/salan-type ligands,[131,132] as well as other metals such as vanadium,[133,134] manganese,[135,136] and iron.[137–139] A highly efficient catalytic system for oxidizing prochiral sulfides was reported by Katsuki (Scheme 18).[139] This method achieves enantioselective oxidation with just 1 mol % of catalyst **79** and inexpensive hydrogen peroxide as a terminal oxidant.[139] It should be noted that

despite the formidable progress of these chemocatalytic methods, substrates bearing two sp^3 carbons adjacent to the sulfur atom remain a major challenge in terms of enantioselectivity.



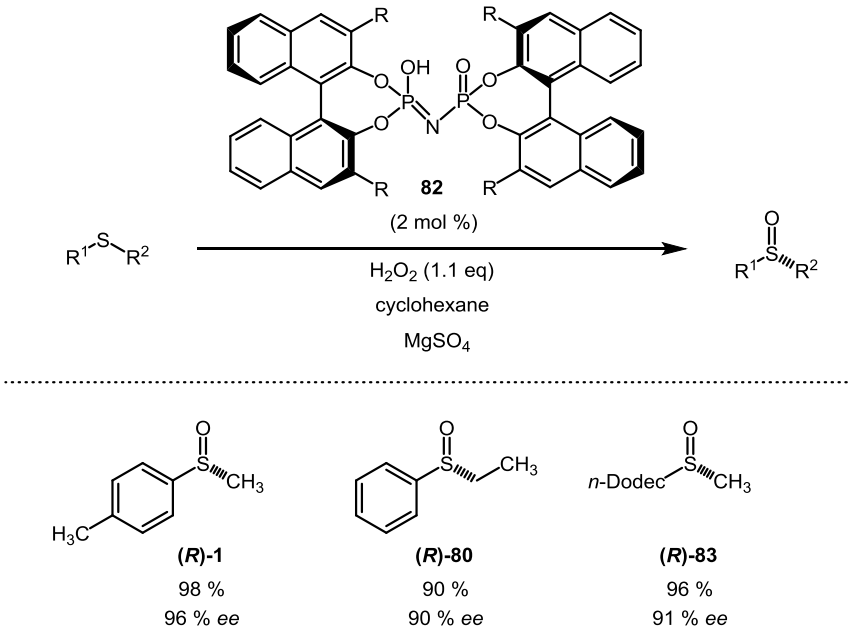
Scheme 18. The salan-based iron catalyst for asymmetric oxidation of prochiral sulfides.

Organocatalysis is another modality that can be utilized for asymmetric oxidations of sulfides.[127] A number of reports took inspiration from natural flavin-containing oxidases.[140–142] Chiral flavins based on paracyclophane chiral units were designed, however, the stereoselectivity achieved was only modest.[127,140] Later, other modifications of planarly chiral flavin salts with increased steric demands and hydrophobic π-π interactions, reached up to 60% *ee*.[140,143] An interesting approach was reported by Cibulka et. al. that covalently linked flavin derivatives with chiral cyclodextrins. Enantioselectivities achieving up to 80% *ee* were obtained, using low catalyst loadings (Scheme 19).[144,145]



Scheme 19. Efficient organocatalyst using flavin-like cofactor merged with cyclodextrin.

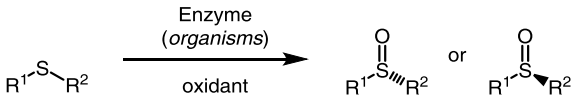
List's group developed a highly efficient organocatalytic method for enantioselective chiral sulfoxide preparation.[146] The activation of hydrogen peroxide with a strong chiral Brønsted acid **82** provided a deep chiral pocket that preorganized sulfide substrates for highly enantioselective oxidation. This method achieved excellent *ee* values (up to 98%) and high yields across various substrates and was utilized in the synthesis of the drug (*R*)-Sulindac (Scheme 20).[146] Many other organocatalytic asymmetric sulfoxidations have been described and are detailed in several comprehensive reviews.[112,127]



Scheme 20. Chiral Brønsted acid-catalyzed asymmetric oxidation of sulfides.

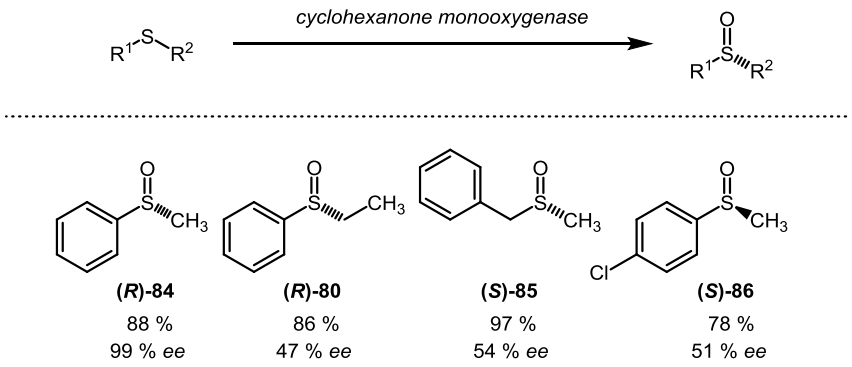
1.5.2.3. Biocatalytic oxidation

Enzymes or whole organisms provide a powerful strategy for asymmetric oxidations and can be utilized for the preparation of chiral sulfoxides (Scheme 21).[147] Methods of recombinant DNA and DNA amplification along with the recent progress in *in vitro* evolution of enzymes fueled the large-scale deployment of enzymes in organic synthesis.[148,149]



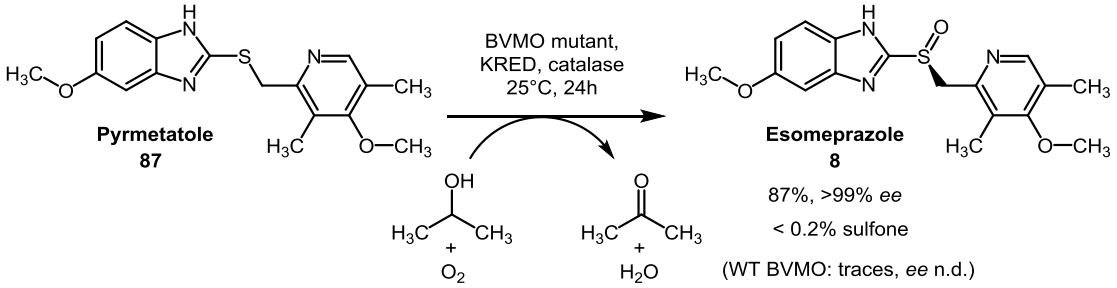
Scheme 21. General scheme of biocatalytic oxidation.

Various oxidases have been used for the asymmetric oxidation of prochiral sulfides to sulfoxides.[147,150,151] Enzymes classes commonly used include flavin monooxygenases (FMO), haloperoxidases, and P-450 cytochromes and other heme-dependent enzymes.[147] Specific examples of natural or evolved enzymes used for this transformation include horseradish peroxidase (HRP),[152] haloperoxidases,[153–155] cyclohexanone monooxygenase (CHMO),[156] 4-hydroxyacetophenone monooxygenase (HAMPO),[157] flavoprotein monooxygenase,[158] lacto-peroxidase, lignin-peroxidase, Baeyer-Villiger oxidase (BVMO),[159,160] myoglobin,[161] and various dioxygenases, e.g. toluene and naphthalene dioxygenase.[162] Recent advances in computational tools and predictive algorithms offer new approaches for the enzyme development, however, challenges related to a narrow substrate scope and enantiocomplementarity remain.[147,156,163,164] For instance, oxidation of prochiral sulfides with CHMO enzyme can provide high yields and enantioselectivity with some model substrates, however relatively small structural changes in the substrate may lead to a significant drop in enantioselectivity or even its reversal (Scheme 22).[156] An enantiocomplementary approach often necessitates the use of entirely different enzymes for each enantiomer, making the development process both time-consuming and costly.



Scheme 22. Biocatalytic oxidation of prochiral sulfides catalyzed by the promiscuous enzyme cyclohexanone monooxygenase.

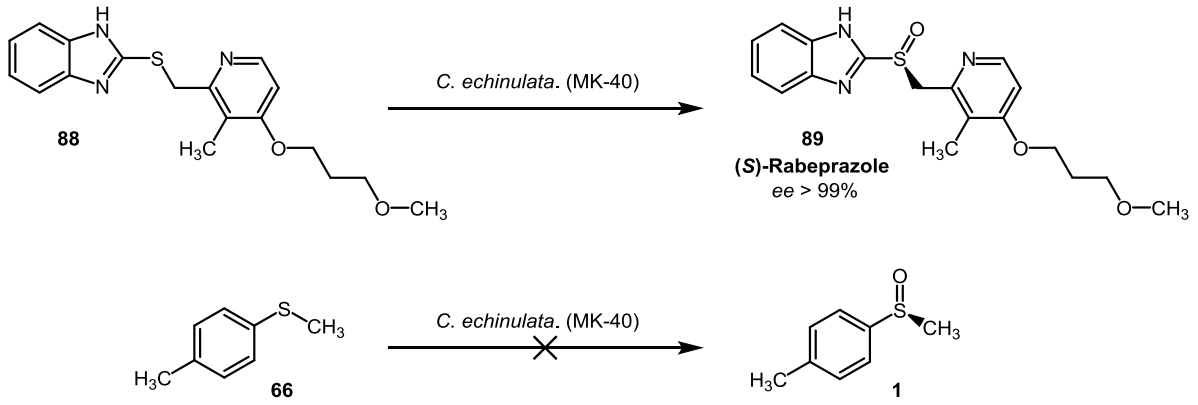
Directed evolution of enzymes enables to tune of the particular enzyme for a given substrate. This feature leads to increasing popularity in industrial applications as it provides an environmentally benign and cost-effective way for the preparation of fine chemicals.[165,166] A prime example is the enzymatic oxidation of Pyrmetazole **87** to Esomeprazole **8** used for the large-scale preparation of the drug. 19 rounds of directed evolution of the natural BVMO enzyme yielded a variant that has an improved activity by 5 orders of magnitude providing the product with a high yield and excellent enantioselectivity (Scheme 23).[160,167]



Scheme 23. Biocatalytic method for the preparation of Esomeprazole using an evolved variant of BVMO enzyme.

Whole-cell catalysis is sometimes preferred over isolated enzymes for stability and cost reasons. Various bacterial,[168] yeast,[169] and fungi strains[170,171] are commonly used for recombinant expression of enzymes since they are relatively easy to cultivate.[147,172,173] The choice of expression system often hinges on factors like growth rate, replication speed, and environmental and nutrient requirements specific to the chosen media. Additionally, factors like cofactor and metabolite availability are crucial for enzyme production, as well as the structure and stability of the target enzyme itself. Another consideration is laboratory setup, including available tools and equipment, familiarity with established protocols, and access to suitable vectors or plasmids for the expression system. On the other hand, bioprospecting for unique strains or organisms, such as extremophiles

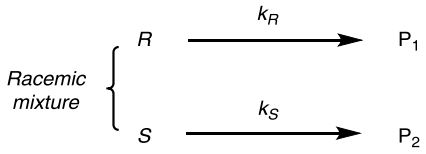
or those from polluted areas, can uncover new systems with improved selectivity and novel reactivity. For example, fungi mold *Cunninghamella echinulata* (MK-40) showed high *ee* (>99%) for (*S*)-Rabeprazole **89** synthesis, though with a narrow substrate range (Scheme 24).[174]



*Scheme 24. The whole organism asymmetric oxidation of Rabeprazole using the strain Cunninghamella echinulata (MK-40). The narrow substrate scope limits its application to other compounds such as **66**.*

1.5.3. Kinetic Resolution of Chiral Sulfoxides

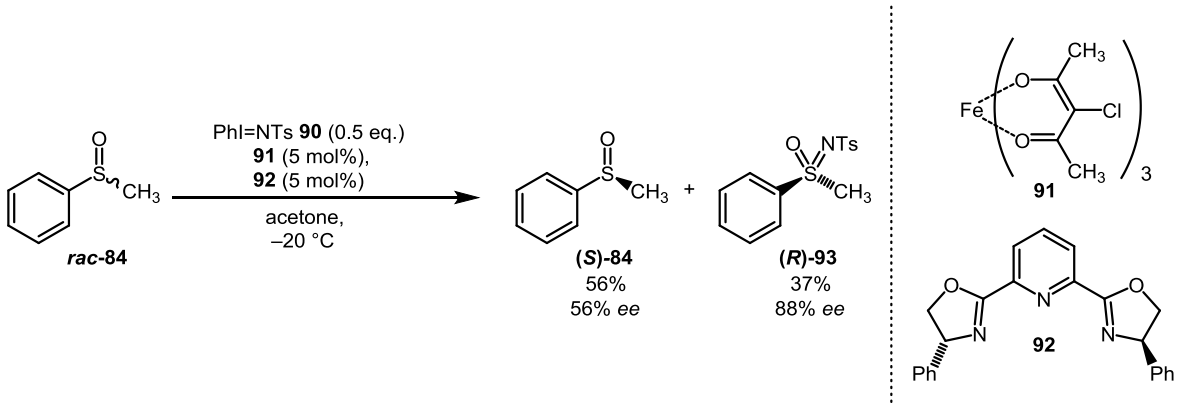
Kinetic resolution involves a chemical process where one enantiomer of a racemic mixture reacts at a faster rate than the other.[175] This preferential reaction results in the conversion of one enantiomer to a product (**P_1** or **P_2**) (e.g. if (*R*)-enantiomer would react faster than *S*: $k_R > k_S$) (Scheme 25).[175] The efficiency of kinetic resolution is characterized by the stereoselective factor *s*, which is defined as a ratio between reaction rate constants (eq.1). Selectivity factor can be also derived from the conversion (*C*) of the reaction and *ee* of the substrate. The first utilization of kinetic resolution was reported by Pasteur in 1857.[176] During his experiments with tartaric acid fermentation, Pasteur observed that the reaction proceeded efficiently with *D*-tartaric acid but very poorly, or not at all, with *L*-tartaric acid. When both enantiomers were combined into a racemic mixture, the fermentation process enabled effective kinetic resolution.[176] This experiment stands out as one of the earliest examples of an enantioselective biocatalytic reaction using a whole living organism.



$$s = \frac{k_R}{k_S} = \frac{\ln[(1-C)(1-ee)]}{\ln[(1-C)(1+ee)]} \quad \text{(eq. 1)}$$

Scheme 25. Representation of kinetic resolution and equation for calculating stereoselectivity (s).

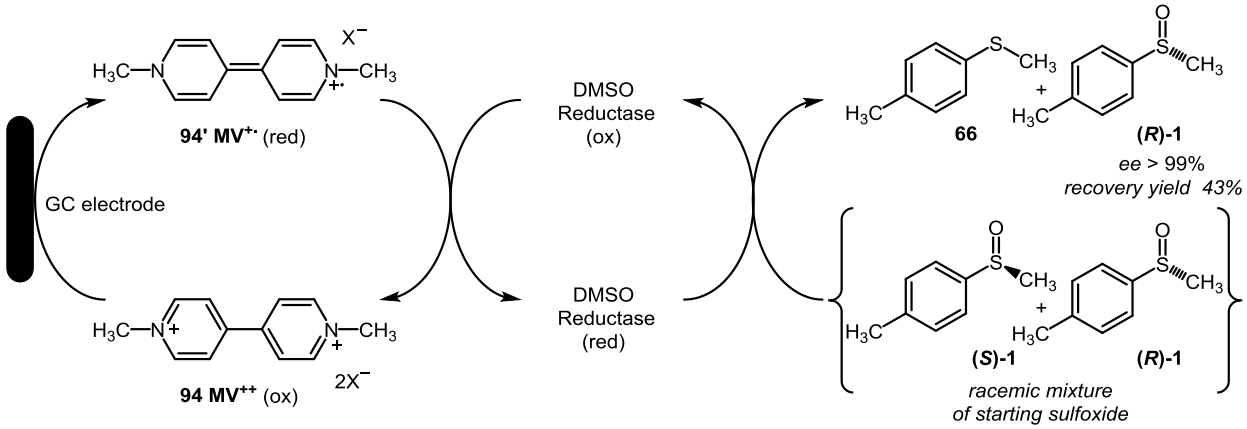
Chemical methods for the kinetic resolution of sulfoxides are limited to the above-mentioned oxidative catalytic systems,[177] including chiral titanium complexes and vanadium-salan systems.[125,134] Kinetic resolution is achieved through the selective oxidation of one sulfoxide enantiomer to sulfone. Another oxidative approach relies on iron-catalyzed asymmetric nitrene transfer, which produces sulfoximines (Scheme 26).[178]



Scheme 26. Iron complex-catalyzed imidative kinetic resolution of racemic sulfoxides.

Kinetic resolution of chiral sulfoxides can be achieved also through a selective reduction to sulfides. However, chemical methods relying on this approach are fundamentally underdeveloped.[179,180] On the other hand, literature reports several enzyme classes that can reduce S=O even in non-natural substrates with considerable stereoselectivity. These enzyme classes include trimethylamine monooxygenase (EC 1.14.13.148), trimethylamine-N-oxide reductase (EC 1.7.2.3), dimethyl sulfide:cytochrome c2 reductase (EC 1.8.2.4), biotin sulfoxide reductase (EC:1.8.4.13), respiratory dimethyl sulfoxide reductase (1.8.5.3) and methionine S-oxide reductases (1.8.4.11-14).[181] Therefore, enzymes are of particular interest for this type of enantioselective transformation. For instance, Yamazaki group utilized DMSO reductase from *Rhodobacter sphareoides f. sp. denitrificans* for kinetic resolution on various aryl alkyl sulfoxides

with high *ee* values reaching 99%.[182] The recovered sulfoxide enantiomers were isolated in the absolute (*R*)-configuration, reflecting the enzyme's (*S*)-selectivity. The method was refined and applied to prepare (*R*)-sulfoxides on a preparative scale, though yields were below 50% (Scheme 27).[183,184]

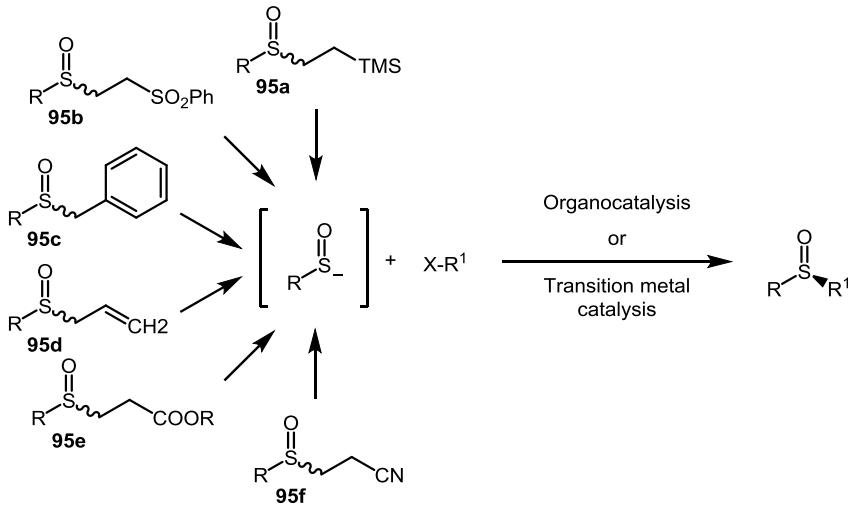


Scheme 27: Electrochemical enzymatic deoxygenation of chiral sulfoxides.

Notably, in the context of enzymatic asymmetric S=O bond reductions, the analysis of substrate specificity for bovine MsrA revealed its ability to selectively reduce various sulfoxides, including MetSO in proteins, *N*-AcMetSO, and *L*-MetSO, DMSO, and *S*-(–)-methyl-*p*-tolyl sulfoxide **1** among others.[185]

1.5.4. Other Methods

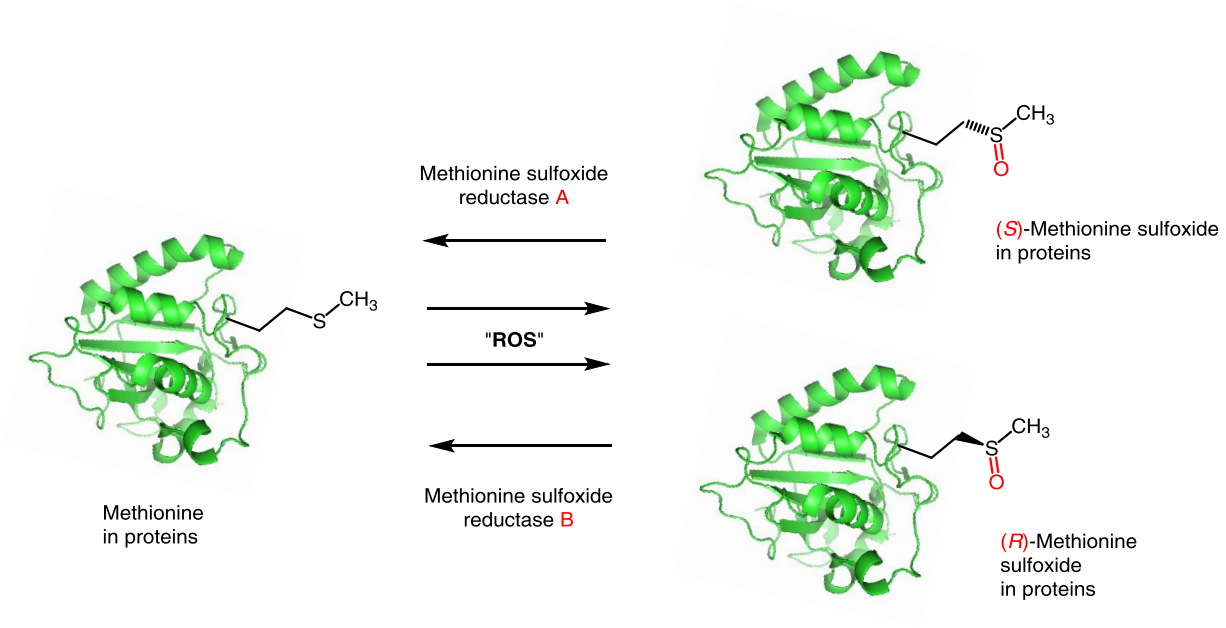
Alternative approaches for enantioselective preparation of sulfoxides include desymmetrization methods, such as asymmetric hydrogenation of vinyl sulfoxides.[186] Recently, increasing attention has been given to asymmetric substitution reactions that use in situ-generated sulfenate anions (Scheme 28). [187,188]



Scheme 28. Asymmetric nucleophilic substitution with sulfenate anion generated from various sulfoxide precursors.

1.6. Methionine Sulfoxide Reductase

Methionine is an essential amino acid obtained from the diet, crucial for various metabolic pathways.[189,190] It is the only amino acid with a single codon, AUG, which also acts as the start codon in eukaryotes (formylmethionine is used in prokaryotes). Methionine is prone to oxidation by reactive oxygen species (ROS),[191,192] forming an equimolar mixture of sulfoxide epimers *R* and *S*.[193] Methionine sulfoxide reductases (Msrs) are a class of enzymes that reverse this oxidation and convert methionine sulfoxide back to methionine and thus helps maintain redox balance and repair damaged proteins.[192,194–196] Msrs exists in two enantiocomplementary forms: MsrA, which reduces the (*S*)-enantiomer, and MsrB, which reduces the (*R*)-enantiomer (Scheme 29).[189,197,198] These enzymes, with mirror-image active sites, have evolved independently through convergent evolution and are encoded by separate genes on different chromosomes in the eukaryotes.[199–205]



Scheme 29. Schematic representation of reversible oxidation of methionines in proteins.[196,206]

Msr enzymes are found across all aerobic life forms[185,200,207–211] displaying a wide range of catalytic activities (e.g., MsrA from *S. cerevisiae*: 7.71 ± 0.03 s^{-1}, and MsrA from *E. coli*: 3.7 ± 0.5s^{-1}, MsrA from *P. trichocarpa* 0.30 ± 0.03 s^{-1}, and MsrB from *A. thaliana* 0.17 ± 0.01 s^{-1} etc.; for free methionine sulfoxide or *N*-acetyl methionine sulfoxide).[212] Regardless of these variations, five amino acids in the active site are proposed to be essential for the activity: cysteine (Cys), glutamic acid (Glu), tryptophan (Trp), phenylalanine (Phe) and tyrosines (Tyr) (Figure 11).[213,214] Site-directed mutagenesis has shown that replacing the catalytic amino acids such as cysteine with serine (Ser) stops the enzyme's activity,[214,215] while introducing selenocysteine (Sec) enhances it.[216,217]

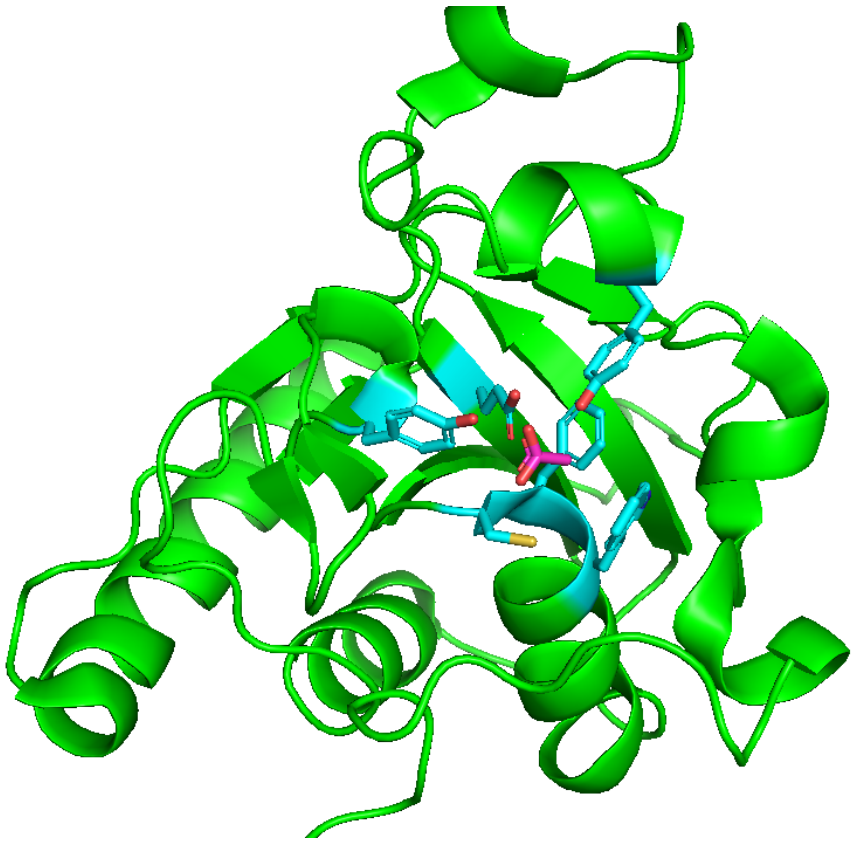
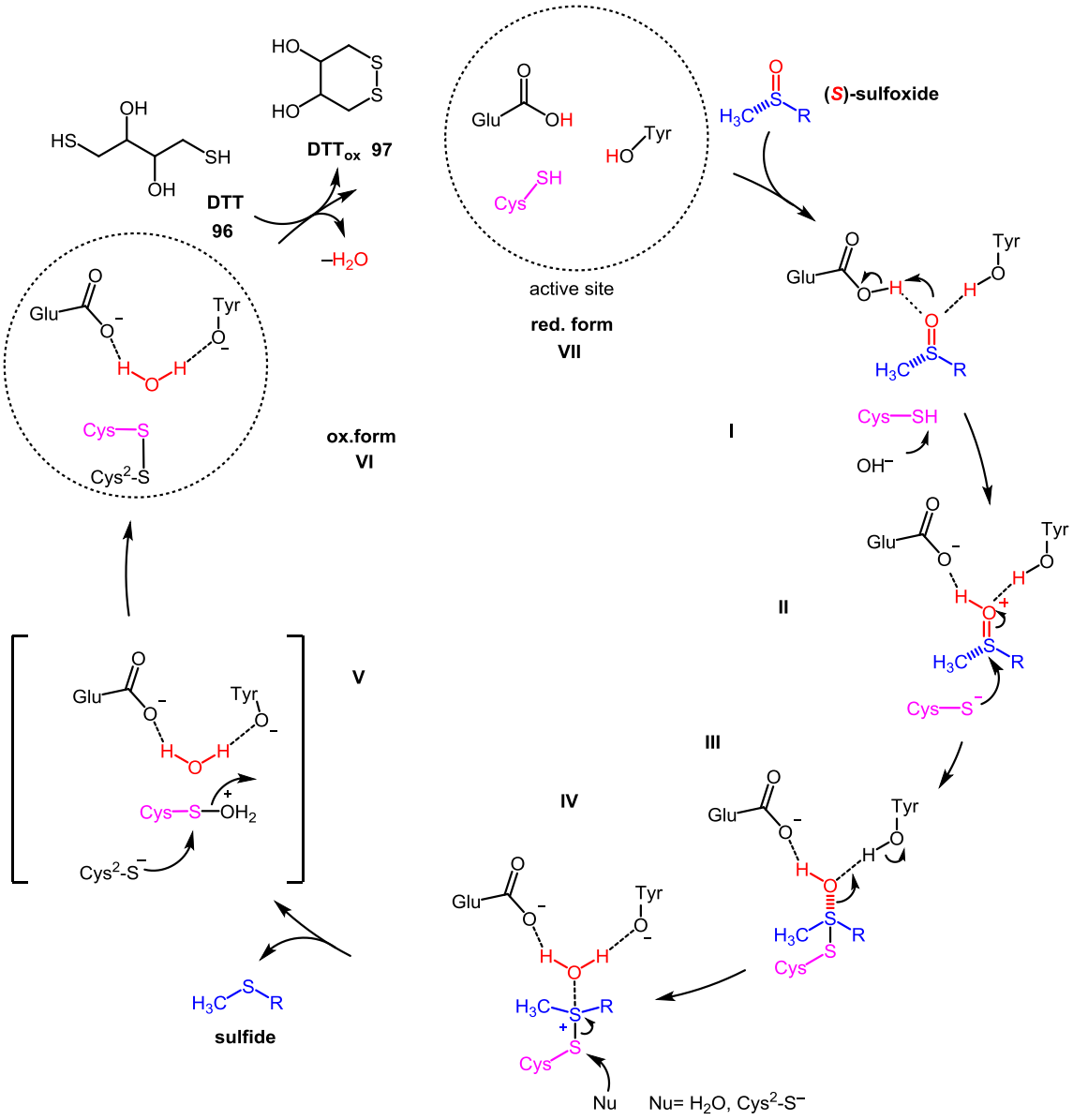


Figure 11. Detailed view of the 3D structure of the active site of MsrA highlighting amino acids (in teal color) important for enzyme function. The acetate ion is shown inside the hydrophobic cavity. [205]

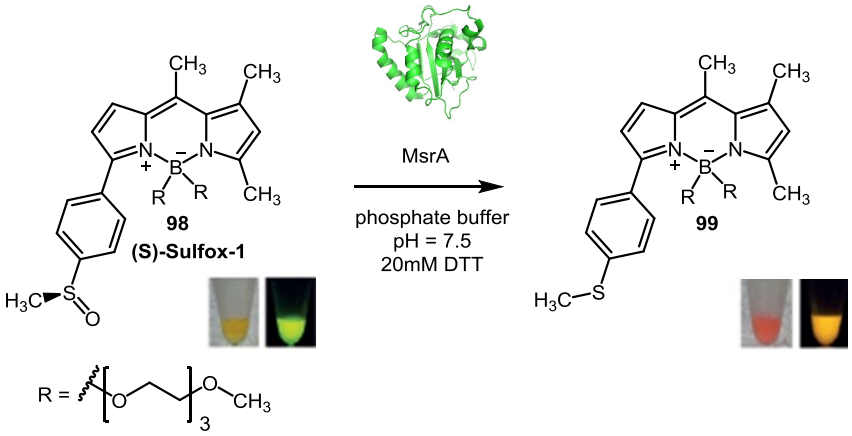
The proposed reaction mechanism is depicted in Scheme 30.[215,218–221] The process begins with the sulfoxide entering the enzyme's active site, where it forms a supramolecular complex (Michaelis complex) through hydrogen bonds with glutamic acid and tyrosine. Tryptophan and phenylalanine contribute to π-π stacking, increase cavity hydrophobicity, and help position the substrate for enantioselective transformation. Once inside, protonation and activation of the S=O bond occurs (**I**), enabling its attack by an active cysteine residue (**II**), leading to a sulfenic acid intermediate. Tyrosine donates a proton (**III**), facilitating water departure and leaving behind a sulfonium ion. This ion is then attacked by a nucleophile (**IV**), which could be water or a second catalytic cysteine, depending on the MsrA variant. This results in either a protonated sulfenic acid or the formation of a disulfide bond (**IV**, **V**). Finally, the resulting sulfide is released from the active site, and the enzyme regenerates (**VI**) back to its reduced form (**VII**). This reduction regeneration step is mediated by the natural thioredoxin/NADPH system *in vivo*, while *in vitro*, reductants like dithiothreitol (DTT), dithioerythritol (DTE), or tris(2-carboxyethyl)phosphine (TCEP) are utilized.



Scheme 30. Proposed catalytic cycle of methionine sulfoxide reductase A.

Oxidative damage is a phenomenon associated with a number of diseases including cancer, Alzheimer's, and other neurodegenerative disorders.[222–224] Methionine sulfoxide reductase A, has been recognized for its ability to mitigate this oxidative damage by repairing oxidized methionine residues in various proteins.[225] Although a broad spectrum of cellular proteins is known to be inactivated by methionine oxidation, there are growing examples where methionine sulfoxide generation serves as a gain-of-function posttranslational modification.[226–228] Furthermore, studies have linked MsrA activity to an extension of life span in organisms such as fruit flies (*Drosophila*).[192,194,195,229,230] Despite the biological importance of MsrA, simple and effective tools for real-time *in vivo* monitoring of its activity were lacking until recently. Our laboratory has developed BODIPY-based ratiometric fluorescence probes **98** named (*S*)-Sulfox-1 and (*R*)-Sulfox-1

that enabled direct and efficient monitoring of MsrA and MsrB activity in living cells (Scheme 31).[206] A variety of other probes has been developed in our laboratory ever since.[231–233] These projects indicated that Msrs are robust enzymes with high enantioselectivity and relatively low specificity. This observation was a starting point for a project described in this thesis.



Scheme 31. Fluorescent probe (S)-Sulfox-1 for the monitoring MsrA activity in vivo and in vitro with a visible color change upon sulfoxide reduction and sulfide formation.[206]

2. Aims of the Work

The major aim of this work was to develop a robust biocatalytic method for the general enantioselective preparation of chiral sulfoxides. There were the following specific sub-goals.

1. Explore the enzyme methionine sulfoxide reductase A (MsrA) for the kinetic resolution of a wide range of chiral sulfoxides including the pharmaceutically important ones.
2. Explore the enzyme methionine sulfoxide reductase B (MsrB) for the enantiocomplementary kinetic resolution of chiral sulfoxides.
3. Develop a chemoenzymatic protocol for deracemization of chiral sulfoxides based on Msr enzymes.
4. Explore the reactivity of *in vitro* evolved variants of Msr enzymes in the kinetic resolution of sulfoxides.

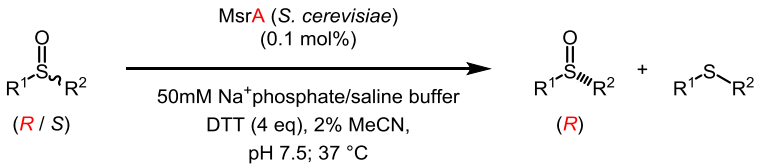
3. Results and Discussion

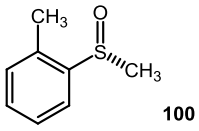
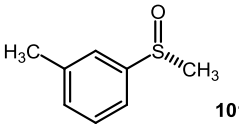
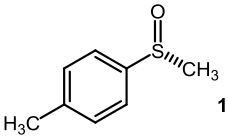
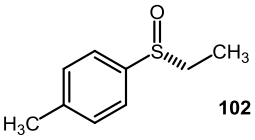
3.1. Enzymatic Kinetic Resolution of Sulfoxides With Methionine Sulfoxide Reductase A

Previous experiments in our lab with chiral fluorescent probes provided expertise in the recombinant expression of MsrA enzymes from various organisms. Having these enzymes in hand prompted us to test this class of enzymes for a new enzymatic kinetic resolution of chiral sulfoxides. The initial experiments were done with MsrA from *S. cerevisiae*. In order to test the substrate scope of the enzyme, a set of four racemic sulfoxides **1**, **100**-**102** was prepared by alkylation of corresponding thiols followed by oxidation. It is worth mentioning that classical oxidation with *m*-CPBA was later substituted with the acetic acid/hydrogen peroxide method that provided much cleaner products with minimal overoxidation to sulfones.

The substrates were chosen to assess the allowed *o*-, *m*-, *p*- substitution pattern on the aromatic ring as well as the change of methyl to larger ethyl substitution. The initial experiments were performed at a 6.5 mM concentration of substrates (~1 g/L for model substrates) in phosphate buffer pH 7.5 with 2 % acetonitrile as a co-solvent. These conditions secured good solubility of substrates while keeping reasonable substrate concentration. Given the catalytic parameters of the enzyme (K_M=560 μM, k_{cat}=7.71 s^{-1} for methionine sulfoxide **5**) and our previous experiment with fluorescent probes, 0.1 mol% of the enzyme was used as a catalytic loading. 4 equivalents of DTT (26 mM) were used as a terminal reductant. Delightfully, all substrates were reduced by MsrA with excellent enantioselectivity. Corresponding (*R*)-sulfoxides were obtained with *ee* values of > 99% at the 50% conversion (Table 1). Substrates **100** and **102** required longer reaction times, which most probably reflects their steric hindrance. It has to be noted that the enzymatic reaction did not proceed any further after the 50% conversion was reached, and the sulfoxide concentration remained constant for several more hours. These experiments demonstrate an exceptional enantioselectivity of MsrA even among other enzymes commonly utilized for kinetic resolution of chiral compounds (e.g. lipases).[234]

Table 1. Pivotal results of kinetic resolution with MsrA (S. cerevisiae).[a]



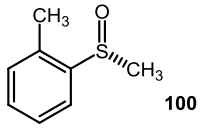
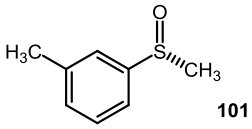
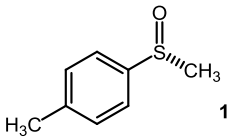
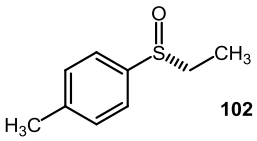
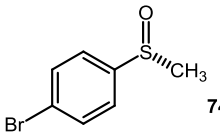
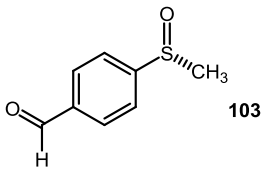
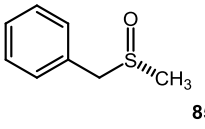
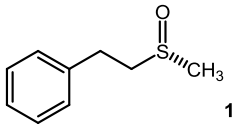
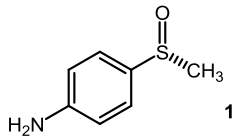
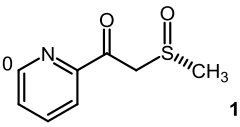
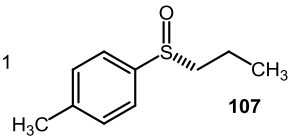
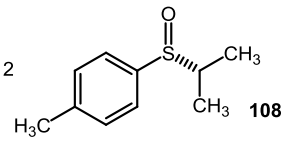
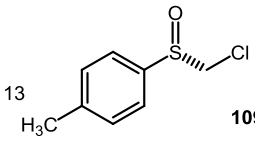
entry	product	conversion[b] (%)	*ee* (%)	*s*
1	 **100**	50	>99	>100
2	 **101**	50	>99	>100
3	 **1**	50	>99	>100
4	 **102**	49	98	>100

(a) Reactions were performed on a 10 µmol scale (6.5 mM substrate solution), the reaction time to complete conversion was from 4h to 10h. (b) conversion measured by HPLC against the blank reaction without an enzyme.

Encouraged by these results, other sulfoxide substrates were synthesized according to the general procedure and tested in MsrA-catalyzed kinetic resolution. The results are summarized in Table 2, substrates **74** and **103** containing electron-accepting bromo and carbaldehyde substituents on the aromatic ring reacted readily providing enantiomerically pure sulfoxides at the 50% conversion. Notably, MsrA enables chemoselective reduction of sulfoxide over aldehyde, which is a non-trivial reaction achieved by purely chemical means. Also, replacing the aryl group in the substrate with phenylmethyl or phenylethyl moiety yielded enantiomerically pure sulfoxides **85** and **104**. As mentioned above, sulfoxides with two adjacent sp^3 carbon substituents are notoriously difficult substrates to obtain in high enantiomeric excess by chemical methods. Therefore, this enzymatic approach provides a new way for the preparation of these difficult substrates. Substrate **105** with electron-donating amino group on the aromatic ring and investigational drug oxisuran **106** were also good substrates for the enzymatic reduction, however difficult isolation of the sulfoxides from the reaction mixture prevented the determination of the

enantiomeric excess. Further optimization would be required to isolate these products. Substrates **107**-**109** with propyl, isopropyl, and chloromethyl substituents indicated limitations of the method in terms of the second substituent on the sulfoxide moiety. Anything larger than the ethyl group seems to shut down the reactivity. This observation is in agreement with the X-ray structure of the enzyme which shows a relatively shallow binding pocket in the active site of the enzyme.[205]

Table 2. Summary table of kinetic resolution with isolated enzyme MsrA (S. cerevisiae)[(a),(c)]

entry	product	conversion[(b)] (%)	*ee* (%)	*s*
1	 **100**	50	>99	>100
2	 **101**	50	>99	>100
3	 **1**	50	>99	>100
4	 **102**	49	98	>100
5	 **74**	50	>99	>100
6	 **103**	50	>99	>100
7	 **85**	50	>99	>100
8	 **104**	50	>99	>100
9	 **105**	48	n.d.[(d)]	n.d.[(d)]
10	 **106**	51	n.d.[(d)]	n.d.[(d)]
11	 **107**	<3%	0	n.d.[(d)]
12	 **108**	<3%	0	n.d.[(d)]
13	 **109**	<3%	0	n.d.[(d)]

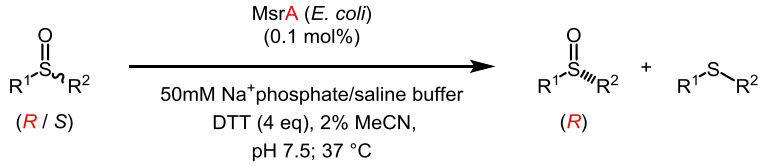
(a) Reactions were performed on a 10 µmol scale (6.5 mM substrate solution), (b) conversion was measured by HPLC against the blank reaction without an enzyme, (c) the reaction time to complete conversion was from 2h to 10h,-(d) n.d. = not determined.

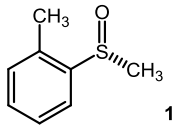
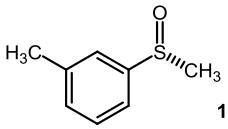
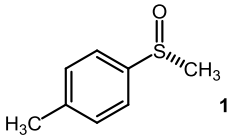
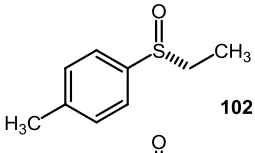
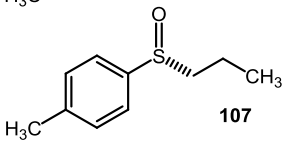
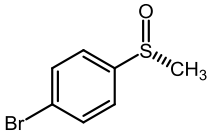
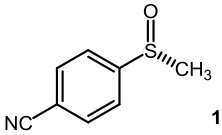
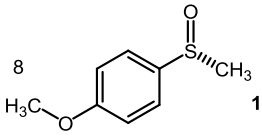
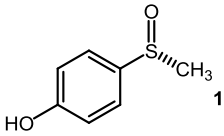
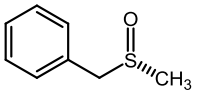
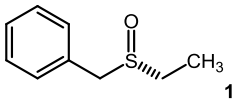
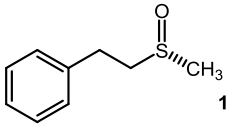
It has been reported that certain MsrAs can also stereoselectively oxidize methionine to methionine sulfoxide.[221] This reverse process would provide the opposite enantiomer than the reduction. Therefore, MsrA (*S. cerevisiae*) was also tested for oxidation of sulfide **66** with various

concentrations of H_2O_2 as a terminal oxidant. However, no sulfoxide product was observed. Therefore, this direction was not pursued further.

During these experiments, MsrA from *E. coli* bacteria was also recombinantly expressed. It turned out that the production of this enzyme is higher yielding (50 mg/L culture vs 24 mg/L culture) and the resulting enzyme preparation is more stable for long-term storage while having similar activity as MsrA from *S. cerevisiae*. Therefore, this enzyme was also tested for kinetic resolution with a panel of chiral sulfoxides (Table 3). Similarly to previous experiments, MsrA (*E. coli*) turned out to be highly efficient for the kinetic resolution of chiral sulfoxides. A variety of sulfoxides with different structural features and functional groups were obtained as pure enantiomers with enantioselectivity factor *s* reaching a value of more than 100 (Table 3). Same as with the yeast enzyme, the largest accepted R^2 substituent on the sulfoxide is the ethyl group. Anything larger, like propyl suppresses the reactivity with the enzyme. Despite this fact, this enzymatic kinetic resolution of sulfoxides belongs to the most general and efficient methods published so far.

Table 3. Summary table of kinetic resolution with isolated homologous enzyme MsrA (E. coli)[(a)]



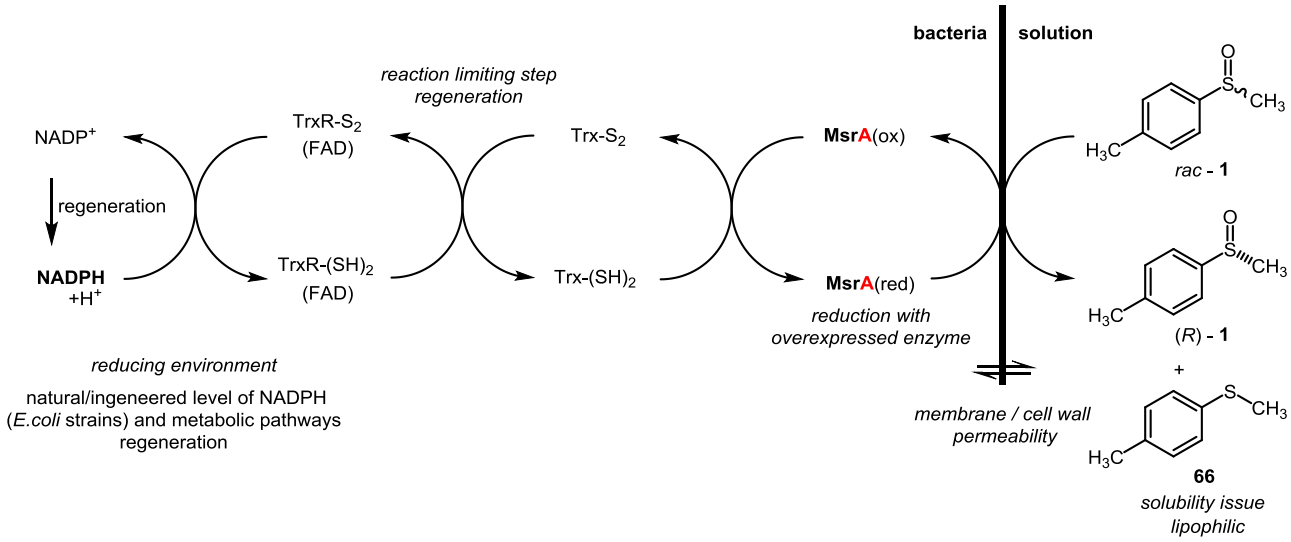
entry	product	conversion (%)	ee (%)	s
1	 **100**	50	>99	>100
2	 **101**	50	>99	>100
3	 **1**	50	>99	>100
4	 **102**	50	>99	>100
5	 **107**	<2%	n.d.	n.d.[(b)]
6	 **74**	50	>99	>100
7	 **110**	50	>99	>100
8	 **111**	50	>99	>100
9	 **112**	50	>99	>100
10	 **85**	50	>99	>100
11[(c)]	 **113**	50	>99	>100
12	 **104**	50	>99	>100

(a) Reactions were performed on a 10 µmol scale (6.5mM substrate solution). (b) n.d. = not determined; (c) the reaction was carried out with 0.15 mol% of MsrA.

3.2. Chemoenzymatic Deracemization of Chiral Sulfoxides

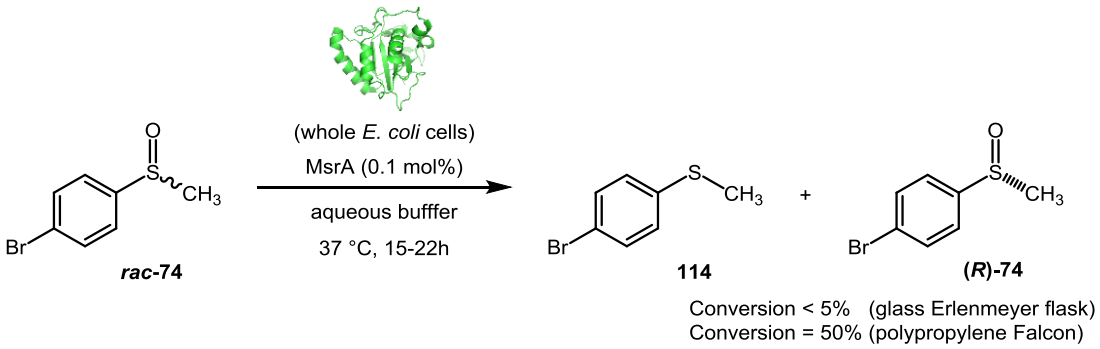
The enzymatic kinetic resolution described above is a new powerful method for the preparation of enantiomerically pure sulfoxides. In order to make the method more practical and economical for the large-scale production of chiral sulfoxides, we sought a way to avoid a relatively expensive DTT reductant as well as the isolation of the enzyme. *E. coli* cells used for the preparation of the MsrA enzyme could provide sufficient reducing power through

the NADPH/TrXR/TrX cascade to drive the reaction forward (Scheme 32). The whole-cell format of the reaction would also eliminate the need for enzyme isolation.



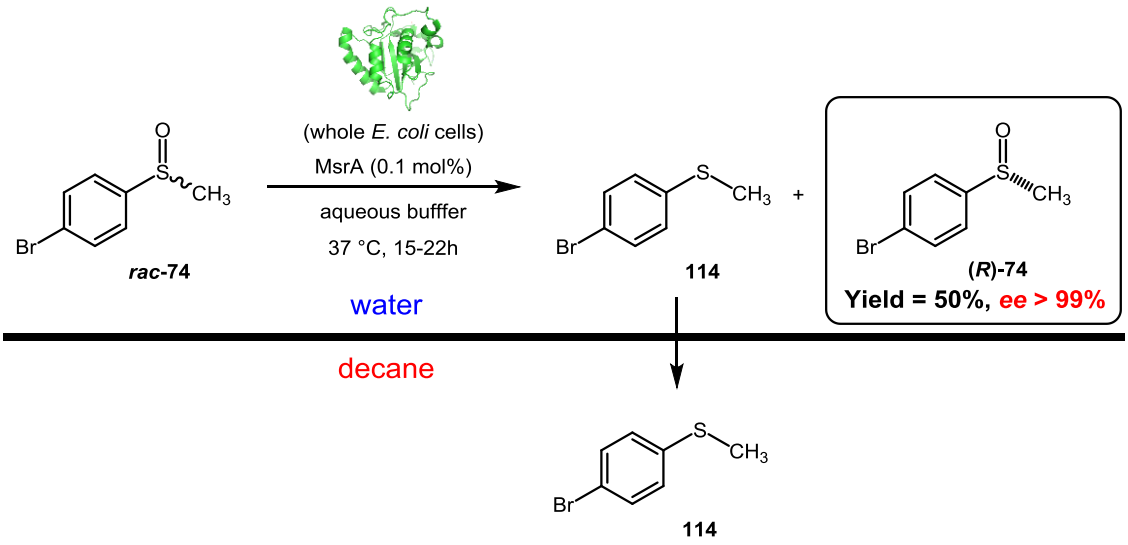
Scheme 32. Schematic representation of thioredoxin-based reducing system of E. coli.

In order to test the whole cell catalysis hypothesis, a number of initial experiments were performed with variations in buffers (LB broth, TBE, M9 minimal), pH (6-9), substrate concentration, carbon source (glucose, glycerol), cells concentration, a type of a reaction vessel, organic cosolvents at different concentrations, etc. As a result of these experiments, an interesting observation was made. A reaction carried out in a 50 mL polypropylene Falcon tube provided enantiopure sulfoxide **74** at roughly 50% conversion, whereas the same reaction performed in a 100 mL glass Erlenmeyer flask showed minimal conversion and enantiomeric excess values (Scheme 33). Investigation of this observation led us to a hypothesis that a resulting lipophilic sulfide product is toxic to cells that are no longer capable of supplementing the reaction with a reductant. The relatively lipophilic surface of a polypropylene tube is likely capable of sequestering the sulfide product thus preventing the toxic effect on *E. coli* cells. To evaluate this hypothesis, cells from an unsuccessful reaction were reacted with the (*S*)-Sulfox-1 probe. No reaction was observed until DTT was introduced, indicating that the cells had lost their natural reducing conditions, likely because of reduced NADPH levels or cell death. Nevertheless, MsrA was still present and functional.



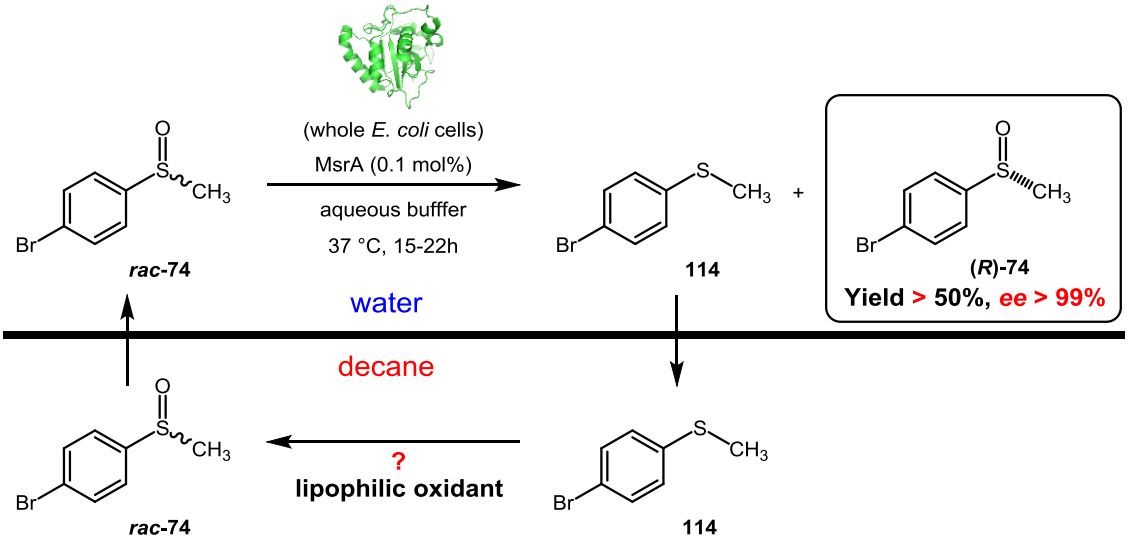
Scheme 33. Preliminary trials involving whole-cell reductions were conducted with E. coli in glass Erlenmeyer flasks or polypropylene Falcon tubes.

Our hypothesis was that the accumulation of lipophilic sulfide, which forms from the 6.5 mM sulfoxide as the reaction progresses, might damage the cell membrane of *E. coli* rather than targeting specific cellular processes, leading to cell toxicity. To test this, we took advantage of the fact that *E. coli* can grow in the presence of water non-miscible organic solvents, like decane (partition coefficient log $K_{OW} > 4$).[235] First, we confirmed that *E. coli* cells can grow on LB agar plates covered with a layer of decane.[236] Then, we tested the solution culture of *E. coli* with 5% (v/v) of decane. The cell growth observed was nearly identical to cell culture without decane co-solvent. Furthermore, the cell culture supplemented with decane co-solvent was also tolerant to the 6.5 mM concentration of model sulfoxide compound **74**. Interestingly, co-solvents like hexane and heptane were incompatible with the bacterial culture's growth. We assumed that the lipophilic sulfide product would partition into the organic phase, thereby not interacting directly with the cell membranes, while the polar, non-toxic sulfoxide would stay in the aqueous buffer and be accessible for reduction. Delightfully, when tested in the kinetic resolution of racemic sulfoxide **74**, the addition of 5% (v/v) decane to the cell culture resulted in enantiopure sulfoxide at 50% conversion, aligning with the *in vitro* results (Scheme 34). To determine enzyme concentration, we quantified MsrA in the cell culture under the reaction conditions (details in the experimental section). The concentration of the enzyme in the whole-cell reaction turned out to be almost identical to the *in vitro* experiment (0.1 mol %), confirming the expected value given the original design of the experiment.



Scheme 34. Whole-cell kinetic resolution with MsrA enabled by the addition of decane in a two-phase system.

This whole-cell biphasic kinetic resolution approach provided us with the opportunity to reoxidize the sulfide product in the organic phase back to sulfoxide and obtain yields beyond the maximum 50% for kinetic resolution. In order to achieve this goal, a suitable lipophilic oxidant was required (Scheme 35).



Scheme 35. The proposed concept of two opposing pathways in a single reaction setup, enabled by a two-phase system. Asymmetric enzymatic reduction takes place in the aqueous phase and racemic chemical oxidation in the organic phase.

The oxidant had to meet two requirements. First, it needed to be lipophilic enough to reside in the organic phase to prevent oxidative damage to the cells. Second, it had to have a sufficient rate of oxidation of sulfide to sulfoxide that would be compatible with the enzymatic

reduction rate. In the initial experiments, *tert*-butyl hydroperoxide **115** and cumyl hydroperoxide **116** were tested due to their large lipophilic groups and ability to oxidize sulfides to sulfoxides (Figure 12).[237] However, their solubility in the aqueous environment was still relatively high, resulting in cell death. Therefore, hydroperoxide **117** with a long aliphatic chain was synthesized (Figure 12).[238] Extending the alkyl chain by an additional nine carbons eliminated toxicity to cells, however, the oxidation rate of sulfide to sulfoxide was very low (<5% in 24h). Therefore, hydroperoxides were abandoned as possible oxidants.

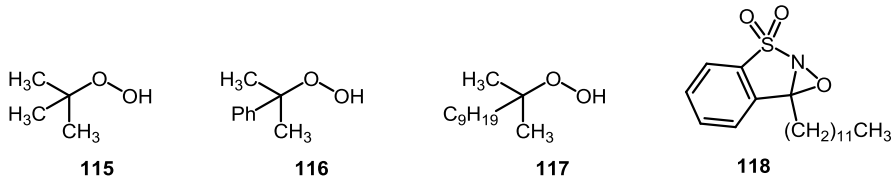
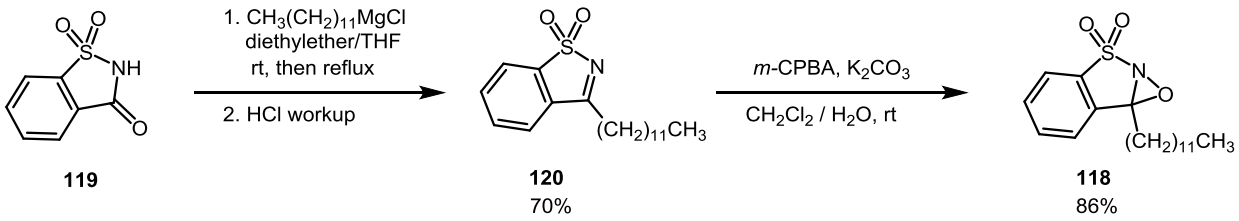


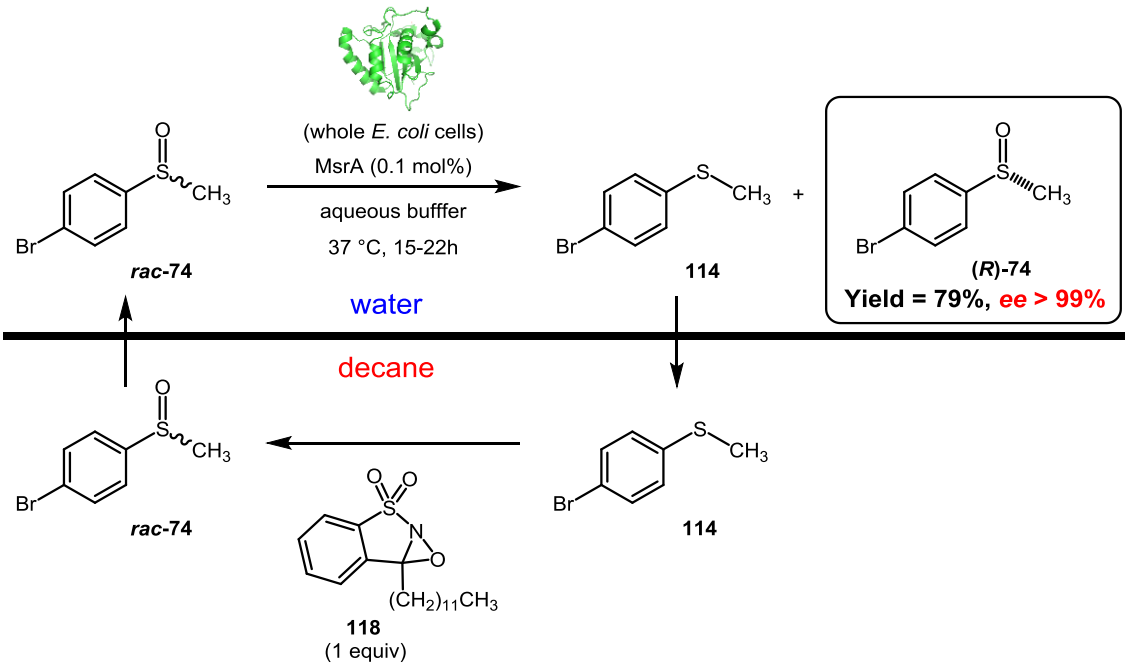
Figure 12. Tested oxidants for biocompatible oxidation of sulfides.

Next, the attention was turned to oxaziridine-based oxidants that are more reactive in the oxidation of sulfides than hydroperoxides. Oxaziridine **118** with twelve-carbon chain was prepared in two simple steps from a cheap starting material saccharin **119** (Scheme 36).[99] It turned out to be compatible with cell culture and exhibited sufficient reactivity toward sulfide oxidation. The imine **120**, a waste product of the oxidation reaction, can be easily separated from the sulfoxide product and regenerated.



Scheme 36. Two-step synthesis of saccharine-based oxaziridine oxidant.

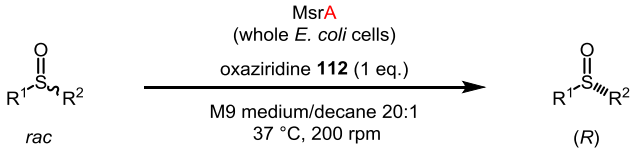
By using oxaziridine oxidant **118**, a preparative scale deracemization of racemic sulfoxide **74** was achieved. One equivalent of oxidant was sufficient to obtain enantiomerically pure sulfoxide **74** in 79% isolated yield (112 mg) (Scheme 37).

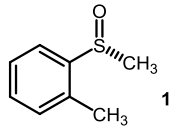
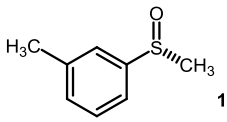
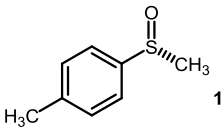
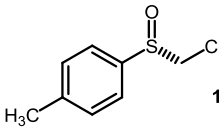
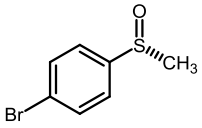
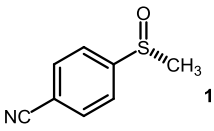
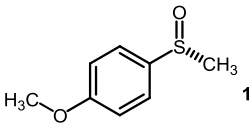
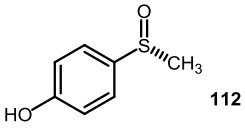
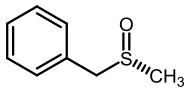
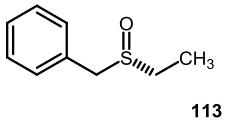
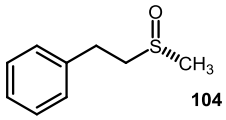
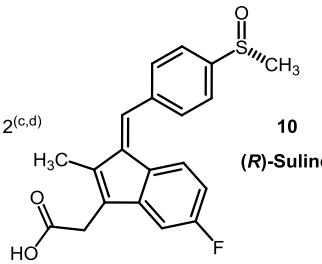


*Scheme 37. Schematic representation of sulfoxide **74** deracemization cycle in a two-phase system with MsrA and oxaziridine oxidant.*

These conditions were utilized to determine the substrate scope of this deracemization protocol. All chiral sulfoxides used in the kinetic resolution were successfully deracemized on a preparative scale (0.65 mmol), all showing excellent enantioselectivity (up to *ee* > 99%) and isolated yields above 50% (Table 4, entry 1 – 11). Notably, some of the lipophilic sulfoxides (e.g. **111**) were isolated in very good yields reaching up to 90%. On the other hand, the yield of more polar substrate **112** with phenolic substituent was 62% as determined by HPLC and 52% of the isolated product. The lower yield of the product may be related to the worse partitioning into the decane phase and slower reoxidation of the sulfide product. For some substrates (**85** and **113**), an optimization of cell (enzyme) loading was required to obtain high yields and enantioselectivities. Thanks to these optimizations, even these challenging alkyl-alkyl sulfoxides **85** and **113** were successfully deracemized with good yields and excellent enantioselectivities.

Table 4. Final table showing the substrate scope of deracemization.[a]



Entry	Product	HPLC yield (isolated yield)	ee
1	 **100**	80% (72 mg, 72%)	99%
2	 **101**	75% (70 mg, 70%)	99%
3	 **1**	68% (62 mg, 62%)	99%
4	 **102**	55% (59 mg, 54%)	99%
5	 **74**	82% (112 mg, 78%)	99%
6	 **110**	74% (65 mg, 61%)	99%
7	 **111**	91% (99 mg, 90%)	99 %
8	 **112**	62% (52 mg, 52%)	99%
9[b]	 **85**	93% (75 mg, 75%)	99%
10[c]	 **113**	72% (64 mg, 59%)	99%
11	 **104**	89% (97 mg, 88%)	99%
12[c,d]	 **10** **(*R*)-Sulindac**	75% (140 mg, 61%)	97%

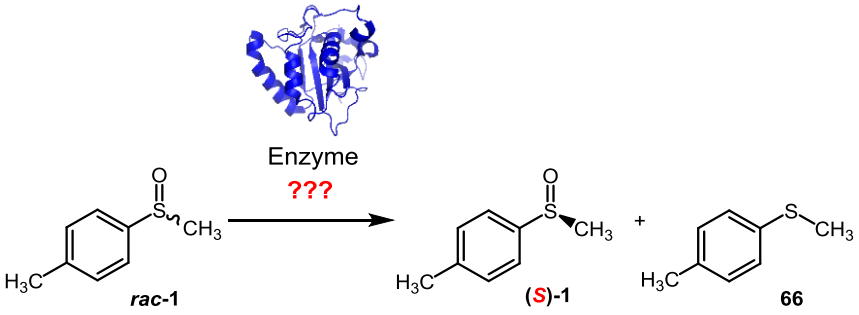
(a) Reactions were performed on a 0.65 mmol scale (6.5 mM substrate), (b) The reaction was carried out with 0.2 mol% of MsrA, (c) The reaction was carried out with 0.3 mol% of MsrA, (d) β-Cyclodextrin (1 equiv) was added to the reaction mixture.

To further demonstrate the applicability of this method, we utilized the deracemization conditions for the asymmetric preparation of the non-steroidal anti-inflammatory drug Sulindac **10** (Table 4, entry 12). Due to Sulindac's poor solubility in both aqueous and organic solvents, further optimization of conditions was needed. In order to boost the solubility, we took advantage of β-cyclodextrin and its ability to complex and solubilize organic compounds. Co-evaporation of racemic Sulindac with one equivalent of β-cyclodextrin from a 50% aqueous ethanol yielded deep yellow powder that was easily soluble in aqueous buffers.[239] Interestingly, Sulindac methyl ester

121 was insoluble under any conditions. Using the optimized conditions, racemic Sulindac **10** was successfully deracemized on a preparative scale to yield the (*R*)-enantiomer of the sulfoxide in 61% isolated yield (140 mg) with 97% *ee*. To the best of our knowledge, this is the first general method for the efficient deracemization of chiral sulfoxides. It should be noted that we are aware of earlier attempts of deracemization of a specific allylic sulfoxide by Viedma ripening and a multistep repetitive kinetic resolution requiring isolation steps.[240,241] However, our protocol provides a general platform for straightforward deracemization of a wide range of sulfoxide substrates achieving yields of up to 90% and enantiomeric excesses up to >99% *ee*. The enzyme MsrA demonstrated unprecedented enantioselectivity as well as a remarkably wide substrate scope. By combining MsrA with oxaziridine oxidant **118** in a biphasic system, we were able to deracemize a variety of aryl alkyl and alkyl alkyl sulfoxides. After these results were published, other sulfoxide deracemization procedures were reported that were inspired by our approach.[242,243]

3.3. Enantiocomplementary Method for the Preparation of (*S*)-Sulfoxides

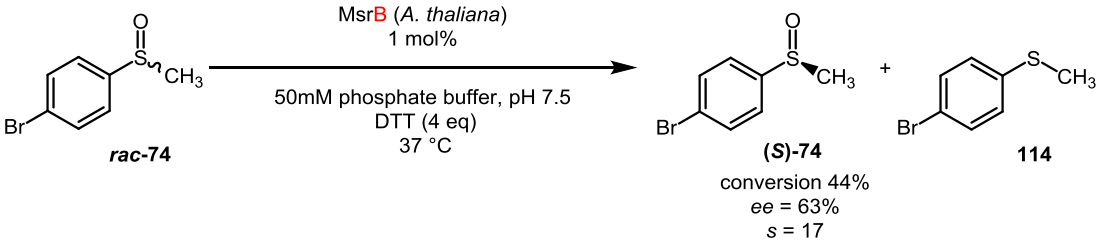
While biocatalysis has become essential for creating enantioenriched chiral molecules, natural enzymes often exhibit high substrate specificity and lack enantiocomplementary counterparts. Directed evolution, a technique introduced by Frances H. Arnold, enables the creation of custom enzymes for chemical synthesis.[244–247] Although enantioselectivity can be altered through this method, the process can be labor-intensive, requiring many repetitive steps of evolution and selection, and depends heavily on efficient screening techniques. [248,249] Our objective was to find an enantiocomplementary alternative to MsrA with a broad substrate scope and applicability (Scheme 38).



Scheme 38. Proposed complementary biocatalytic kinetic resolution to obtain the opposite enantiomer.

As mentioned in the introduction, MsrB enzymes offer a natural enantiocomplementary

alternative that we set out to explore. First, recombinant expression of MsrBs with the highest reported activity was attempted. MsrAB from *H. pylori* was reported to have both A and B domains with the A domain being catalytically inactive while the B domain having strong (*R*)-sulfoxide reducing activity.[250] After several attempts, this enzyme was successfully expressed and isolated. Surprisingly, it was observed that the enantioselectivity of the enzyme was exactly opposite to the reported data.[250] This observation was confirmed on multiple substrates. Interestingly, the original article discusses the structural features of the domains in relation to their catalytic activity/inactivity. Based on our results, these conclusions seem incorrect. We reached out to the authors, however our inquiry remained without an answer. Similar results were obtained with multidomain MsrAB from *N. gonorrhoeae*.[202] Therefore, four other MsrB enzymes were expressed in *E. coli*: MsrB2 from *A. thaliana*,[207] CBS-1 from *H. sapiens*,[210] MsrB from *N. gonorrhoeae*,[202] and MsrB from *T. kodakarensis.*[251] Expression of all four enzymes was confirmed by SDS-PAGE. CBS-1 from *H. sapiens* was difficult to isolate from bacterial culture and MsrB from *T. kodakarensis* showed negligible activity. The other two enzymes showed measurable desired reactivity towards model sulfoxides **74**. Because of the worked-out procedure for the expression and isolation of MsrB2 from *A. thaliana*, this enzyme was selected for further experiments. It should be noted that the overall activity of MsrB2 was significantly lower compared to MsrA. Therefore, in a prospective experiment with sulfoxide **74**, a ten-fold excess of the enzyme was required to push the reaction to a significant conversion (Scheme 39). Despite the increased enzyme loading (1 mol %), the reaction required 44 hours to reach 44% conversion. The enantiomeric excess of the remaining (*S*)-**74** was determined to be 63%, giving only a modest stereoselectivity factor *s* of 17. Together with relatively high enzyme loading, this method does not provide a practical enantiocomplementary alternative to MsrA. It should be noted that the phenomenon of lower activity and high substrate specificity of natural MsrBs as compared to MsrAs was observed previously and seems to be general.[252]

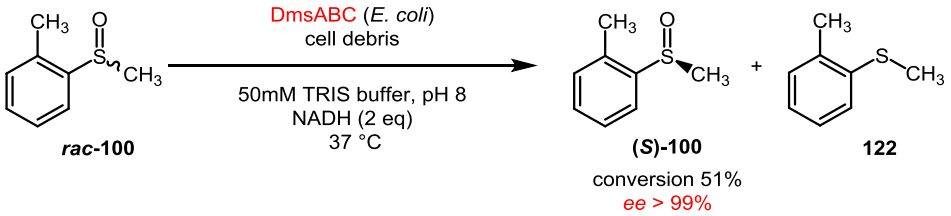


Scheme 39. Kinetic resolution with enantioselective MsrB2 (A. thaliana).

Based on these experiments, it was decided that the MsrB class of enzymes is not suitable for a general kinetic resolution providing the opposite enantiomers to MsrA. Accidentally, it was observed in our lab that stressed stationary phase *E. coli* cell cultures possess high (*R*)-sulfoxide-reducing activity as observed with (*R*)-Sulfox-1 fluorescent probe **98**. It was determined that the activity is located in a membrane fraction. Furthermore, to observe the activity in the membrane fraction, NADH was required. Supplementing the membrane fraction with DTT as a reductant did not lead to a measurable activity. Also, pyridine N-oxide was shown to be a competitive substrate to (*R*)-Sulfox-1. Based on these observations and information from databases, it was hypothesized that the enzyme responsible for this activity is dimethyl sulfoxide reductase (DMSO reductase, DmsABC). Efforts to isolate this multi-subunit membrane enzyme by a reported procedure or its modification proved unsuccessful.[253,254] The final proof came from the knock-out experiment with *E. coli* knock-out strain missing the catalytic subunit A of DmsABC.[255] This strain lacked the activity, however, when transformed with a plasmid with *dmsABC* gene cluster with an upstream natural promoter region, the activity was restored. These experiments unambiguously assigned the observed activity to the DmsABC enzyme. DmsABC is an enzyme complex consisting of three subunits A, B, and C. Subunit C is the membrane anchor, subunit B mediates electron transfer, and subunit A contains a catalytically active molybdopterin cofactor. It is a known enzyme, however, its upregulation in stressed *E. coli* cells and its enantiospecificity towards (*R*)-sulfoxides was described by our lab for the first time. After further literature research, as far as the stereoselectivity of reduction is concerned, there were several reports on enantioselective reduction of sulfoxides in whole *E. coli* cells, where DmsABC was suspected for the activity; however, no direct evidence was provided.[241,256,257] This accidental discovery provided the desired enantiocomplementary enzymatic activity to MsrA.

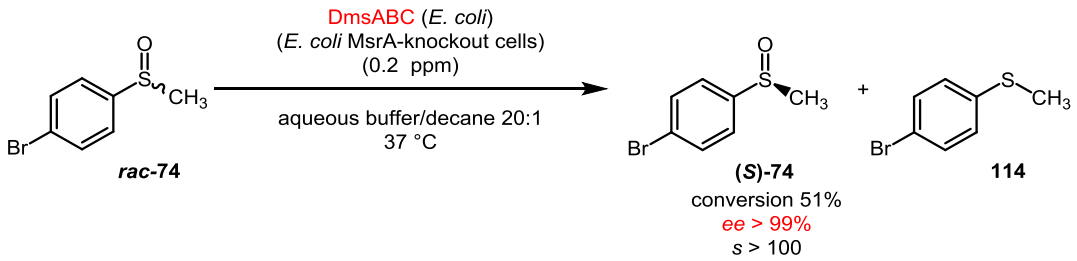
3.3.1. Application of Dimethylsulfoxide Reductase for Enantiocomplementary Kinetic Resolution of Sulfoxides

In the initial experiment, a membrane fraction of lysed overgrown *E. coli* cells was resuspended in Tris buffer, and racemic sulfoxide **100** and NADH (2 equivs) were added. After 18 hours the conversion of the reaction reached 51% and the isolated sulfoxide **100** showed to have the desired *S* configuration with *ee* > 99% (Scheme 40).



Scheme 40. Initial experiments with DmsABC in cell membrane fraction.

The promising initial results prompted us to test DmsABC further. However, the reproducibility of the experiments was inconsistent. The lack of reproducibility was probably due to the differences in the membrane fraction preparation. The activity of the enzyme was sensitive to the conditions of cell lysis by sonication. Since it was difficult to control the activity in the membrane fraction, it was decided to go for the whole-cell format of the reaction, similar to the previous experiments with MsrA. Also, natural NADH concentration in cells should provide enough reducing power to drive the reaction without an external reductant. We used a mutant *E. coli* strain with *msrA* gene knock-out (KEIO collection ID JW4178) to prevent unwanted activity.[258] It has been shown that the natural activity of enantiocomplementary MsrA is also upregulated at the stationary phase,[259] which would potentially reduce the yield and selectivity of the reaction. *E. coli* cell culture was overgrown to OD_{600} 3-3.5, in which DmsABC activity is naturally upregulated, the cells were resuspended in fresh nutrient medium buffer (M9 minimal) with 5% (v/v) of decane and incubated with the model substrate ***rac*-74**. The resulting mixture was incubated at 37°C and conversion and enantiomeric excess were monitored by HPLC. To the our delight, the reaction achieved 51% conversion in just 1 hour, with an excellent enantiomeric excess (*ee* > 99%) for the (*S*)-enantiomer, showing a high selectivity factor (*s* > 100) (Scheme 41).

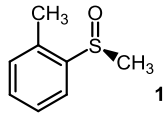
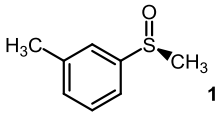
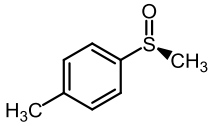
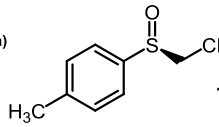
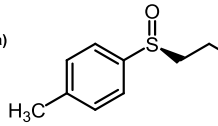
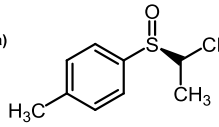
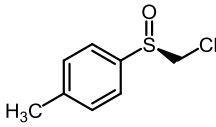
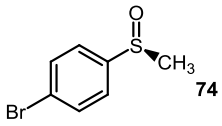
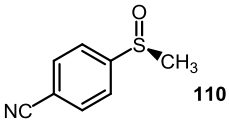
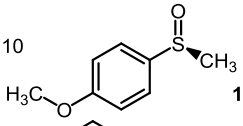
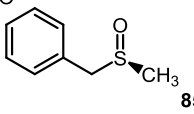
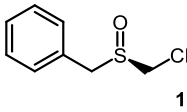
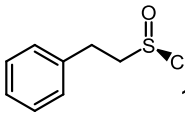
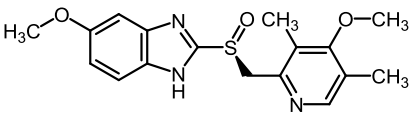


Scheme 41. Kinetic resolution with DmsABC in the whole-cell format.

In order to assess the amount of DmsABC reductase in the reaction mixture, proteomic analysis of the harvested cells was performed. The relative intensity of all proteins was related to the total protein concentration. Based on this analysis the amount of DmsABC reductase in the reaction mixture was estimated to be 0.00002 mol% or 0.2 ppm. This level of activity is in

agreement with the previously reported data for the purified enzyme and documents the exceptional catalytic performance of this molybdenum-containing enzyme.[254] We then explored the substrate scope of the reaction, successfully resolving a broad array of aryl alkyl and alkyl alkyl sulfoxides with excellent enantioselectivity (*s* > 100) (Table 5).

Table 5. The substrate scope of DmsABC-catalyzed kinetic resolution of chiral sulfoxides.

entry	product	conversion / *t*	*ee*	*s*
1[(a)]	 **100**	49 % / 2 h	98%	> 100
2	 **101**	50 % / 1 h	> 99%	> 100
3	 **1**	50 % / 2 h	> 99%	> 100
4[(a)]	 **102**	52 % / 2 h	99%	> 100
5[(a)]	 **107**	39 % / 2 h	26%	3
6[(a)]	 **108**	9 % / 2 h	0	–
7	 **109** [(a)]	52 % / 2 h	> 99%	> 100
8	 **74**	51 % / 1 h	> 99%	> 100
9	 **110**	52 % / 1 h	99%	> 100
10	 **111**	48 % / 1 h	98%	> 100
11	 **85**	48 % / 1 h	99%	> 100
12	 **113**	50 % / 1 h	> 99%	> 100
13	 **104**	52 % / 1 h	99%	> 100
14[(b)]	 **8** **Esomeprazole**	29 % / 2 h	40%	> 100

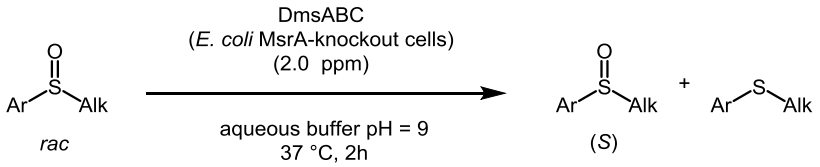
(a) 0.4 ppm of DmsABC, (b) 2.0 ppm of DmsABC (details in experimental section).

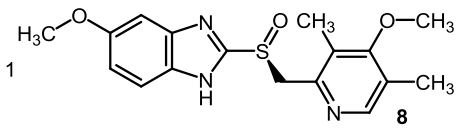
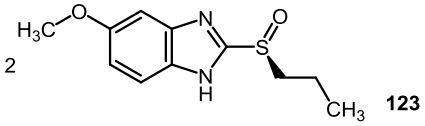
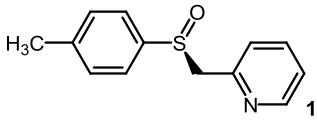
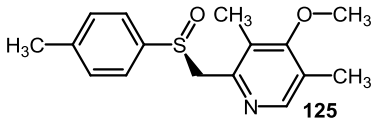
Similar to MsrA, the DmsABC reductase efficiently resolved various aryl methyl/ethyl sulfoxides. Notably, chiral sulfoxides with two adjacent sp^3 carbons, which are typically challenging for chemical catalysts, were resolved with high enantioselectivity. Propyl tolyl sulfoxide **107**, which is unreactive with MsrA, can be processed by DmsABC reductase but with low enantioselectivity (*s* = 3). The isomeric isopropyl tolyl sulfoxide **108** showed even lower reactivity, indicating the enzyme's sensitivity to branching at the alpha carbon of the sulfoxide group. Interestingly, chloromethyl tolyl sulfoxide **109**, also unreactive with MsrA, was a good substrate for DmsABC

reductase, yielding the product with high enantioselectivity (*s* > 100). The chloromethyl group can facilitate further synthetic transformations via nucleophilic substitution or reactions with organometallic species, thereby broadening the range of accessible enantiomerically pure chiral sulfoxides.[58,61,63,64]

Given that the enzyme produces (*S*)-enantiomers of sulfoxides, we tested Omeprazole as a substrate to obtain the widely used drug Esomeprazole **8**. Notably, racemic Omeprazole was resolved with an excellent selectivity factor (*s* > 100). This unexpected reactivity led us to examine substrates **123**, **124,** and **125** to better understand the substitution pattern necessary for achieving high enantioselectivity, especially in comparison to substrate **107** (Table 6). However, these substrates did not exhibit high enantioselectivity, suggesting that other steric or electronic factors may contribute to the high enantioselectivity observed for substrate **8**.

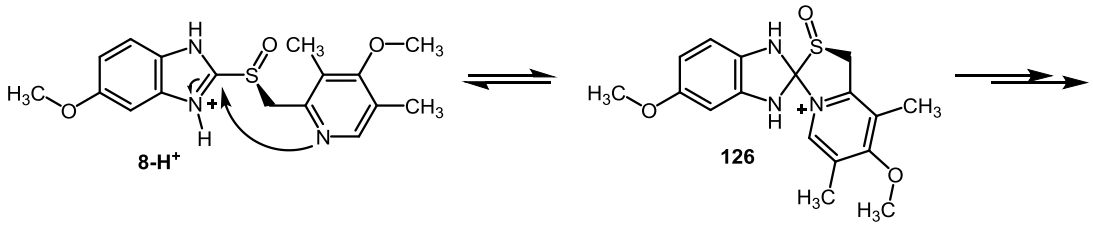
Table 6. Investigation of substitution effects using new sulfoxide substrates with a shared backbone.



entry	product	conversion	ee	s
1	 **8**	29%	40%	>100
2	 **123**	42%	28%	3
3	 **124**	20%	11%	3
4	 **125**	5% (2h) 16% (18h)	n.d. 14%	n.d. 7

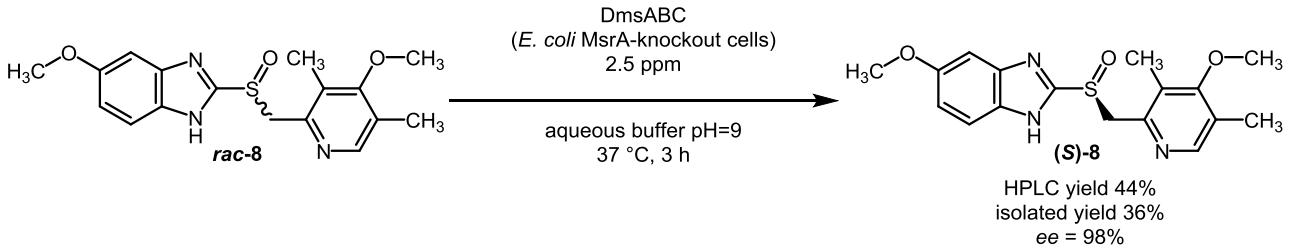
The working hypothesis is that Omeprazole can form a spirocyclic structure **126** which is also proposed in the first step of activation of the drug in the stomach's parietal cells (Scheme 42).[27–29,260–263] This rigid spirocyclic form has a very different spatial organization that can probably fit better in the active site of the enzyme. It would be interesting to test this hypothesis

with stable spirocyclic compounds with a sulfoxide moiety in different positions.



Scheme 42. The initial step of Esomeprazole (pro)drug mechanism of covalent inhibition of the proton pump (ATPase).

To further show the utility of this enzymatic kinetic resolution, we scaled up the process to resolve Omeprazole into the blockbuster drug Esomeprazole. Given the low solubility of Omeprazole in the reaction buffer, we increased the pH to 9, which enhanced its solubility and minimized the formation of unwanted side products.[27–29,167,260–263] Notably, the use of a decane co-solvent was not necessary under these conditions. From 50 mg of Omeprazole, we obtained Esomeprazole **8** with a 38% isolated yield (56% conversion) and an enantiomeric excess of 98% (Scheme 43). This proof-of-concept experiment was conducted in a 100 mL reaction volume without extensive optimization. For larger-scale production of Esomeprazole, increasing the reaction volume or further optimizing conditions (e.g., using a flow reactor) would be necessary.



Scheme 43. Preparative-scale kinetic resolution of Omeprazole.

We developed a new enantiocomplementary enzymatic protocol for highly efficient kinetic resolution of chiral sulfoxides. This method demonstrates excellent enantioselectivity across a wide range of substrates, surpassing many other enantioselective enzymes. Although DmsABC reductase is a complex multidomain, multi-cofactor enzyme system, we successfully developed a simple whole-cell format that can be easily utilized for an efficient resolution providing a blockbuster drug Esomeprazole. With a catalyst loading as low as 0.2 ppm, this approach highlights the power of biocatalysis. This study also highlights the potential for discovering new enzymatic activities in well-known organisms like *E. coli*. Additionally, with Alphafold technology now allowing

accurate predictions of protein structures,[264] including those of DmsABC domains, we can use docking tools to explore new substrates and refine our understanding of enzyme selectivity for future drug development or directed evolution (Figure 13).[265] Notably, in related projects in our lab it was discovered that DmsABC can deoxygenate other substrates than sulfoxides with substantial enantioselectivity.

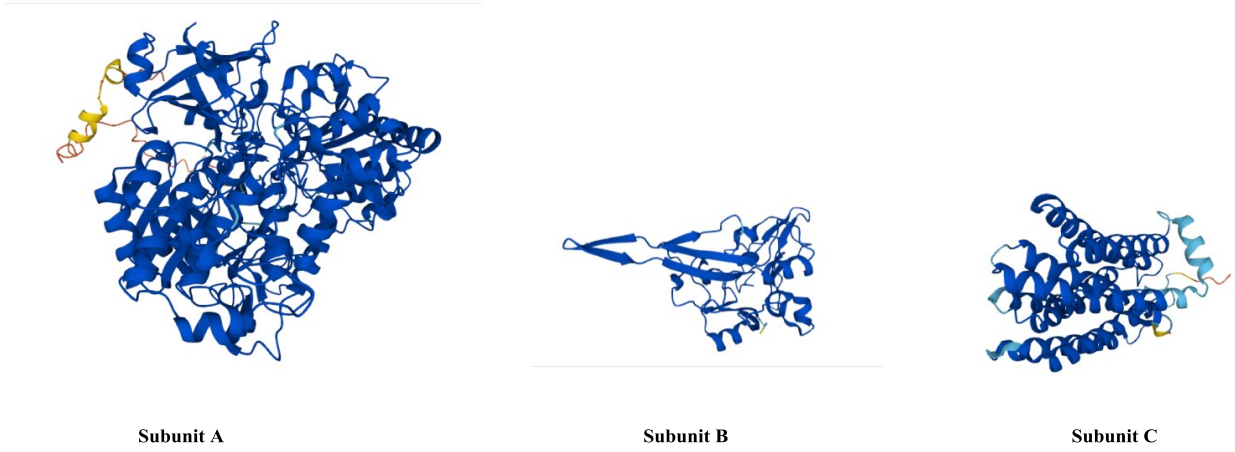
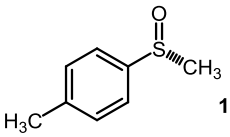
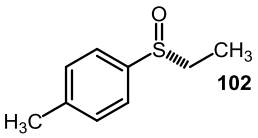
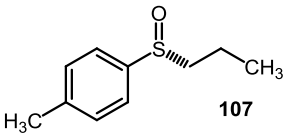


Figure 13. The spatial arrangement of subunits of the multidomain protein dmsABC. The structures are taken from the UniProt database, they were generated using Alphafold.[265]

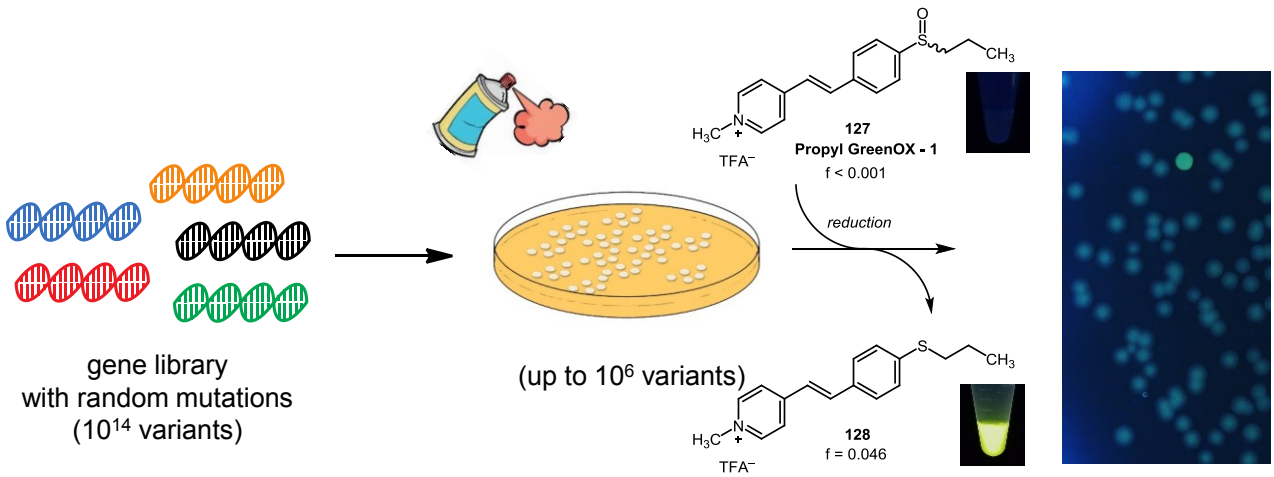
3.4. Expanding the Substrate Scope of MsrA by Directed Evolution

Despite the success of the kinetic resolution using MsrA, certain limitations in terms of substrate scope were observed and are described above. The enzyme exhibited a broad tolerance to substituents on one side of the sulfoxide, but on the other side, only smaller groups like methyl or ethyl were accepted. Larger substituents, such as propyl, prevent the reaction (Table 7). This limitation prompted us to explore methods of directed evolution to expand the substrate scope for MsrA.

Table 7. Substituent limitations in MsrA-based kinetic resolution.

Entry	Product	Conversion	*ee*	*s*
1	 **1**	50 %	99 %	>100
2	 **102**	50 %	99 %	>100
3	 **107**	< 3 %	n.d.	n.d.

Our lab has successfully developed a straightforward high-throughput assay for the directed evolution of sulfoxide reductases, utilizing a novel fluorescent probe **127**, which carries a propyl substituent on the sulfinyl group.[232] The main advantage of this probe is its ability to be applied directly on agar plates containing thousands of *E. coli* colonies expressing variants of MsrA (Scheme 44). This enables a broad and unbiased screening of large sequence spaces without the need for site-directed mutagenesis. This methodology overcomes the mutual bottleneck of high-throughput assays, and could be extended to the evolution of other enzymes with similar activities, even without prior knowledge of their 3D structures or active site locations.



Scheme 44. Visual representation of a high-throughput assay for directed evolution of MsrA.

By using this method, two new variants of MsrA were obtained that were capable of reducing propyl-substituted probe **127**. The sequencing of the variants revealed that both contain three mutations (Figure 14). Interestingly, they share the same amino acid mutation in the active

site. Conserved phenylalanine was substituted for leucine in both variants. Later it was found out that this mutation is solely responsible for the activity. Therefore, a variant MsrA (F52L) with this single mutation was prepared by my colleagues, and I further tested it.

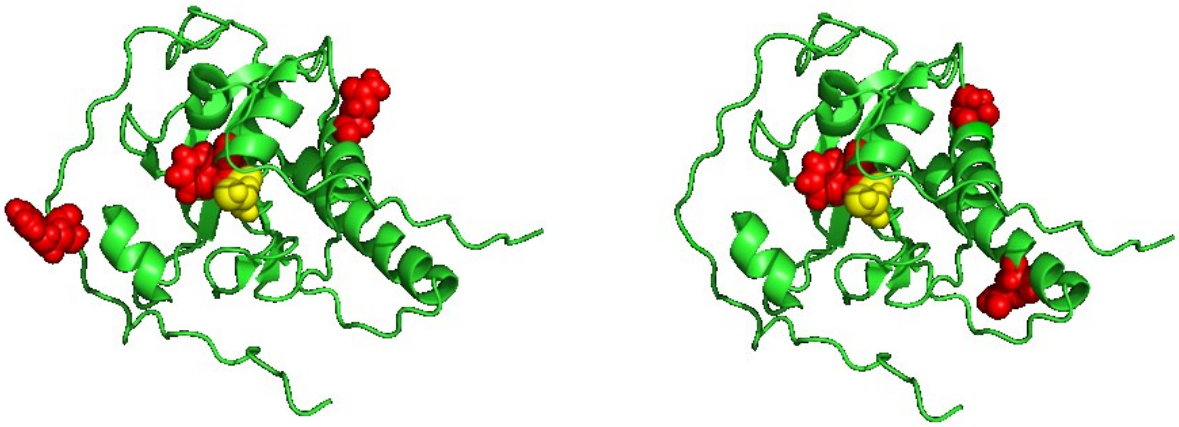
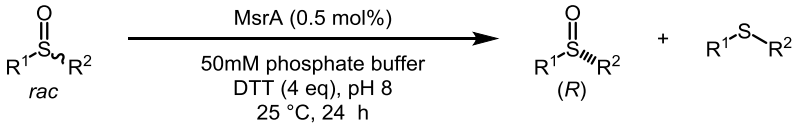
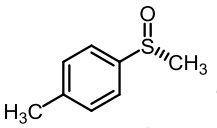
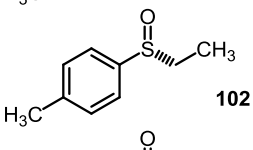
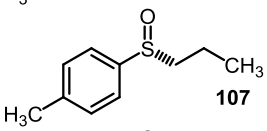
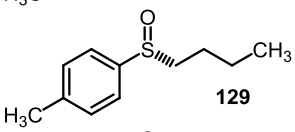
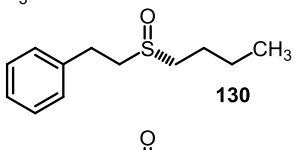
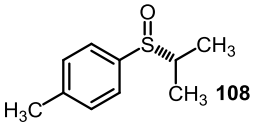
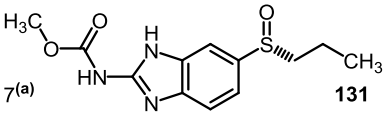


Figure 14. Two new variants of *MsrA with mutations are highlighted in red. The active site cysteine is shown in yellow.*

First, the temperature optimum was determined for the new variant. Three temperatures (25°C, 30°C, and 37°C) were screened and the lowest 25°C turned out to provide the best results in terms of reactivity. This observation may be due to the lower thermal stability of the new variant as it contains the mutation in the inner lipophilic region of the enzyme. Next, a series of substrates was tested in kinetic resolution with the new variant MsrA (F52L) along with the wild-type MsrA as a control (Table 8). As expected, the wild-type MsrA only resolved substrates **1** and **102**, which have smaller methyl and ethyl groups. In contrast, the F52L mutant successfully resolved substrates **107** and **129**, which feature larger propyl and butyl substituents on the sulfoxide moiety. Remarkably, the F52L clone was also able to resolve the challenging substrate **130**, containing two bulky alkyl groups, with excellent enantioselectivity. Additionally, the pharmaceutically important albendazole-(*R*)-sulfoxide **131**, the active metabolite of the anti-parasitic drug albendazole,[266–269] was obtained with high enantioselectivity (94% *ee*). However, the F52L mutant showed limitations with substrate **108**, where branching at the alpha carbon of the sulfoxide moiety reduced its expanded substrate scope.

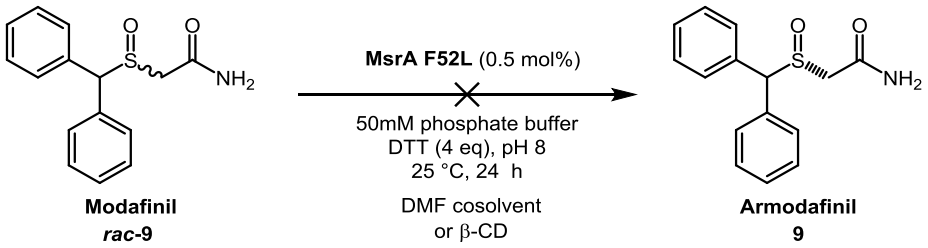
Table 8. The substrate scope of MsrA F52L and MsrA WT in kinetic resolution



Entry	Product	MsrA WT Conversion (*ee*)	MsrA F52L Conversion (*ee*)
1	 **1**	50 % (99 %)	50 % (99 %)
2	 **102**	50 % (99 %)	50 % (99 %)
3	 **107**	<3 %	50 % (99 %)
4	 **129**	<3 %	50 % (99 %)
5	 **130**	<3 %	50 % (99 %)
6	 **108**	<3 %	<3 %
7[(a)]	 **131**	6 % (6 %)	49 % (94 %)

(a) 1.5 mol% of MsrA used

Additionally, the kinetic resolution of modafinil **9** (the racemate of the marketed drug Armodafinil (*R*)-**9**) was attempted. However, no significant conversion was observed (Scheme 45). This lack of reactivity is probably due to the branching effect of the amide group that does not allow the substrate to fit properly into the enzyme's active site. Recently, it has been reported that a variant of MsrA having substitution of phenylalanine in the active site to alanine (our variant has Phe to Leu), can react with substrates bearing ester groups. These directed evolution experiments show that further expansion of the substrate scope of Mrs enzymes is feasible. [270]



Scheme 45. The resolution of modafinil was unsuccessful.

4. Conclusion

In conclusion, our research has achieved three major advances in the biocatalytic resolution of chiral sulfoxides. We first developed a novel deracemization method using the enzyme MsrA, which delivered yields of up to 90% and enantiomeric excesses above 99% ee. The wide substrate scope of MsrA was evident in its ability to deracemize a variety of aryl alkyl and alkyl alkyl sulfoxides when combined with an oxaziridine oxidant in a biphasic system, highlighting its potential for diverse practical applications. Recently, several new studies about deracemization have emerged, as summarized in a comprehensive review.[271] Some of these advancements include combining MsrA with other enzymes in a cascade with cofactor regeneration, offering an alternative method for the synthesis of chiral sulfoxides,[242] or with photocatalysis.[243]

Secondly, we introduced a highly efficient enantiocomplementary enzymatic protocol for kinetic resolution using DmsABC reductase. Despite the complexity of this enzyme system, we simplified the process by employing a whole-cell format, achieving excellent enantioselectivity and resolving important sulfoxides including the blockbuster drug Esomeprazole. This study emphasizes the power of biocatalysis and opens up new possibilities for discovering enzymatic activities in familiar organisms like *E. coli*.

Lastly, through the application of directed evolution, we expanded the substrate scope of the natural MsrA enzyme. The evolved MsrA now effectively resolves challenging chiral sulfoxides, including pharmaceutically relevant compounds such as albendazole-(*R*)-sulfoxide. These advancements collectively represent significant contributions to the field, offering new methodologies and expanding the potential for biocatalytic applications in pharmaceuticals and beyond.

5. Experimental part

5.1. General Methods, Instuments and Materials

5.1.1. Chemical Part

Starting materials and reagents were used as obtained from the suppliers unless otherwise stated. Solvents were dried and distilled according to general procedures before use in the reactions. Analytical TLC was performed using a precoated silica gel 60 Å F254 plates (0.2 mm thickness) and visualized by irradiation with UV light at 254 nm or by dipping in stain solution ($KMnO_4$, AMC) followed by heating. Preparative column chromatography was carried out using silica gel 60 Å (particle size 0.063–0.200mm). Purifications by HPLC were performed under the following conditions: Agilent ZORBAX SBC18 column(5 µL, 9.4 × 150 mm); UV/Vis detection at λ_{obs} = 254 and 300 nm; flow rate 4 mL/min; gradient elution methods (0.1% TFA in H_2O – CH_3CN from 5:95 to 0:100, from 50:50 to 0:100, during 20-25 min (method a-b); or alternatively without TFA (method c-d); or 0.1% ammonium formate in H_2O – CH_3CN from 50:50 to 0:100 in 20 min (method e).^{1}H and ^{13}C NMR spectra were recorded on Varian UNITY INOVA-300, Bruker Avance-400 and Bruker Avance III - 600 instruments. ^{1}H NMR spectra were recorded at 300 MHz, 400 MHz, or 600 MHz. ^{13}C NMR spectra were obtained at 101 MHz and were ^{1}H-decoupled. Deuterated solvents were used as obtained from the suppliers. Chemical shifts (δ) are reported in ppm relative to the solvents ($CDCl_3$: δC = 77.0 ppm; $(CH_3)_2SO$: δC = 40.2 ppm; CD_3OD: δC = 49.0 ppm) or the non-deuterated solvent residual peak ($CHCl_3$: δH = 7.26 ppm; $(CH_3)_2SO$: δH = 2.50 ppm; CHD_2OH: δH = 3.31 ppm) or to the internal TMS standard (δH = 0 ppm). Chemical shifts for ^{19}F NMR are reported in terms of chemical shift in reference to the device's internal standard (fluorobenzene set to δ -112.96 ppm). The spectra were processed and analyzed with Mestrenova software (x64-14.1.0-24037). Accurate mass measurements (HRMS) were obtained by ESI on Bruker Compact Q-TOF spectrometer or an Agilent 6530 Q-TOF MS spectrometer. Analytical HPLC was performed under the following conditions: Agilent Eclipse plus C18 column (3.5 µL, 4.6×100 mm); UV/Vis detection at λ_{obs} = 254 nm, 220 nm or 300 nm, flow rate 0.4 mL/min; gradient elution method (0.1% aqueous formic acid – CH_3CN from 95:5 to 0:100 in 13 min. Alternatively, conditions without formic acid were used. Enantiomeric excesses were determined by HPLC with chiral stationary phase analysis using Daicel Chiralpak (IA, IB, IC) and Chiralcel OD-H columns. A mixture of *n*-heptane/propan-2-ol was used as the eluent. The detailed conditions are given in the characterization part of the products. The spectra measured on HPLC with a chiral stationary phase

were compared with those of a racemic standard. Retention times of the same compounds may vary depending on the quality and batch of solvents used. The absolute configurations of the products were determined by the comparison of HPLC with chiral stationary phase retention times and optical rotation values with the literature. Optical rotations were measured on an automatic polarimeter Autopol III. The samples were measured in chloroform or methanol, with the concentrations *c* reported in g/100 mL. The specific optical rotation of the chiral substances prepared is given in units of 10^{-1}deg cm^2g^{-1}. Selectivity factor *s* was calculated according to formula $s = \ln[(1-c)(1-ee)]/\ln[(1-c)(1+ee)]$.[175] Infrared spectra were recorded on a Thermo Nicolet Avatar 370 FT-IR spectrophotometer or with ATR- IR spectrophotometer. All chemical structures were drawn in ChemDraw v18.2.

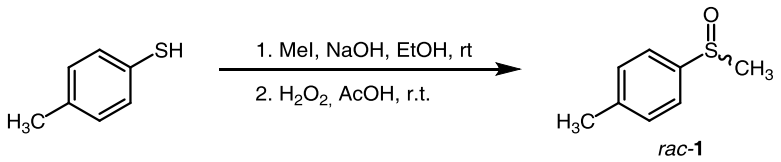
5.1.2. Biochemical Part

Plasmids with Msr genes were cloned in-house or were obtained from Genescript or as a gift from Dr. Moskovitz lab.[259] All buffers and nutrient media (LB Broth, M9 minimal, LB agar, etc.) were prepared according to standard protocols [272,273] and were sterilized in autoclave or by passage through sterilization filters. All biochemical experiments with buffers, cells, and isolated enzymes were carried out using aseptic techniques with sterile vials, flasks, and tips. Plasmid transformations into competent *E. coli* cells were performed according to the standard protocols provided by the suppliers. *E. coli* cells stocks were stored in 50% glycerol solution at –78°C and were transported on dry ice and defrosted on ice. Antibiotics (ampicillin, kanamycin, chloramphenicol, tetracyclin) were prepared as concentrated aqueous stock solutions (1000×, 500×). The optical density (OD_{600}) of cultures was measured on Biodrop. Cells were lysed by an ultrasound probe while keeping them on ice with alternating cycles of burst/rest at 15s/15s duration for 5 or 10 min, or otherwise stated. Isolated enzymes were stored in glycerolic storage buffers at –20°C, –78°C), or in the fridge (4-5°C) for short-term storage. The SDS-PAGE gels consisted of a 12% resolving gel (acrylamide 12%, bis-acrylamide 0.4%, Tris-HCl 375 mM, pH 8.8, SDS 0.1%, ammonium persulfate 0.05%, tetramethylethylenediamine 0.005% v/v) and a 4% stacking gel (acrylamide 4%, bis-acrylamide 0.1%, Tris-HCl 126 mM, pH 6.8, SDS 0.1%, ammonium persulfate 0.05%, tetramethylethylenediamine 0.01% v/v). The SDS-PAGE running buffer contained Tris-HCl 25 mM (pH 8.8), glycine 192 mM, and SDS 0.1%. Samples were prepared by mixing with denaturing SDS-SB(DTT) loading buffer, which included Tris-HCl (50 mM) pH 6.8, SDS (0.1%), sodium borate (25 mM), DTT (100 mM), glycerol (10%), bromophenol blue (0.1%). The samples were either heated at 95°C for 5 minutes before loading or loaded directly onto the SDS-PAGE gel.

The electrophoresis was carried out at a constant voltage of 200 V. Following the separation, the gels were stained using commercial Coomassie Brilliant Blue. Precision Plus Protein™ was used as a protein mass marker (M). Proteomic analyses were performed by Pavel Talacko and Karel Harant in Biocev. Protein structures were visualized by PyMOL (v.2.5.7).

5.2. Synthesis of Organic Compounds

5.2.1. General Procedure for the Synthesis of Racemic Sulfoxides

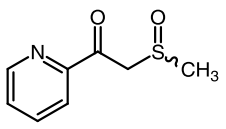


rac-**1**

The reaction sequence was carried out according to literature methods.[274,275] Representative procedure: Methyl iodide (852 mg, 6.0 mmol) was added dropwise to a solution of 4-methylbenzenethiol (500 mg, 4.0 mmol) and NaOH (240 mg, 6.0 mmol) in EtOH (10 mL) and the reaction mixture was stirred at room temperature for 4-5 h. The resulting mixture was diluted with water (10 mL) and extracted with CH_2Cl_2 (3 × 40 mL). The organic phase was washed with H_2O and brine, dried over $MgSO_4$, and concentrated by rotary evaporation under reduced pressure to obtain a crude product, which was taken to the next step without further purification. The latter product was dissolved in glacial acetic acid (4 mL) and H_2O_2 (30 % in water, 1.6 mL, 16.0 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 1 h. Then, the mixture was basified with NaOH (2M aqueous solution) and extracted with CH_2Cl_2 (3 × 30 mL). The organic phase was washed with brine, dried over $MgSO_4$, and concentrated by rotary evaporation under reduced pressure. The crude product was purified by column chromatography on silica gel (cyclohexane/acetone = 1/1) to afford sulfoxide ***rac*-1** in 85% yield (525 mg) as a white solid.

Several commercially available sulfides served as convenient starting points. For the alkylation reaction, commercially available iodo, bromo, or chloroalkyls were used, or tosylates, prepared from alcohols.[276] The spectra of prepared *rac*-Sulfoxides **1**, **74**, **85**, **100**-**102**, **104**, **107**-**108**, **110**-**113**, **129**-**130** are in agreement with the reported data.[146,170,206,274,277–285]

5.2.2. Synthesis of Racemic Sulfoxide 106

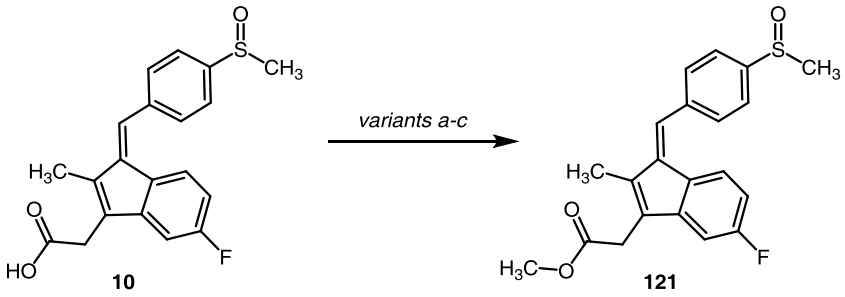


***rac*-Oxisuran (*rac*-106)**.[286] Prepared according to described procedures.[287,288] Picolinic acid (2g, 16.25 mmol) was refluxed in the mixture of methanol (50 mL) and sulfuric acid (5 mL) for 5 h. Then, the reaction mixture was cooled down to

rt, and pH was carefully adjusted with 10% solution of K_2CO_3 to reach pH = 8, and extracted with dichloromethane (3 × 50 mL) Combined organic phase was washed with brine (20 mL), dried over $MgSO_4$ and evaporated to obtain crude product methyl picolinate (1.85 mg, 83%) as yellowish oil which solidified in the fridge. The product was used in the next step without further purification.

Sodium hydride (60% dispersion in oil, 90 mg, 2.25 mmol) was overlayed with DMSO and heated at 80°C (oil bath) under an argon atmosphere for 45 min. The reaction mixture was cooled down to rt and methyl picolinate (274 mg, 2 mmol) as a solution in DMSO (1 mL) was added dropwise while cooling to not exceed 30°C. The reaction mixture was stirred for an additional 3 hours at rt. The resulting brown solution was poured onto cold water and pH was adjusted with 1M HCl solution to pH = 8, and extracted with dichloromethane (3 × 20 mL). The combined organic phase was washed with cold water (10 mL), and cold brine (10 mL), dried over $MgSO_4$, and evaporated. The orange oily residue was purified by column chromatography (dichloromethane/methanol 20:1) to give a mixture of product and DMSO. Further purification by recrystallization from hexane/ethyl acetate (filtered hot), provided pure product ***rac*-106** as a yellowish solid in 13% yield. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.77 – 8.69 (m, 1H), 8.12 – 8.05 (m, 1H), 7.92 – 7.84 (m, 1H), 7.58 – 7.50 (m, 1H), 4.80 (d, *J* = 13.7 Hz, 1H), 4.57 (d, *J* = 13.7 Hz, 1H), 2.77 (s, 3H). The spectrum is in agreement with the reported data.[286]

5.2.3. Synthesis of Sulindac Methyl Ester 121



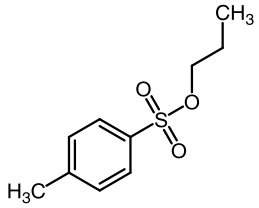
Procedure A. Large-scale synthesis of **121** was achieved by Fischer esterification.[289] ***Rac*-10** (356 mg, 1 mmol) was dissolved in dry methanol (25 mL) to form a yellow solution. Ten drops of concentrated H_2SO_4 were added, and the reaction mixture was refluxed for 90 minutes using a Dean-Stark apparatus. After cooling, the solvent was evaporated, and the residue was dissolved in dichloromethane. The solution was then neutralized with 2M aqueous NaOH solution, washed with water and brine, and dried over $MgSO_4$. Evaporation of the solvent yielded ester **121** in 93% yield (345 mg). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 – 7.69 (m, 2H), 7.69 – 7.64 (m, 2H), 7.19 – 7.11 (m,

2H), 6.88 (dd, *J* = 8.9, 2.4 Hz, 1H), 6.56 (ddd, *J* = 9.2, 8.4, 2.4 Hz, 1H), 3.71 (s, 3H), 3.57 (s, 2H), 2.81 (s, 3H), 2.21 (s, 3H). The spectrum is in agreement with the reported data.[289]

Procedure B. Small-scale protocol was utilized for derivatization after kinetic resolution. **(*R*)-10** (3.6 mg) was dissolved in dry methanol (1 mL). A 2M solution of (trimethylsilyl)diazomethane in hexane was added in three portions (110 µL total) over 35 minutes at room temperature. After 1 hour, nearly complete conversion was confirmed by TLC. The reaction mixture was evaporated, and the residue was purified by small-scale chromatography using a Pasteur pipette (silica gel, dichloromethane/methanol 40:1). The purified product was then used for HPLC with chiral stationary phase determination of enantiomeric excess.

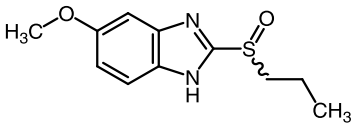
Procedure C. An alternative small-scale protocol was also utilized for derivatization after kinetic resolution. **(*R*)-10** (20 mg) was dissolved in anhydrous DMF (0.5 mL), and K_2CO_3 (20 mg) was added. The reaction mixture was stirred under argon for 5 minutes at room temperature (rt), after which MeI (12 µL) was added in a single portion. The mixture was stirred for an additional 1 hour, then the solvent was evaporated under vacuum. The residue was purified by small-scale chromatography (silica gel, dichloromethane/methanol 30:1). The purified product was subsequently analyzed by HPLC with chiral stationary phase to determine its enantiomeric excess (*ee*).

5.2.4. Synthesis of Tosylate 132



Tosylate **132** was synthesized according to a modified reported procedure.[276] To a solution of tosyl chloride (5.1 g, 26.8 mmol) in dichloromethane (100 mL), propanol (4 mL, 53.2 mmol) was added, followed by the dropwise addition of triethylamine (3.8 mL, 27.3 mmol). The reaction mixture was stirred until the complete disappearance of the starting material. It was then diluted with dichloromethane, washed with water and brine, dried over $MgSO_4$, and evaporated. The crude residue was purified by column chromatography (silica gel, dichloromethane/cyclohexane 1:1), yielding propyl 4-methylbenzenesulfonate **132** in 84% yield (4.8 g). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.83 – 7.74 (m, 2H), 7.38 – 7.31 (m, 2H), 3.98 (t, *J* = 6.5 Hz, 2H), 2.44 (s, 3H), 1.71 – 1.61 (m, 2H), 0.89 (t, *J* = 7.4 Hz, 3H). The spectrum is in agreement with the reported data.[290]

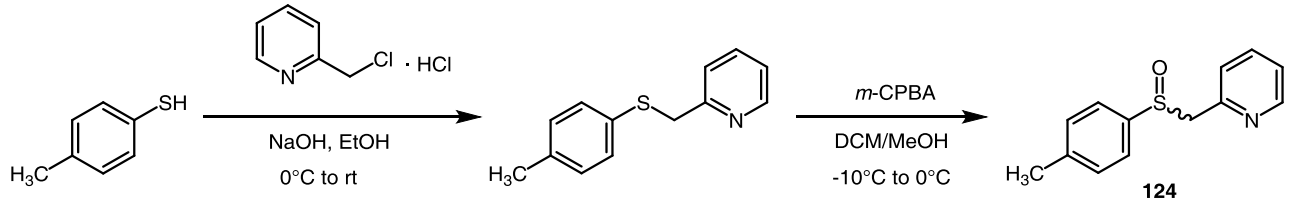
5.2.5. Synthesis of Sulfoxide 123

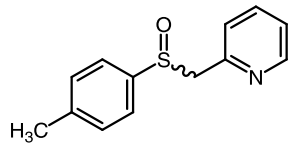


***rac*-5-Methoxy-2-(propylsulfinyl)-1H-benzo[d]imidazole (*rac*-123).** Compound ***rac*-123** was prepared according to modified protocols.[27,291] To a solution of 5-methoxybenzimidazole-2-thiol (360 mg, 2 mmol) in ethanol (10 mL) and water (2 mL) with sodium hydroxide (160 mg, 4 mmol), Propyltosylate (419 mg, 2 mmol) was added portionwise at rt and stirred for 2 h. Then, the temperature was raised to 70°C and the reaction mixture was stirred overnight. Then, the reaction mixture was extracted with dichloromethane (3 × 40 mL), and the organic phase was washed with water (10 mL) and brine (20 mL), dried over anhydrous $MgSO_4$, and concentrated by rotary evaporation under reduced pressure to provide crude sulfide, which was used in the next step without further purification.

To a stirred solution of a crude sulfide in dichloromethane (20 mL) and methanol (1 mL), a solution of *m*-chloroperbenzoic acid (≤77%, 450 mg, 2 mmol) in dichloromethane (10 mL) was added dropwise at –30 °C and stirred at this temperature for 1 h. The reaction mixture was diluted with dichloromethane (70 mL), washed with saturated aqueous solution of $NaHCO_3$ (20 mL), 10% aqueous solution of NaS_2O_3 (10 mL), water (10 mL) and brine (10 mL), dried over $MgSO_4$ and concentrated by rotary evaporation under reduced pressure. Purification of the crude product by column chromatography (silica gel, dichloromethane/methanol 10:1 then 5:1) afforded ***rac*-123** in 47 % yield (222 mg) as a viscous oil. **^{1}H NMR** (400 MHz, $(CD_3)_2SO$): 13.42 (bs, 1H), 7.53 (d, *J* = 8.9 Hz, 1H), 7.08 (s, 1H), 6.91 (dd, *J* = 8.9, 2.5 Hz, 1H), 3.80 (s, 3H), 3.26-3.13 (m, 2H), 1.80-1.67 (m, 1H), 1.60-1,47 (m, 1H), 0.98 (t, 3H). **^{13}C NMR** (101 MHz, $(CD_3)_2SO$): 156.9, 153.4, 137.4, 119.2, 113.7, 97.8, 55.9, 55.6, 15.5, 13.3. **HRMS (ESI)**: *m*/*z* $[M+H]^+$ calcd for $C_{11}H_{15}N_2O_2S^+$ 239.0849; found: 239.08487. **IR**(KBr + acetone): 3070, 1625, 1413, 1305, 1272, 1204, 1156, 1024.

5.2.6. Synthesis of Sulfoxide 124



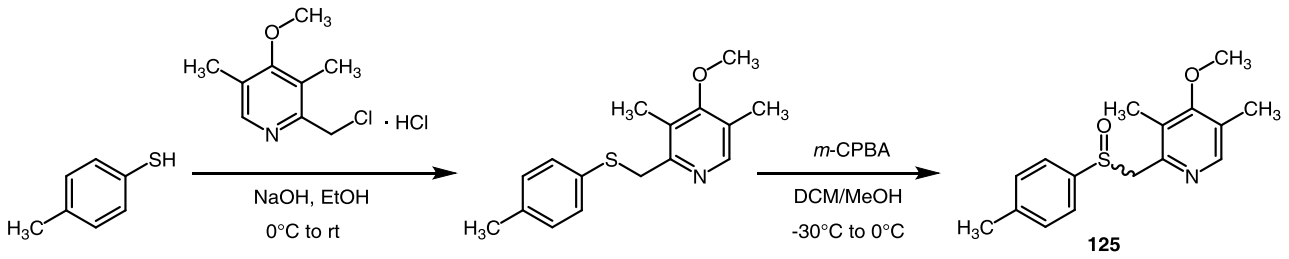


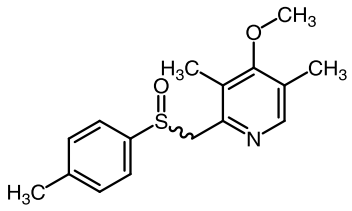
***rac*-2-((*p*-Tolylsulfinyl)methyl)pyridine (*rac*-124).**[292] To a solution of 4-methylbenzenethiol (249 mg, 2 mmol) in ethanol (10 mL) and sodium hydroxide (320 mg, 8 mmol) 2-(chloromethyl)pyridine hydrochloride

(324 mg, 2 mmol) in water (5 mL) was added dropwise at 0°C, then the temperature was raised to rt and stirred for 4 h. The reaction mixture was filtered, extracted with dichloromethane (3 × 40mL), and the organic phase was washed with water (10 mL) and brine (20 mL), dried over anhydrous $MgSO_4$, and concentration by rotary evaporation under reduced pressure provided yellow oil, which was used in the next step without further purification.

To a stirred solution of a crude sulfide in dichloromethane/methanol 9:1 (20 mL), a solution of *m*-chloroperbenzoic acid (≤77%, 450 mg, 2 mmol) was added dropwise at 0°C and stirred at this temperature for 2 h. The reaction mixture was diluted with dichloromethane (80 mL), washed with saturated aqueous solution of $NaHCO_3$ (20 mL), 10% aqueous solution of NaS_2O_3 (10 mL), water (10 mL) and brine (10 mL), dried over $MgSO_4$ and concentrated by rotary evaporation under reduced pressure. Purification of the crude product by column chromatography (silica gel, dichloromethane/methanol 10:1) afforded ***rac*-124** in 70 % yield (325 mg) as an off-white solid. **^{1}H NMR** (400 MHz, CD_3Cl): 8.59-8.52 (m, 1H), 7.70-7.62 (m, 1H), 7.44-7.39 (m, 2h), 7.29-7.19 (m, 4H), 4.26 (d, *J* = 12.5 Hz, 1H), 4.21 (d, *J* = 12.5 Hz, 1H), 2.39 (s, 3H). The spectrum is in agreement with the reported data.[292]

5.2.7. Synthesis of Sulfoxide 125

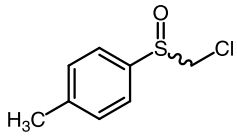




***rac*-4-Methoxy-3,5-dimethyl-2-((p-tolylsulfinyl)methyl)pyridine (*rac*-125)**. To a solution of 4-methylbenzenethiol (373 mg, 3 mmol) in ethanol (15 mL) and sodium hydroxide (480 mg, 12 mmol) 2-(chloromethyl)-4-methoxy-3,5-dimethylpyridine hydrochloride (663 mg, 3 mmol) was added in portions as a solid at 0°C, then the temperature was raised to rt and stirred for 18 h. The reaction mixture was filtered, extracted with dichloromethane (3 × 40mL), and the organic phase was washed with water (10 mL) and brine (20 mL), dried over anhydrous $MgSO_4$, and concentration by rotary evaporation under reduced pressure provided yellow oil, which was used in the next step without further purification.

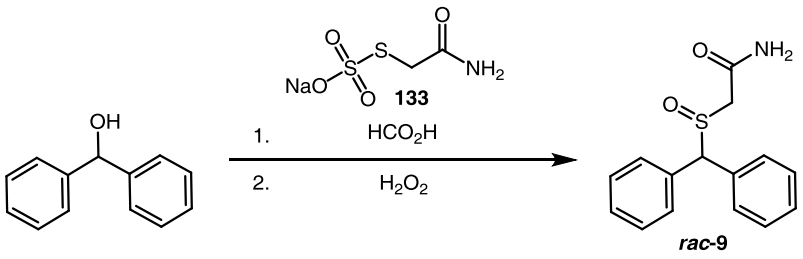
To a stirred solution of a crude sulfide in dichloromethane/methanol 9:1 (15 mL), a solution of *m*-chloroperbenzoic acid (≤77%, 675 mg, 3 mmol) in dichloromethane (15 mL) was added dropwise at –30°C and stirred at this temperature for 2 h. The reaction mixture was diluted with dichloromethane (70 mL), washed with 10% aqueous solution of NaS_2O_3 (15 mL), saturated aqueous solution of $NaHCO_3$ (20 mL), water (15 mL) and brine (15 mL), dried over $MgSO_4$ and concentrated by rotary evaporation under reduced pressure. Purification of the crude product by column chromatography (adsorbed on silica gel with methanol, elution mixture cyclohexane/ethyl acetate 1:1 to 0:1) yielded 89 % of ***rac*-125** as a white solid (770 mg). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.22 (s, 1H), 7.48 – 7.40 (m, 2H), 7.30 – 7.21 (m, 2H), 4.43 (d, J = 12.5 Hz, 1H), 4.07 (d, J = 12.5 Hz, 1H), 3.71 (s, 3H), 2.39 (s, 3H), 2.24 (s, 3H), 2.09 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 164.3, 149.7, 149.6, 141.7, 140.6, 129.7, 127.2, 125.9, 124.2, 63.8, 59.9, 21.5, 13.4, 11.5. **HRMS (ESI+)**: *m*/*z* $[M+H]^+$ calcd for $C_{16}H_{20}NO_2S^+$ 290.1209; found: 290.1209 **IR(KBr)**: 1589, 1565, 1470, 1395, 1269, 1090, 1072, 1048, 1000.

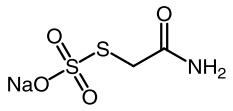
5.2.8. Synthesis of Sulfoxide 109



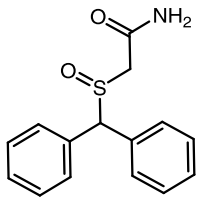
(*rac*)-1-((Chloromethyl)sulfinyl)-4-methylbenzene (*rac*-109).[293] Prepared according to reported procedures.[293–295] To a solution of *rac*-**1** (308 mg, 2 mmol) in dry dichloromethane (10 mL), NCS (280 mg, 2.1 mmol) was added in small portions at room temperature under argon. The reaction was stirred for 2 days, then diluted with dichloromethane, and washed with 4% aqueous solution of KI, 10% aqueous solution of $Na_2S_2O_3$, and brine, then dried over $MgSO_4$. After evaporation, the residue was purified by column chromatography (cyclohexane/acetone 4:1 to 3:1), yielding ***rac*-109** as a white solid in 85% yield (320 mg). Adding base (K_2CO_3) gave me poorer results while using excess NCS led to over-chlorination. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.65 – 7.55 (m, 2H), 7.42 – 7.31 (m, 2H), 4.36 (s, 2H), 2.42 (s, 3H). The spectrum is in agreement with the reported data.[293]

5.2.9. Synthesis of Sulfoxide 9 (Modafinil)





Sodium S-(2-amino-2-oxoethyl) sulfurothioate 133.[296] The reagent was prepared according to the literature procedure.[296] Chloroacetamide (2 g, 21.4 mmol) and sodium thiosulfate (5 g, 31.6 mmol) were dissolved in water (10 mL) and heated at 70°C for several hours. The mixture was cooled to rt and then placed into fridge overnight. White needle crystals were collected, washed with methanol, and then dried on the vacuum at 70°C, yielding 3.4 g (81%) of product **133**. The reagent was used for the next step without further purification. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.05 (s, 2H).

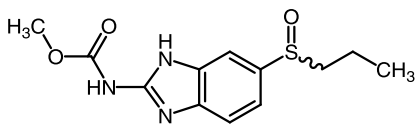


***rac*-Modafinil (*rac*-9).**[296] Modafinil was prepared according to the literature procedure.[296] Mixture of benzhydrol (368 mg, 2 mmol), crude reagent **125** (560 mg), formic acid (2 mL, 80%) and water (0.5 mL) was heated at 80°C for 2h (still not fully dissolved). Additional water (5 mL) was added. The viscous reaction mixture was cooled down to 0°C and H_2O_2 (0.25 mL, 35 %) was added dropwise and stirred for 2h at rt. The reaction mixture was suspended with additional water (5 mL), and the solid was filtered off with frit S4, washed with water, and dried under vacuum. The crude product was purified by column chromatography (ethyl acetate, then ethyl acetate/methanol 9:1) providing sulfoxide Modafinil ***rac*-9** in 48% yield (265 mg) as off-white solid and sulfide **134** in 39% yield (202 mg) as a white solid.

Sulfoxide ***rac*-9: ^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.54 – 7.47 (m, 2H), 7.47 – 7.32 (m, 8H), 7.05 (bs, 1H), 5.62 (bs, 1H), 5.19 (s, 1H), 3.49 (d, *J* = 14.4 Hz, 1H), 3.10 (d, *J* = 14.4, 1H). The spectrum is in agreement with the reported data.[279]

Sulfide **134**: **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.46 – 7.37 (m, 4H), 7.37 – 7.30 (m, 4H), 7.29 – 7.21 (m, 2H), 6.51 (bs, 1H), 5.63 (bs, 1H), 5.18 (s, 1H), 3.09 (s, 2H). The spectrum is in agreement with the reported data.[297]

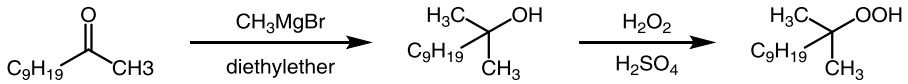
5.2.10. Synthesis of Sulfoxide 131 (Albendazole Sulfoxide)



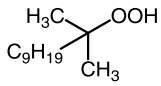
***rac*-Albendazole sulfoxide (*rac*-131).** Albendazole (135 mg, 0.5 mmol) was dissolved in dichloromethane (3 mL) and acetic acid (0.5 mL). H_2O_2 (35% in water, 0.21 mL) was added dropwise at room temperature, and the reaction mixture was stirred for 65 minutes. Then, a saturated aqueous solution of $NaHCO_3$ (30 mL) was added slowly, followed by extraction with dichloromethane (3 × 30 mL). The combined organic layers were washed with water, then brine, and dried over $MgSO_4$. After evaporation, the crude residue was purified by column

chromatography (silica gel, cyclohexane/acetone 1:1 to 0:1, followed by repurification with dichloromethane/methanol 40:1 to 20:1), yielding ***rac*-131** as an off-white solid (94 mg, 67%). ^{1}H NMR (400 MHz, DMSO) δ 12.40 – 11.76 (bs, 1H), 11.76 – 11.05 (bs, 1H), 7.69 (d, J = 1.7 Hz, 1H), 7.55 (d, J = 8.3 Hz, 1H), 7.32 (dd, J = 8.3, 1.7 Hz, 1H), 3.77 (s, 3H), 2.90 – 2.69 (m, 2H), 1.69 – 1.54 (m, 1H), 1.54 – 1.40 (m, 1H), 0.95 (t, J = 7.4 Hz, 3H). The spectrum is in agreement with the reported data.[298]

5.2.11. Synthesis of Oxidant 117



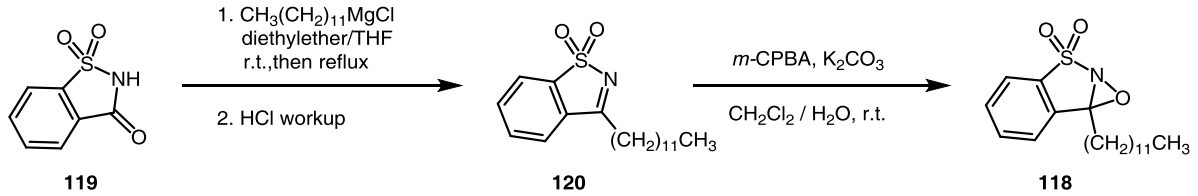
117

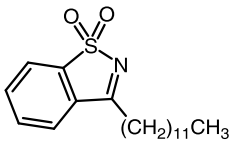


2-**Hydroperoxy-2-methylundecane (117)**.[238] Peroxide **117** was prepared according to the literature procedure.[238] To a dry flask, 3M MeMgBr solution in Et_2O (1.1 mL, 3.3 mmol) was added and cooled to – 78 °C. Then a solution of 2-undecanone (0.61 mL, 3 mmol) in Et_2O (2.2 mL) was added dropwise and then stirred for an additional 1 h at – 78 °C and another 45 min at rt. The reaction was quenched by the addition of 1M HCl (1 mL) and extracted with Et_2O (2 × 15 mL). The combined organic phase was washed with a saturated aqueous solution of $NaHCO_3$ (10 mL) and brine (10 mL), dried over $MgSO_4$, and evaporated to obtain crude product 2-methylundecan-2-ol as a colorless oil (483 mg, 87%), which was used in the next step without further purification.

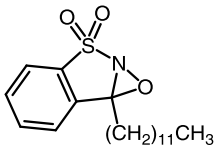
A solution of 2-methylundecan-2-ol (200 mg, 1.1 mmol) in dioxane (1 mL) was added dropwise to a cooled mixture of H_2SO_4 (96%; 0.23 mL), water (0.16 mL), and H_2O_2 (30%; 1.34 mL) at –10 °C. The reaction was heated to 60 °C for 24 hours. Monitoring by TLC (cyclohexane/ethyl acetate 4:1) showed incomplete conversion, so an additional portion of H_2O_2 (30%; 0.67 mL) was added at –10 °C, and the mixture was warmed again to 60 °C and stirred for another 24 hours. After cooling, the reaction was quenched cautiously with a saturated K_2CO_3 solution to adjust the pH to basic. The mixture was extracted with cyclohexane (3 × 20 mL), and the combined organic phases were washed with saturated aqueous solution of $NaHCO_3$ and brine, dried over $MgSO_4$, and evaporated. The solid residue was purified by column chromatography (cyclohexane/ethyl acetate 8:1) to give the product **117** as a pure oil in 51% yield (110 mg). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.12 (s, 1H), 1.38 – 1.17 (m, 16H), 1.21 (s, 6H), 0.92 – 0.84 (m, 3H). The spectrum is in agreement with the reported data.[238]

5.2.12. Synthesis of Oxidant 118





3-Dodecylbenzo[*d*]isothiazole 1,1-dioxide (120). Compound **120** was prepared by a modification of the reported procedure.[299] Saccharin **119** (2.75 g, 15 mmol, recrystallized from acetone) was dissolved in dry THF (30 mL) under argon and a 1M solution of dodecylmagnesium bromide in Et_2O (34 mL, 34 mmol) was added dropwise at room temperature. The reaction mixture was stirred overnight at room temperature, followed by reflux for 4 h. The resulting mixture was cooled to room temperature, quenched with 1M HCl (20 mL), and extracted with CH_2Cl_2 (4 × 150 mL). The organic phase was washed with brine, dried over $MgSO_4$, and concentrated by rotary evaporation under reduced pressure to obtain colorless oil, which solidified on standing. Dodecane impurity was subsequently distilled off under reduced pressure and the residue was purified by column chromatography on silica gel (cyclohexane/CH_2Cl_2 1:2) providing **120** in 70% yield (3.50 g) as an off-white solid. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.94 – 7.90 (m, 1H), 7.77 – 7.66 (m, 3H), 3.00 – 2.92 (m, 2H), 1.92 – 1.85 (m, 2H), 1.52 – 1.43 (m, 2H), 1.37 –1.22 (m, 16H), 0.91 – 0.85 (t, J = 6.8 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 176.3, 139.8, 133.8, 133.5, 131.4, 123.8, 122.5, 31.9, 31.2, 29.63, 29.62, 29.59, 29.40, 29.35, 29.3, 29.2, 25.4, 22.7, 14.1. **HRMS (ESI)**: m/z $[M+H]^+$ calcd for $C_{19}H_{30}NO_2S^+$ 336.1992; found: 336.2008. **IR(KBr)**: 2920, 2851, 1607, 1559, 1329, 1174, 1126.



7b-Dodecyl-7b*H*-benzo[*d*][1,2]oxaziridin[2,3-*b*]isothiazole 3,3-dioxide (118)

Compound **118** was prepared by the reported procedure.[99] To a solution of **120** (127 mg, 0.38 mmol) in CH_2Cl_2 (4 mL) a saturated aqueous solution of K_2CO_3 (4 mL) was added followed by a solution of 3-chloroperoxybenzoic acid (≤ 77%, 95 mg, 0.42 mmol) in CH_2Cl_2 (4 mL). The reaction mixture was stirred for 2 h at room temperature. The resulting mixture was extracted with CH_2Cl_2 (3 × 20 mL) and the organic phase was washed with water (10 mL) and brine, dried over $MgSO_4$, and concentrated by rotary evaporation under reduced pressure. The residue was purified by column chromatography on silica gel (cyclohexane/CH_2Cl_2 1:1) to afford **118** in 86% yield (114 mg) as a white solid. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.82 – 7.68

(m, 4H), 2.59 (ddd, *J* = 15.1, 8.8, 6.4 Hz, 1H), 2.23 (ddd, *J* = 15.1, 9.1, 7 Hz, 1H), 1.62 – 1.51 (m, 2H), 1.46 – 1.34 (m, 2H), 1.33 – 1.19 (m, 16H), 0.88 (t, *J* = 6.8 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) *δ* 134.9, 134.0, 133.9, 132.5, 125.7, 124.1, 86.3, 31.9, 29.63 (2xC), 29.57, 29.40, 29.36, 29.3, 29.2, 28.6, 23.6, 22.7, 14.1. **HRMS (ESI)**: *m*/*z* $[M+H]^+$ calcd for $C_{19}H_{30}NO_3S^+$ 352.1941; found: 352.1957. **IR(KBr)**: 2917, 2848, 1365, 1183, 1162.

5.3. Preparation of the Enzymes

5.3.1. Preparation of MsrA from *E. coli*

A glycerol stock of *E. coli* BL21(DE3) with *msrA* gene in pGEX vector[259] was used for the preculture (2 mL) that was inoculated to 200 mL of LB medium with ampicillin (100 mg/L). The bacterial culture was incubated at 37 °C, 200 rpm until OD_{600} ≈ 1 was reached. Then the production of MsrA was induced with 0.5 mM IPTG and the culture was incubated at 37 °C, 200 rpm for an additional 2 h. Then, cells were harvested by centrifugation (4000 × g for 30 min at 4 °C). The cell pellet was resuspended in PBS buffer (10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 2.7 mM KCl, 140 mM NaCl, pH 7.4) and lysed by sonication on ice. Cell debris was removed by centrifugation (10 000 × g for 30 min at 4 °C), the soluble fraction was loaded onto a glutathione agarose column (Protino Glutathione Agarose 4B, Macherey-Nagel) and the resulting suspension was gently mixed for 30 min at ambient temperature. The column was washed with 5 × 10 bed volumes of PBS and finally, the protein was eluted with 3 × 1 bed volume of elution buffer (50 mM Tris-HCl, 10 mM glutathione, pH 8). Purity was determined via SDS-PAGE. The elution buffer was exchanged with storage buffer (20 mM Tris-HCl buffer, 1 mM DTT, 10% glycerol, 0.1 M NaCl, pH = 8.0) with Amicon Ultra-4 centrifugal filter (3000 MWCO). This procedure typically yielded ≈ 50 mg of protein per liter of culture. Protein concentration was determined from the absorbance at 280 nm (using the calculated extinction coefficient).

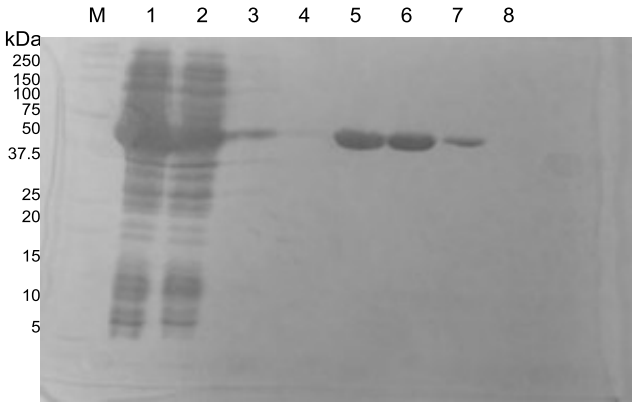


Figure 15. SDS-PAGE of MsrA (E. coli) purification. (M)-protein mass marker, (1)- lysate, (2)-flow through, (3-4)-washes, (5-7)-elutions.

5.3.2. Preparation of MsrA from *S. cerevisiae*

A glycerol stock of *E. coli* BL21(DE3) with *msrA* gene in pQE30 vector[300] was used for the preculture (4 mL) that was inoculated to 500 mL of LB medium with ampicillin (100 mg/L). The bacterial culture was incubated at 37 °C, 200 rpm until OD_{600} ≈ 0.5 was reached. Then the production of MsrA was induced with 0.5 mM IPTG and the culture was incubated at 37 °C, 200 rpm for an additional 4 h. Then, cells were harvested by centrifugation (4000 × g for 30 min at 4 °C). The cell pellet was resuspended in LEW buffer (50 mM NaH_2PO_4, 300 mM NaCl, pH 8) and lysed by sonication on ice. Cell debris was removed by centrifugation (10 000 × g for 30 min at 4 °C), and the soluble fraction was loaded onto the affinity column (Protino Ni-IDA Resin, Macherey-Nagel). The column was washed with 2 × 4 bed volumes of LEW buffer, and the protein was eluted with 3 × 1.5 bed volumes of elution buffer (50 mM NaH_2PO_4, 300 mM NaCl, 250 mM imidazole, pH 8). Purity was determined via SDS-PAGE. The elution buffer was exchanged with storage buffer (20 mM Tris-HCl buffer, 1 mM DTT, 10% glycerol, 0.1 M NaCl, pH = 8.0) with Amicon Ultra-4 centrifugal filter (3000 MWCO). This procedure yielded approximately 24 mg of protein per liter of culture. Protein concentration was determined from the absorbance at 280 nm (using the calculated extinction coefficient).

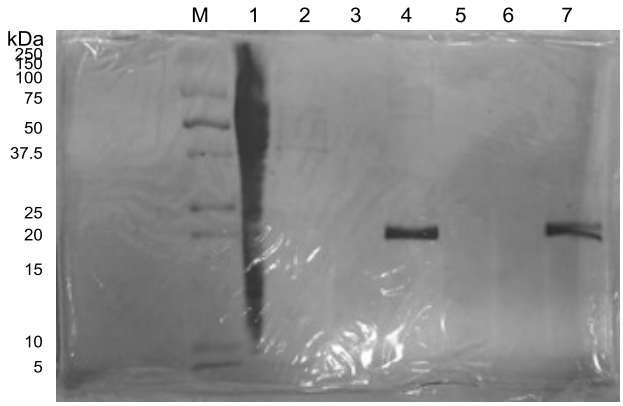


Figure 16. SDS-PAGE of MsrA (S. cerevisiae). (M)-protein mass marker, (1)-flow through, (2-3)-washes, (4)-elution, (5-7)-repeated at lower loadings.

5.3.3. MsrB from *H. sapiens* (CBS-1),[210] *N. gonorrhoeae* (pilB),[202] *T. kodakarensis* (MsrB)[251] and *A. thaliana* (MsrB2)[207]

Genes encoding MsrB domains from *H. sapiens* (CBS-1), *N. gonorrhoeae* (pilB), and *T. kodakarensis* (MsrB) in pET16b vectors (from Genescript), as well as MsrB2 from *A. thaliana* in the pQE30 vector, were transformed into chemically competent *E. coli* BL21(DE3) cells (Agilent) following the manufacturer's protocol. An aliquot of the transformed cells was plated onto LB agar containing ampicillin (100 mg/L) and grown overnight at 37 °C. One colony from the corresponding MsrB-expressing strain was used to inoculate an overnight preculture, which (0.4 mL) was then used to inoculate 100 mL of LB medium with ampicillin (100 mg/L). The bacterial culture was incubated at 37 °C, 200 rpm, until OD_{600} reached approximately 0.4. MsrB production was induced by the addition of 0.2 mM IPTG (for MsrB2 from *A. thaliana*) or 0.5 mM IPTG (for all other MsrBs). The culture was incubated overnight at 22 °C (MsrB2 from *A. thaliana*) or at 37 °C (for all other MsrBs), with shaking at 200 rpm. The cells were harvested by centrifugation (4 000 × g for 30 minutes at 4 °C). The cell pellet was resuspended in LEW buffer (50 mM NaH2PO4, 300 mM NaCl, pH 8) and lysed by sonication. After centrifugation (10 000 × g for 30 minutes at 4 °C), the soluble fraction was loaded onto a Protino Ni-IDA resin column (Macherey-Nagel) and purified by gravity flow at ambient temperature. The column was washed with 2 × 8 mL of LEW buffer, and the protein was eluted with 2 × 2 mL of elution buffer (50 mM NaH2PO4, 300 mM NaCl, 250 mM imidazole, pH 8). Purity was determined via SDS-PAGE. The elution buffer was exchanged for storage buffer (20 mM Tris-HCl, 1 mM DTT, 10% glycerol, 0.1 M NaCl, pH 8.0) using an Amicon Ultra-4 centrifugal filter (3 000 MWCO), yielding approximately 10 mg of protein. The CBS-1 reductase from *H. sapiens* provided only a very low yield of the desired product, so it was excluded

from further experiments. Protein concentration was determined from the absorbance at 280 nm (using the calculated extinction coefficient).

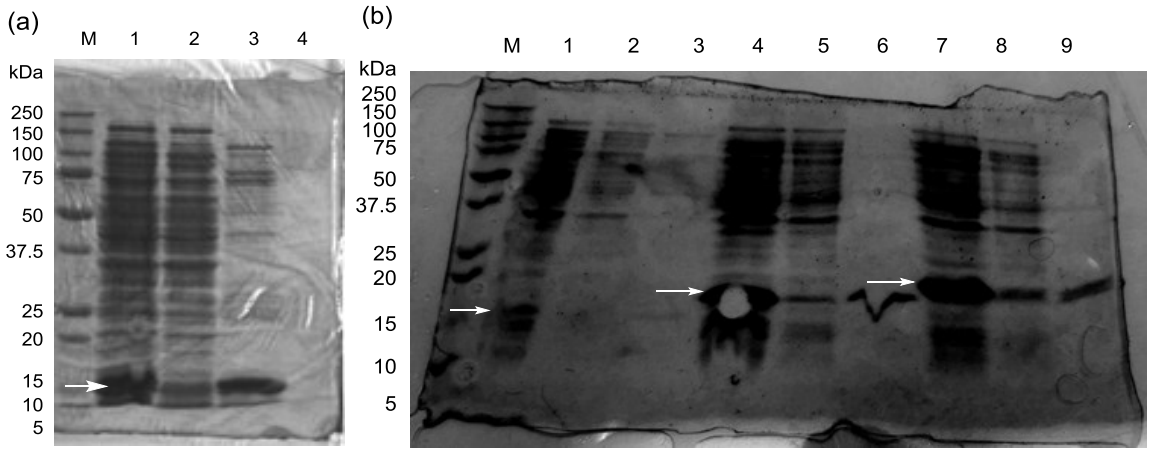
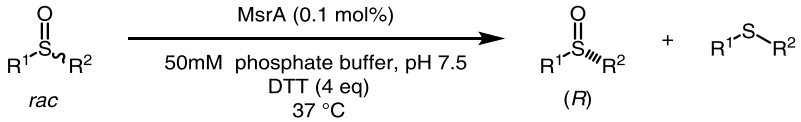
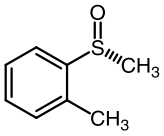


Figure 17. (a) SDS-PAGE MsrB2 (A. thaliana): (M)-protein mass marker, (1)-lysate, (2)-flow through, (3)-elution; (b): SDS-PAGE MsrBs: (M)-protein mass marker; CBS-1 (H. sapiens): (1)-lysate, (2)- flow through, (3)- elution; PilB (N. gonorrhoeae): (4)-lysate, (5)- flow through, (6)- elution; MsrB (T. kodakarensis): (7)-lysate, (8)- flow through, (9)- elution.

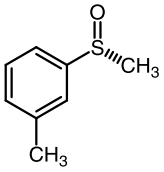
5.4. General Procedure for the Kinetic Resolution of Racemic Sulfoxides with Isolated Enzymes MsrA



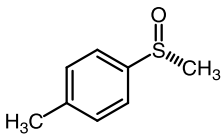
A 1.5 mL Eppendorf tube was charged with individual solutions of DTT (1448 µL of 27 mM, 4 equivs., 26 mM final concentration) in phosphate buffer (50 mM Na_2HPO_4, 50 mM NaH_2PO_4, 50 mM NaCl, pH = 7.5) and a racemic sulfoxide (30 µL of 324 mM, 9.7 µmol, 6.5 mM final concentration) in CH_3CN. The resulting mixture was gently mixed and the reaction was initiated by the addition of MsrA (22 µL of 444 µM, 0.1 mol%, 6.5 µM final concentration) in a storage buffer. The reaction mixture was incubated at 37 °C at 250 rpm. The course of the reaction was monitored by analytical HPLC. After the reaction reached 50% conversion (typically 1 - 6 h, or otherwise stated), the reaction mixture was extracted with toluene (2 × 4 mL), washed with H_2O (2 mL), brine (2 mL), dried under $MgSO_4$ and concentrated by rotary evaporation under reduced pressure. The enantiomeric excess of the crude product was determined by HPLC with the chiral stationary phase.



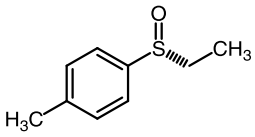
(*R*)-1-Methyl-2-(methylsulfinyl)benzene ((*R*)-100).[301] $C_8H_{10}OS$ (154.23 g/mol), MsrA (*S. cerevisiae*): conversion 50% in 9 h, *ee* > 99%, *s* > 100. The enantiomeric excess was determined by HPLC (IC chiralpak: heptane/propan-2-ol (80:20); flow rate 1 mL/min; 25 °C, 220 nm, t_r= 22.9 and 24.5 min); MsrA (*E. coli*): conversion 50%, *ee* > 99%, *s* > 100. The enantiomeric excess was determined by HPLC (IC chiralpak: heptane/propan-2-ol (80:20); flow rate 1 mL/min; 25 °C, 220 nm, t_r= 22.4 and 23.9 min).



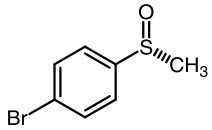
(*R*)-1-Methyl-3-(methylsulfinyl)benzene ((*R*)-101).[302] $C_8H_{10}OS$ (154.23 g/mol), MsrA (*S. cerevisiae*): conversion 50%, *ee* > 99%, *s* > 100. The enantiomeric excess was determined by HPLC (IB chiralpak: heptane/propan-2-ol (90:10); flow rate 0.25 mL/min; 25 °C; 220 nm; t_r= 51.1 and 53.4 min).; MsrA (*E. coli*): conversion 50%, *ee* > 99%, *s* > 100. The enantiomeric excess was determined by HPLC (IC chiralpak: heptane/propan-2-ol (80:20); flow rate 1 mL/min; 25 °C; 220 nm; t_r= 23.2 and 27.8 min).



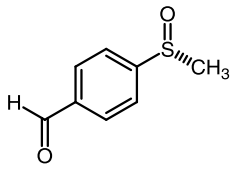
(*R*)-1-Methyl-4-(methylsulfinyl)benzene ((*R*)-1).[146] $C_8H_{10}OS$ (154.23 g/mol), MsrA (*S. cerevisiae*): conversion 50%, *ee* > 99%, *s* > 100. The enantiomeric excess was determined by HPLC (IA chiralpak: heptane/propan-2-ol 95:5; flow rate 0.25 mL/min; 25 °C; 220 nm; t_r= 65.2 and 67.9 min); MsrA (*E. coli*): conversion 50%, *ee* > 99%, *s* > 100. The enantiomeric excess was determined by HPLC (IA chiralpak: heptane/propan-2-ol 95:5; flow rate 0.25 mL/min; 25 °C; 220 nm; t_r= 69.1 and 72.5 min).



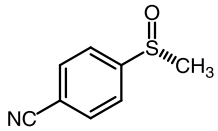
(*R*)-1-(Ethylsulfinyl)-4-methylbenzene ((*R*)-102).[278] $C_9H_{12}OS$ (168.25 g/mol), MsrA (*S. cerevisiae*): conversion 49% in 10 h, *ee* = 98%, *s* > 100. The enantiomeric excess was determined by HPLC (IA chiralpak: heptane/propan-2-ol (95:5); flow rate 0.5 mL/min; 25 °C; 220 nm; t_r= 27.1 and 29.2 min); MsrA (*E. coli*): conversion 50% in 10 h, *ee* > 99%, *s* > 100. The enantiomeric excess was determined by HPLC (IA chiralpak: heptane/propan-2-ol (95:5); flow rate 0.5 mL/min; 25 °C; 220 nm; t_r= 28.7 and 31.4 min).



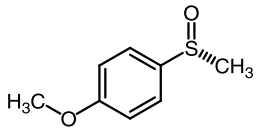
(*R*)-1-Bromo-4-(methylsulfinyl)benzene ((*R*)-74).[206] C_7H_7BrOS (219.10 g/mol), MsrA (*S. cerevisiae*): conversion 50%, *ee* > 99%, *s* > 100. The enantiomeric excess was determined by HPLC (IC chiralpak: heptane/propan-2-ol (80:20); flow rate 1 mL/min; 25 °C; 220 nm; t_r= 18.2 and 19.6 min).; MsrA (*E. coli*): conversion 50%, *ee* > 99%, *s* > 100. The enantiomeric excess was determined by HPLC (IC chiralpak: heptane/propan-2-ol (80:20); flow rate 1 mL/min; 25 °C; 220 nm; t_r= 18.2 and 19.7 min).



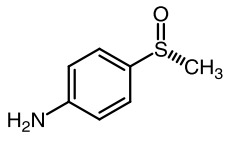
(*R*)-4-(Methylsulfinyl)benzaldehyde ((*R*)-103). $C_8H_8O_2S$ (168.21 g/mol), MsrA (*S. cerevisiae*): conversion 50%, *ee* > 99%, *s* > 100;. The enantiomeric excess was determined by HPLC (IC chiralpak: heptane/propan-2-ol (80:20); flow rate 1 mL/min; 25 °C; 220 nm; t_r= 50.1 and 54.4 min). MsrA (*E. coli*): conversion 51%, *ee* = 99%, *s* > 100. The enantiomeric excess was determined by HPLC (IC chiralpak: heptane/propan-2-ol (80:20); flow rate 1 mL/min; 25 °C; 220 nm; t_r= 46.5 and 50.5 min).



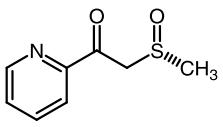
(*R*)-4-(Methylsulfinyl)benzonitrile ((*R*)-110).[146] C_8H_7NOS (165.21 g/mol), MsrA (*E. coli*): conversion 50%, (*ee* > 99%), *s* > 100. The enantiomeric excess was determined by HPLC (IC chiralpak: heptane/propan-2-ol (80:20); flow rate 1 mL/min; 25 °C; 220 nm; t_r= 41.5 and 45.6 min).



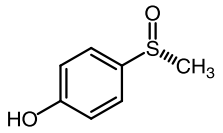
(*R*)-1-Methoxy-4-(methylsulfinyl)benzene ((*R*)-111).[146] $C_8H_{10}O_2S$ (170.23 g/mol), MsrA (*E. coli*): conversion 50%, *ee* > 99%, *s* > 100. The enantiomeric excess was determined by HPLC (ODH chiralcel: heptane/propan-2-ol (90:10); flow rate 1 mL/min; 25 °C; 220 nm; t_r= 13.3 and 15.9 min).



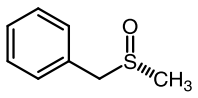
4-(Methylsulfinyl)aniline (105). C_7H_9NOS (155.22 g/mol), MsrA (*S. cerevisiae*) conversion 48%, *ee* = n.d., *s* = n.d. The enantiomeric excess not determined due to inneficient extraction from the reaction mixture.



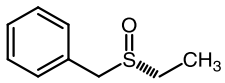
Oxisuran (**106**).[286] $C_8H_9NO_2S$ (183.23 g/mol), MsrA (*S. cerevisiae*) conversion 51%, *ee* = n.d., *s* = n.d. The enantiomeric excess not determined due to inneficient extraction from the reaction mixture.



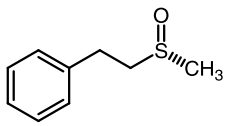
(*R*)-4-(Methylsulfinyl)phenol ((*R*)-112).[303] $C_7H_8O_2S$ (156.20 g/mol), MsrA (*E. coli*): conversion 50%, *ee* > 99%, *s* > 100. Reaction mixture was evaporated on vaccum, suspended in propan-2-ol and filtered. The enantiomeric excess was determined by HPLC (IC chiralpak: heptane/propan-2-ol (80:20); flow rate 1 mL/min; 25 °C ; 220 nm; t_r= 13.5 and 14.6 min).



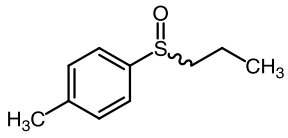
(*R*)-((Methylsulfinyl)methyl)benzene ((*R*)-85).[280] $C_8H_{10}OS$ (154.23 g/mol), MsrA (*S. cerevisiae*): conversion 50%, *ee* > 99%, *s* > 100. The enantiomeric excess was determined by HPLC (IA chiralpak: heptane/propan-2-ol (95:5); flow rate 1 mL/min; 25 °C; 25 °C; 220 nm; t_r= 22.1 and 24.1 min).; MsrA (*E. coli*): conversion 50%, *ee* > 99%, *s* > 100. The enantiomeric excess was determined by HPLC (IA chiralpak: heptane/propan-2-ol (95:5); flow rate 1 mL/min; 25 °C; 25 °C; 220 nm; t_r= 22.1 and 23.9 min).



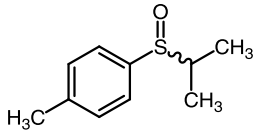
(*R*)-((Ethylsulfinyl)methyl)benzene ((*R*)-113).[100] $C_9H_{12}OS$ (168.25 g/mol), The reaction was carried out with 0.15 mol% of MsrA (*E. coli*), conversion 50% in 18 h, (*ee* > 99%), *s* > 100. The enantiomeric excess was determined by HPLC (IC chiralpak: heptane/propan-2-ol (80:20); flow rate 1 mL/min; 25 °C; 220 nm; t_r= 22.2 and 24.3 min).



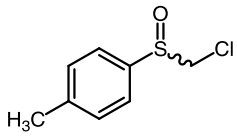
(*R*)-(2-(Methylsulfinyl)ethyl)benzene ((*R*)-104).[170] $C_9H_{12}OS$ (168.25 g/mol), MsrA (*S. cerevisiae*): conversion 50%, (*ee* > 99%), *s* > 100. The enantiomeric excess was determined by HPLC (IA chiralpak: heptane/propan-2-ol (95:5); 1 mL/min; 25 °C; 220 nm; t_r= 23.7 and 26.6 min).; MsrA (*E. coli*): conversion 50%, (*ee* > 99%), *s* > 100. The enantiomeric excess was determined by HPLC (IA chiralpak: heptane/propan-2-ol (95:5); 1 mL/min; 25 °C; 220 nm; t_r= 22.9 and 25.7 min).



***rac*-1-Methyl-4-(propylsulfinyl)benzene ((*R*)-107)**.[282] $C_{10}H_{14}OS$ (182.28 g/mol), MsrA (*S. cerevisiae*): conversion < 3%, *ee* = n.d., *s* = n.d.; MsrA (*E. coli*) conversion < 2%, *ee* = n.d., *s* = n.d. The enantiomeric excess not determined due to low conversion.

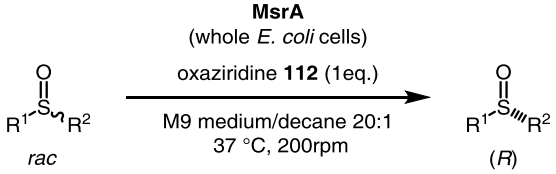


***rac*-1-(Isopropylsulfinyl)-4-methylbenzene ((*R*)-108)**.[283] $C_{10}H_{14}OS$ (182.28 g/mol), MsrA (*S. cerevisiae)* conversion < 3%, *ee* = n.d., *s* = n.d. The enantiomeric excess not determined due to low conversion.



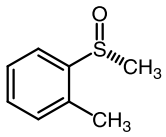
***rac*-1-((Chloromethyl)sulfinyl)-4-methylbenzene ((*R*)-109)**.[293] C_8H_9ClOS (188.67 g/mol), MsrA (*S. cerevisiae)* conversion < 3%, *ee* = n.d., *s* = n.d. The enantiomeric excess not determined due to low conversion.

5.5. General Procedure for Deracemisation of Sulfoxides

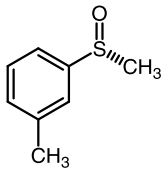


A 2 mL preculture of *E. coli* BL21(DE3) with *msrA* gene in pGEX vector was used to inoculate 200 mL LB medium with ampicillin (100 mg/L). The bacterial culture was incubated at 37 °C, 200 rpm until OD_{600} of 0.8-1.0 was reached. Then, the production of the enzyme was induced with 0.5 mM IPTG, and the culture was incubated at 37 °C, 200 rpm for an additional 2 h until OD_{600} of 2.5 was reached. Then, cells were harvested by centrifugation (3 500 × g, 30 min, 4 °C). The cell pellet was washed with M9 minimal medium (48 mM Na_2HPO_4, 22 mM KH_2PO_4, 8.6 mM NaCl, 18.7 mM NH_4Cl, 2 mM $MgSO_4$, 0.1 mM $CaCl_2$, 0.4% glycerol, pH 6.95) and centrifuged (3500 × g, 30 min, 4 °C). Racemic sulfoxide (0.65 mmol) in M9 minimal medium (80 mL) and cells resuspended in M9 minimal medium (20 mL) were added to a 1L Erlenmeyer flask followed by a solution of oxaziridine **6** (230 mg, 0.65 mmol) in decane (5 mL). The reaction mixture was incubated at 37 °C, 200 rpm for 22 h. The conversion of the reaction was determined by analytical HPLC. Then, a 10% aqueous solution of $Na_2S_2O_3$ (40 mL) was added and the reaction mixture was centrifuged (3 500 × g, 30

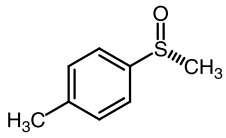
min, 4 °C). The supernatant was extracted with EtOAc (4 × 200 mL), the organic phase was washed with H_2O (50 mL) and brine (50 mL), dried over $MgSO_4$, and concentrated by rotary evaporation under reduced pressure. The crude product was purified by column chromatography on silica gel (typically cyclohexane/acetone = 1:1). The enantiomeric excess of the purified product was determined by HPLC with the chiral stationary phase. The absolute configuration of the sulfoxides was determined by comparison of the HPLC retention times and the optical rotation with the reported data. Sulindac ***rac*-10** was solubilized using the following protocol.[239] Sulindac ***rac*-10** (233 mg, 0.64 mmol) and **β-CD** (800 mg, 0.64 mmol, with approximately 10% water content) were suspended in 50% ethanol (50 mL) in a 250 mL flask. The mixture was placed on a rotary evaporator, heated to 50°C, and rotated without applying reduced pressure until all solids were dissolved. The solvent was then evaporated under reduced pressure. The resulting yellow residue was fully soluble in M9 minimal buffer (80 mL) used for deracemization.



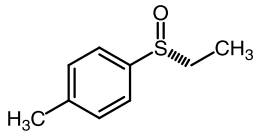
(*R*)-1-Methyl-2-(methylsulfinyl)benzene ((*R*)-100).[301] $C_8H_{10}OS$ (154.23 g/mol), 72 mg; white solid, purified by column chromatography on silica gel (acetone/cyclohexane = 1/1), 72% yield; $[\alpha]_D^{25}$ +244.4 (*c* = 0.49, $CHCl_3$); lit: $[\alpha]_D^{25}$ +146.8 (*c* = 1.03, acetone) for (*R*), *ee* = 60%;[301] **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.96 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.45 (ddd, *J* = 7.8, 7.4, 1.4 Hz, 1H), 7.39 (ddd, *J* = 7.4, 7.4, 1.5 Hz, 1H), 7.20 (dd, *J* = 7.4, 1.4 Hz, 1H), 2.69 (s, 3H), 2.38 (s, 3H). The spectrum is in agreement with the reported data.[277] The enantiomeric excess (*ee* > 99%) was determined by HPLC (IC chiralpak: heptane/propan-2-ol (80:20); flow rate 1 mL/min; 25 °C, 220 nm, t_r= 22.6 and 24.1 min).



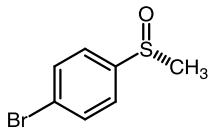
(*R*)-1-Methyl-3-(methylsulfinyl)benzene ((*R*)-101).[302] $C_8H_{10}OS$ (154.23 g/mol), 70 mg; colorless oil, purified by column chromatography on silica gel (acetone/cyclohexane = 1/1), 70% yield; $[\alpha]_D^{25}$ +155.7 (*c* = 0.49, $CHCl_3$); lit: $[\alpha]_D^{25}$ +140.0 (*c* 1.0, CH_3OH) for (*R*), *ee* > 99%;[302] **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.50 – 7.49 (m, 1H), 7.42 – 7.38 (m, 2H), 7.33–7.29 (m, 1H), 2.72 (s, 3H), 2.44 (s, 3H). The spectrum is in agreement with the reported data.[274] The enantiomeric excess (*ee* > 99%) was determined by HPLC (IC chiralpak: heptane/propan-2-ol (80:20); flow rate 1 mL/min; 25 °C; 220 nm; t_r= 23.1 and 27.7 min).



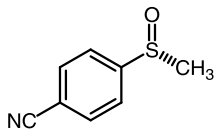
(*R*)-1-Methyl-4-(methylsulfinyl)benzene ((*R*)-1).[146] $C_8H_{10}OS$ (154.23 g/mol), 62 mg, white solid, purified by column chromatography on silica gel (acetone/cyclohexane = 1/1), 62% yield; $[\alpha]_D^{25}$ +146.1 (*c* = 0.51, $CHCl_3$); lit: $[\alpha]_D^{25}$ +138.8 (*c* = 0.66, acetone) for (*R*), *ee* =96%;[146] **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.59 – 7.50 (m, 2H), 7.37 – 7.30 (m, 2H), 2.71 (s, 3H), 2.42 (s, 3H). The spectrum is in agreement with the reported data.[146] The enantiomeric excess (*ee* > 99%) was determined by HPLC (IA chiralpak: heptane/propan-2-ol 95:5; flow rate 0.25 mL/min; 25 °C; 220 nm; t_r= 69.1 and 72.5 min).



(*R*)-1-(Ethylsulfinyl)-4-methylbenzene ((*R*)-102).[278] $C_9H_{12}OS$ (168.25 g/mol), 59 mg, colorless oil, purified by column chromatography on silica gel (acetone/cyclohexane = 1/2), 54% yield; $[\alpha]_D^{25}$ +203.8 (*c* = 0.52, $CHCl_3$); lit: $[\alpha]_D^{25}$ +71.0 (*c* = 0.3, $CHCl_3$) for (*R*), *ee* = 35%;[304] **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.54 – 7.47 (m, 2H), 7.36 – 7.29 (m, 2H), 2.88 (dq, *J* = 13.2, 7.4 Hz, 1H), 2.76 (dq, *J* = 13.2, 7.4 Hz, 1H), 2.42 (s, 3H), 1.19 (t, *J* = 7.4 Hz, 3H). The spectrum is in agreement with the reported data.[278] The enantiomeric excess (*ee* = 99%) was determined by HPLC (IA chiralpak: heptane/propan-2-ol (95:5); flow rate 0.5 mL/min; 25 °C; 220 nm; t_r= 28.7 and 31.3 min).

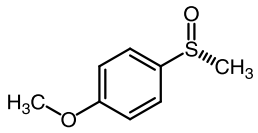


(*R*)-1-Bromo-4-(methylsulfinyl)benzene ((*R*)-74).[206] C_7H_7BrOS (219.10 g/mol), 112 mg, white solid, purified by column chromatography on silica gel (acetone/cyclohexane = 1/1), 79% yield; $[\alpha]_D^{25}$ +131.3 (*c* = 0.50, $CHCl_3$, lit: $[\alpha]_D^{25}$ +131.5 (*c* = 1.3, $CHCl_3$) for (*R*), *ee* > 99%;[206] **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.71 – 7.64 (m, 2H), 7.56 – 7.49 (m, 2H), 2.72 (s, 3H). The spectrum is in agreement with the reported data.[206] The enantiomeric excess (*ee* > 99%) was determined by HPLC (IC chiralpak: heptane/propan-2-ol (80:20); flow rate 1 mL/min; 25 °C; 220 nm; t_r= 18.3 and 19.7 min).

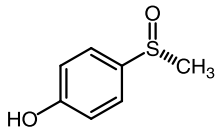


(*R*)-4-(Methylsulfinyl)benzonitrile ((*R*)-110).[146] C_8H_7NOS (165.21 g/mol), 65 mg, white solid, purified by column chromatography on silica gel (acetone/cyclohexane = 1/1), 61% yield; $[\alpha]_D^{25}$ +172.4 (c = 0.40, $CHCl_3$), lit: $[\alpha]_D^{25}$ +108.7 (*c* = 0.67, acetone) for (*R*), *ee* = 95%;[146] **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.86 – 7.81 (m, 2H), 7.79 – 7.75 (m, 2H), 2.76 (s, 3H). The spectrum is in agreement with the reported data.[146] The

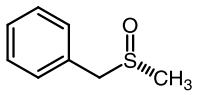
enantiomeric excess (*ee* > 99%) was determined by HPLC (IC chiralpak: heptane/propan-2-ol (80:20); flow rate 1 mL/min; 25 °C; 220 nm; t_r= 42.2 and 46.2 min).



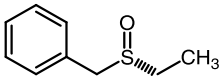
(*R*)-1-Methoxy-4-(methylsulfinyl)benzene ((*R*)-111).[146] $C_8H_{10}O_2S$ (170.23 g/mol), 99 mg, colorless oil, purified by column chromatography on silicagel (acetone/cyclohexane = 1/1), 90% yield; $[\alpha]_D^{25}$ +162.7 (*c* = 0.50, $CHCl_3$), lit:$[\alpha]_D^{25}$ +154.2 (*c* = 0.48, $CHCl_3$) for (*R*), *ee* = 95%;[146] **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.66 – 7.55 (m, 2H), 7.07 – 6.99 (m, 2H), 3.86 (s, 3H), 2.70 (s, 3H). The spectrum is in agreement with the reported data.[146] The enantiomeric excess (*ee* > 99%) was determined by HPLC (ODH chiralcel: heptane/propan-2-ol (90:10); flow rate 1 mL/min; 25 °C; 220 nm; t_r= 13.8 and 15.9 min).



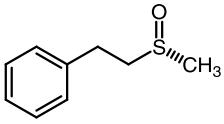
(*R*)-4-(Methylsulfinyl)phenol ((*R*)-112).[303] $C_7H_8O_2S$ (156.20 g/mol), 52 mg, white solid, purified by column chromatography on silica gel (acetone/cyclohexane/MeOH = 1/1/0.05), 52% yield; $[\alpha]_D^{25}$ +122.9 (*c* = 0.35, CH_3OH), lit:$[\alpha]_D^{25}$ +124.0 (*c* = 2.8, CH_2Cl_2) for (*R*), *ee* = 98%;[303] **^{1}H NMR** (400 MHz, $CDCl_3$) 7.54 – 7.47 (m, 2H), 6.99 – 6.92 (m, 2H), 2.76 (s, 3H). The spectrum is in agreement with the reported data.[279] The enantiomeric excess (*ee* > 99%) was determined by HPLC (IC chiralpak: heptane/propan-2-ol (80:20); flow rate 1 mL/min; 25 °C ; 220 nm; t_r= 13.0 and 14.1 min).



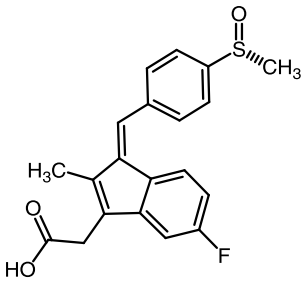
(*R*)-((Methylsulfinyl)methyl)benzene ((*R*)-85).[280] $C_8H_{10}OS$ (154.23 g/mol), the deracemization was carried out with twice the amount of *E. coli* cells (OD_{600} =10, 0.2 mol% MsrA), at the end of the reaction 2-mercaptoethanol (90µL, 1.1 mmol) was added instead of $Na_2S_2O_3$ and the decane phase was removed prior to further work up. Purification by column chromatography on silica gel (acetone/cyclohexane/methanol = 1/1/0.05), afforded **(*R*)-85** in 75% yield (75 mg) as an off-white solid; $[\alpha]_D^{25}$ +60.0 (*c* = 0.5, $CHCl_3$), lit:$[\alpha]_D^{25}$ +50.1 (*c* = 1.0, $CHCl_3$) for (*R*), ee > 98%;[305] **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.42 – 7.27 (m, 5H), 4.06 (d, *J* = 12.8 Hz, 1H), 3.92 (d, *J* = 12.8 Hz, 1H), 2.45 (s, 3H). The spectrum is in agreement with the reported data.[280] The enantiomeric excess (*ee* = 99%) was determined by HPLC (IA chiralpak: heptane/propan-2-ol (95:5); flow rate 1 mL/min; 25 °C; 25 °C; 220 nm; t_r= 21.0 and 22.6 min).



(*R*)-((Ethylsulfinyl)methyl)benzene ((*R*)-113).[100] $C_9H_{12}OS$ (168.25 g/mol), the deracemization was carried out with three times the amount of *E. coli* cells (OD_{600} =15, 0.3 mol% MsrA), at the end of the reaction 2-mercaptoethanol (90µL, 1.1 mmol) was added instead of $Na_2S_2O_3$ and the decane phase was removed prior to further work up. Purification by column chromatography on silica gel (acetone/cyclohexane = 1/2) afforded (*R*)-**113**. in 59% yield; (64 mg) as an yellowish oil; $[\alpha]_D^{25}$ +81.4 (*c* = 0.51, $CHCl_3$), lit: $[\alpha]_D^{22}$ +24.0 (*c* = 1.0, ethanol) for (*R*), *ee* > 45%;[100] **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.41 – 7.27 (m, 5H), 4.03 (d, *J* = 12.9 Hz, 1H), 3.94 (d, *J* = 12.9 Hz, 1H), 2.65 (dq, *J* = 13.2, 7.6 Hz, 1H), 2.55 (dq, *J* = 13.2, 7.6 Hz, 1H), 1.33 (t, *J* = 7.6 Hz, 3H). The spectrum is in agreement with the reported data.[274] The enantiomeric excess (*ee* = 99%) was determined by HPLC (IC chiralpak: heptane/propan-2-ol (80:20); flow rate 1 mL/min; 25 °C; 220 nm; t_r= 24.6 and 27.1 min).



(*R*)-(2-(Methylsulfinyl)ethyl)benzene ((*R*)-104).[170] $C_9H_{12}OS$ (168.25 g/mol), 97 mg, white solid, purified by column chromatography on silica gel (acetone/cyclohexane = 1/1→1/0), 88% yield; $[\alpha]_D^{25}$ –81.9 (*c* = 0.52, $CHCl_3$), lit: $[\alpha]_D^{25}$ +59.0 (*c* = 1.03, $CHCl_3$; for (*S*), *ee* = 30%;[170] **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.36 – 7.22 (m, 5H), 3.18 – 2.89 (m, 4H), 2.58 (s, 3H). The spectrum is in agreement with the reported data.[170] The enantiomeric excess (*ee* > 99%) was determined by HPLC (IA chiralpak: heptane/propan-2-ol (95:5); 1 mL/min; 25 °C; 220 nm; t_r= 23.1 and 25.7 min).



(*R*)-Sulindac ((*R*)-10).[146] $C_{20}H_{17}FO_3S$ (356.41 g/mol). Sulindac was treated with β-cyclodextrin before the deracemization according to the reported procedure[239]. The deracemization was carried out with three times the amount of *E. coli* cells (OD_{600} = 15, 0.3 mol% MsrA), at the end of the reaction 2-mercaptoethanol (90 µL, 1.1 mmol) was added instead of $Na_2S_2O_3$ and the decane phase was removed prior to further work up. Purification by column chromatography on silica gel (acetone/cyclohexane/methanol = 1/1/0.05 to 1/1/0.2) afforded **(*R*)-10** in 61% yield (140 mg) as an yellow solid; $[\alpha]_D^{25}$ +95.1 (*c* = 0.51, $CHCl_3$), lit: $[\alpha]_D^{25}$ +52.2 (*c* = 1.0, CH_3OH) for (*R*), *ee* = 99%;[146] **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.75 – 7.69 (m, 2H), 7.68 – 7.62 (m,

2H), 7.16 (s, 1H), 7.13 (dd, *J* = 8.4, 5.1 Hz, 1H), 6.89 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.56 (ddd, *J* = 8.8, 8.8, 2.4 Hz, 1H), 3.60 (s, 2H), 2.82 (s, 3H), 2.21 (s, 3H). The spectrum is in agreement with the reported data.[146] Enantiomeric excess (*ee* = 97%) was determined by HPLC from a corresponding methyl ester **115**.[146] (IC chiralpak: heptane/propan-2-ol (60:40); 1 mL/min; 25 °C; 220 nm; t_r= 48.0 and 52.2min).

5.5.1. Quantification of MsrA in the Cell Culture

2 mL preculture of *E. coli* BL21(DE3) with *msrA* gene in pGEX vector was used to inoculate 200 mL LB medium with ampicillin (100 mg/L). The bacterial culture was incubated at 37 °C, 200 rpm until OD_{600} of 0.8 was reached. Production of the enzyme was induced with 0.5 mM IPTG and the culture was incubated at 37 °C, 200 rpm for an additional 2 h until OD_{600} of 2.5 was reached. Then, cells were harvested by centrifugation (3500 × g, 30 min, 4 °C). The cell pellet was washed with M9 minimal medium (48 mM Na_2HPO_4, 22 mM KH_2PO_4, 8.6 mM NaCl, 18.7 mM NH_4Cl, 2 mM $MgSO_4$, 0.1 mM $CaCl_2$, 0.4% glycerol, pH 6.95) and centrifuged (3500 × g, 30 min, 4 °C). The cells were resuspended in M9 minimal medium (100 mL, OD_{600} = 5), and 200 µL aliquot was withdrawn. The cells from the aliquot were harvested by centrifugation (3500 × g, 4 min, 4 °C). The cell pellet was resuspended in 200 µL of SDS loading buffer (50mM Tris-Cl pH 6.8, 100mM DTT, 2% SDS, 0.1% bromophenol blue, 10% glycerol) and heated at 95°C for 6 min. The resulting solution was aliquoted and analyzed by SDS-PAGE and compared with the pure MsrA enzyme of known concentration and the bacterial cell culture of the same OD_{600} = 5 with the reference plasmid pUC19 instead of *msrA* gene containing pGEX (Figure 18). Quantification of MsrA was carried out using FIJI software.[306]

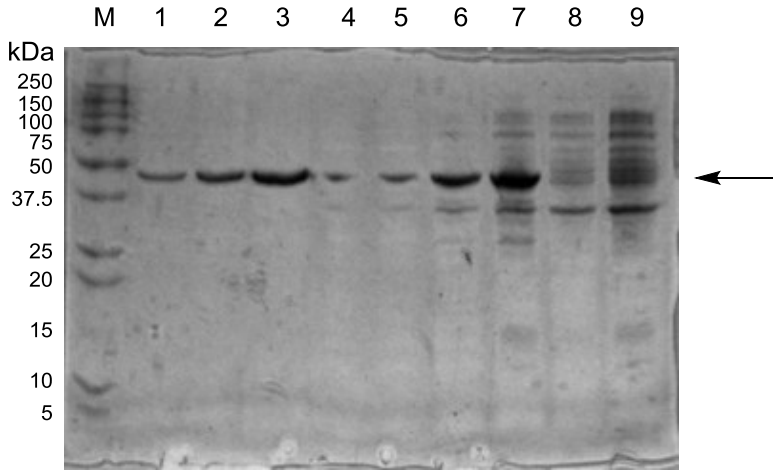
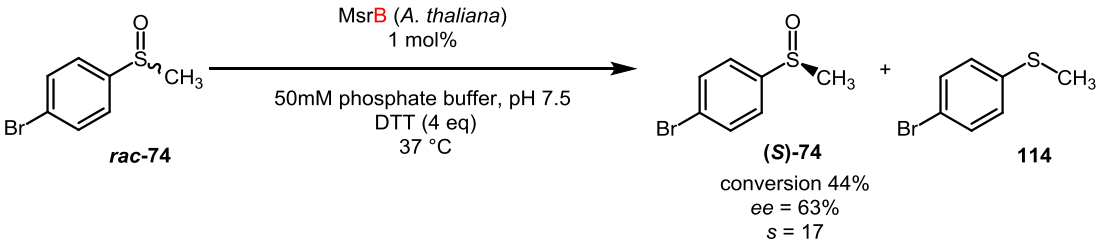


Figure 18. SDS-PAGE MsrA quantification: (M)-protein mass marker, (1)-2 µg of MsrA, (2)-4 µg of MsrA, (3)-8 µg of MsrA, (4)-2 µL aliquot of cells (5)-4 µL aliquot of cells, (6)-8 µL aliquot of cells, (7)-16 µL aliquot of cells, (8)-8 µL aliquot

of cells with reference plasmid (pUC19) (9)-16 µL aliquot of cells with reference plasmid (pUC19).

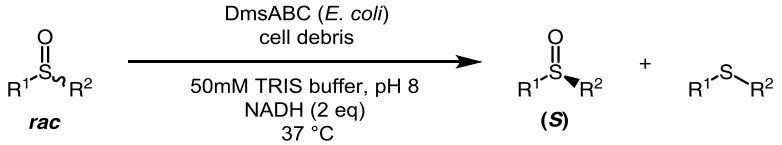
5.6. Procedure for the Kinetic Resolution of Racemic Sulfoxides with Isolated Enzymes MsrBs (*N. gonorrhoeae* (pilB), *T. kodakarensis* (MsrB) and *A. thaliana* (MsrB2))



A 1.5 mL Eppendorf vial was charged with individual solutions of DTT (1440 µL of 27 mM, 4 equivs., 26 mM final concentration) in phosphate buffer (50 mM Na_2HPO_4, 50 mM NaH_2PO_4, 50 mM NaCl, pH 7.5) and a racemic sulfoxide (30 µL of 324 mM, 9.7 µmol, 6.5 mM final concentration) in CH_3CN. The resulting mixture was gently mixed and the reaction was initiated by the addition of the corresponding MsrB (1 mol%, 65 µM final concentration) in a storage buffer. The reaction mixture was incubated at 37 °C at 250 rpm. The course of the reaction was monitored by analytical HPLC. After 44 h the reaction with the highest conversion of 44% (MsrB2 *A. thaliana*) was selected for further analysis (the conversion of the reactions with the other MsrBs was below 10%). The reaction mixture was extracted with toluene (2 × 4 mL), washed with H_2O (2 mL), brine (2 mL), dried under $MgSO_4$, and concentrated by rotary evaporation under reduced pressure. The enantiomeric excess of the crude product was determined by HPLC with chiral stationary phase (*ee* = 63%) providing *s* = 17.

5.7. Procedure for the Kinetic Resolution of Racemic Sulfoxides with DmsABC Reductase

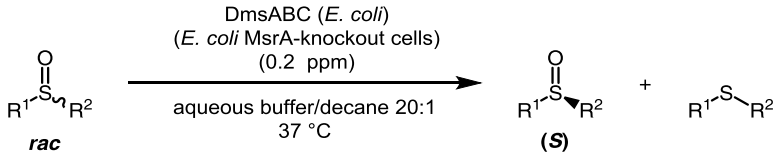
5.7.1. Procedure with the Membrane Fraction



A 2 mL preculture of *E. coli* (*msrA* knock out - KEIO collection ID JW4178)[258] was used to inoculate 400 mL LB medium with kanamycin (50 mg/L). The bacterial culture was incubated at 37 °C, 200 rpm until OD_{600} = 3.5 was reached. Then, 50 mL aliquot of the bacterial culture was withdrawn, and cells were harvested by centrifugation (4 200 × g, 30 min, 4 °C). The cell pellet was washed with Tris buffer (50 mM, pH 8.0) and centrifuged (4 200 × g, 30 min, 4 °C). Cells were resuspended

in Tris buffer (5.9 mL), and lysed by ultrasound on ice for 5-6 min (15 s burst/15 s rest) and divided to Eppendorf vials (each 1.5 mL). Cell membrane fraction (in one vial) was obtained by centrifugation (16 900 × g for 30 min at 4 °C), resuspended in Tris buffer (1470 µL) with NADH (2 equiv., final concentration 13 mM,) and a racemic sulfoxide (30 µL of 324 mM, 9.7 µmol, 6.5 mM final concentration) in CH_3CN was added. The reaction mixture was incubated at 37 °C, 200 rpm for 18-24 h. The conversion of the reaction was determined by analytical HPLC. Then, the reaction mixture was centrifuged (16 900 × g, 2 min, 4 °C). The supernatant was extracted with EtOAc (2 × 2 mL), the organic phase was washed with H_2O (1 mL) and brine (1 mL), dried over $MgSO_4$, and concentrated by rotary evaporation under reduced pressure. The enantiomeric excess was determined by HPLC with chiral stationary phase. The absolute configuration of the sulfoxides was determined by comparison of the HPLC retention times and the optical rotation with the reported data.

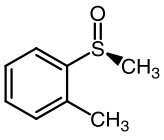
5.7.2. General Procedure with the Whole *E. coli* Cells



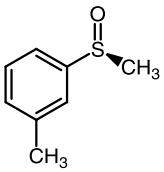
A 2 mL preculture of *E. coli* (*msrA* knock out - KEIO collection ID JW4178)[258] was used to inoculate 400 mL LB medium with kanamycin (50 mg/L). The bacterial culture was incubated at 37 °C, 200 rpm until OD_{600} of 3 was reached. Then, 47 mL aliquot of the bacterial culture was withdrawn, and cells were harvested by centrifugation (4 200 × g, 30 min, 4 °C). The cell pellet was washed with M9 minimal medium (48 mM Na_2HPO_4, 22 mM KH_2PO_4, 8.6 mM NaCl, 18.7 mM NH_4Cl, 2 mM $MgSO_4$, 0.1 mM $CaCl_2$, 0.4% glycerol, pH 7.0) and centrifuged (4 200 × g, 30 min, 4 °C). Cells resuspended in M9 minimal medium (2 mL) were added to a mixture of racemic sulfoxide (0.065 mmol) in M9 minimal medium (8 mL) in 100 mL Erlenmeyer flask and then decane (0.5 mL) was added. The final OD_{600} in the reaction mixture was 14 (0.2 ppm of DmsABC) unless otherwise stated. The reaction mixture was incubated at 37 °C, 200 rpm. The conversion of the reaction was determined by analytical HPLC. Then, the reaction mixture was centrifuged (16 900 × g, 2 min, 4 °C). The supernatant was extracted with EtOAc (4 mL), the organic phase was washed with H_2O (1 mL) and brine (1 mL), dried over $MgSO_4$, and concentrated by rotary evaporation under reduced pressure. The enantiomeric excess was determined by HPLC with

chiral stationary phase. The absolute configuration of the sulfoxides was determined by comparison of the HPLC retention times and the optical rotation with the reported data.

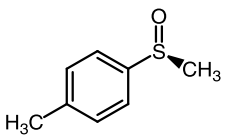
A modified protocol was utilized for substrates **8**, **123**-**125**. Due to the low solubility and acidic instability of the substrates,[167] pH of M9 minimal buffer was adjusted to 9.0, and methanol (2 %) was added to the reaction. Decane as co-solvent was omitted in these experiments. Also, reactions were carried out at a 14.5 µmol scale.



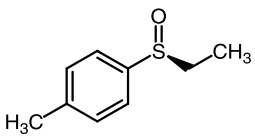
(*S*)-1-Methyl-2-(methylsulfinyl)benzene ((*S*)-100).[307] $C_8H_{10}OS$ (154.23 g/mol), final OD_{600} = 28 (0.4 ppm of DmsABC), conversion 50% in 2 h, *s* > 100. The enantiomeric excess (*ee* = 98%) was determined by HPLC (IC chiralpak: heptane/propan-2-ol (80:20); flow rate 1 mL/min; 25 °C, 254 nm, t_r= 23.2 and 24.8 min)



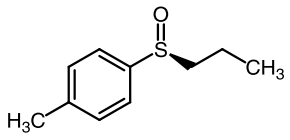
(*S*)-1-Methyl-3-(methylsulfinyl)benzene ((*S*)-101).[307] $C_8H_{10}OS$ (154.23 g/mol), conversion 50% in 1 h, *s* > 100. The enantiomeric excess (*ee* > 99%) was determined by HPLC (IC chiralpak: heptane/propan-2-ol (80:20); flow rate 1 mL/min; 25 °C; 254 nm; t_r= 24.4 and 29.9 min).



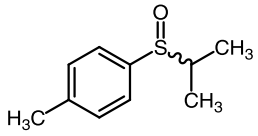
(*S*)-1-Methyl-4-(methylsulfinyl)benzene ((*S*)-1).[307] $C_8H_{10}OS$ (154.23 g/mol), conversion 50% in 2 h, *s* > 100. The enantiomeric excess (*ee* > 99%) was determined by HPLC (ODH chiralcel: heptane/propan-2-ol (98:2); flow rate 1 mL/min; 25 °C; 254 nm; t_r= 32.1 and 35.7 min).



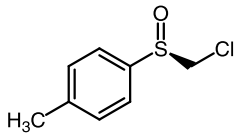
(*S*)-1-(Ethylsulfinyl)-4-methylbenzene ((*S*)-102).[307] $C_9H_{12}OS$ (168.25 g/mol), final OD_{600} = 28 (0.4 ppm of DmsABC), conversion 52% in 2 h, *s* > 100. The enantiomeric excess (*ee* = 99%) was determined by HPLC (IA chiralpak: heptane/propan-2-ol (95:5); flow rate 0.5 mL/min; 25 °C; 254 nm; t_r= 27.4 and 29.2 min).



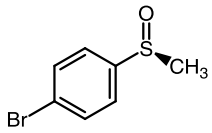
(*S*)-1-(Propylsulfinyl)-4-methylbenzene ((*S*)-107).[282] $C_{10}H_{14}OS$ (182,28 g/mol), final OD_{600} = 28 (0.4 ppm of DmsABC), conversion 39% in 2 h, *s* = 3. The enantiomeric excess (*ee* = 26%) was determined by HPLC (IA chiralpak: heptane/propan-2-ol (95:5); flow rate 1 mL/min; 25 °C; 254 nm; t_r= 12.9 and 13.7 min).



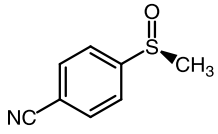
1-(Isopropylsulfinyl)-4-methylbenzene (108).[283] $C_{10}H_{14}OS$ (182,28 g/mol), final OD_{600} = 28 (0.4 ppm of DmsABC), conversion 9 % in 2 h, *s* = 0. The enantiomeric excess (*ee* = 0%) was determined by HPLC (IA chiralpak: heptane/propan-2-ol (95:5); flow rate 1 mL/min; 25 °C; 254 nm; t_r= 12.0 and 12.8 min).



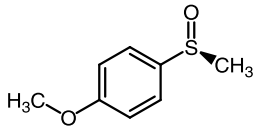
(*R*)-1-((chloromethyl)sulfinyl)-4-methylbenzene ((*R*)-109).[293] C_8H_9ClOS (188,67 g/mol), final OD_{600} = 28 (0.4 ppm of DmsABC), conversion 52% in 2 h, *s* > 100. The enantiomeric excess (*ee* = 99%) was determined by HPLC (IC chiralpak: heptane/propan-2-ol (80:20); flow rate 1 mL/min; 25 °C; 254 nm; t_r= 17.8.7 and 19.4 min). ; $[\alpha]_D^{25}$ –139.0 (*c* = 0.5, $CHCl_3$), lit:$[\alpha]_D^{25}$ +136 (*c* = 1.0, $CHCl_3$) for (*S*), *ee* = 95%).[293]



(*S*)-1-Bromo-4-(methylsulfinyl)benzene ((*S*)-74).[307] C_7H_7BrOS (219.10 g/mol), conversion 51% in 1 h, *s* > 100. The enantiomeric excess (*ee* > 99%) was determined by HPLC (IC chiralpak: heptane/propan-2-ol (80:20); flow rate 1 mL/min; 25 °C; 254 nm; t_r= 18.6 and 20.2 min).

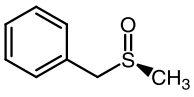


(*S*)-4-(Methylsulfinyl)benzonitrile ((*S*)-110).[307] C_8H_7NOS (165.21 g/mol), conversion 52% in 1 h, *s* > 100. The enantiomeric excess (*ee* = 99%) was determined by HPLC (IC chiralpak: heptane/propan-2-ol (80:20); flow rate 1 mL/min; 25 °C; 254 nm; t_r= 47.0 and 51.3 min).

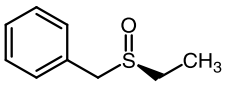


(*S*)-1-Methoxy-4-(methylsulfinyl)benzene ((*S*)-111).[307] $C_8H_{10}O_2S$ (170.23 g/mol), conversion 48% in 1 h, *s* > 100. The enantiomeric excess (*ee* = 98%)

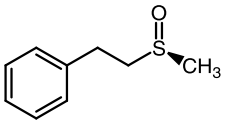
was determined by HPLC (ODH chiralcel: heptane/propan-2-ol (90:10); flow rate 1 mL/min; 25 °C; 254 nm; t_r= 17.0 and 18.1 min).



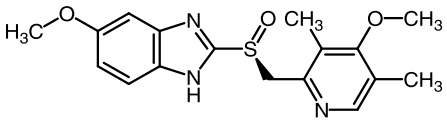
(*S*)-((Methylsulfinyl)methyl)benzene ((*S*)-85).[307] $C_8H_{10}OS$ (154.23 g/mol), conversion 48% in 1 h, *s* > 100. The enantiomeric excess (*ee* = 99%) was determined by HPLC (IA chiralpak: heptane/propan-2-ol (95:5); flow rate 1 mL/min; 25 °C; 25 °C; 220 nm; t_r= 21.9 and 24.0 min).



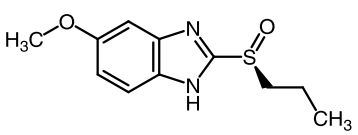
(*S*)-((Ethylsulfinyl)methyl)benzene ((*S*)-113).[307] $C_9H_{12}OS$ (168.25 g/mol), conversion 49% in 1 h, *s* > 100. The enantiomeric excess (*ee* = 99%) was determined by HPLC (IC chiralpak: heptane/propan-2-ol (80:20); flow rate 1 mL/min; 25 °C; 220 nm; t_r= 24.0 and 26.5 min).



(*S*)-(2-(Methylsulfinyl)ethyl)benzene ((*S*)-104).[307] $C_9H_{12}OS$ (168.25 g/mol), conversion 51% in 1 h, *s* > 100. The enantiomeric excess (*ee* = 99%) was determined by HPLC (IA chiralpak: heptane/propan-2-ol (95:5); 1 mL/min; 25 °C; 220 nm; t_r= 23.1 and 25.2 min).

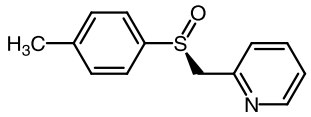


Esomeprazole ((*S*)-8).[167] $C_{17}H_{19}N_3O_3S$ (345,42 g/mol), 14.5 µmol scale, final OD_{600} = 40 (2.0 ppm of DmsABC), conversion 29% in 2 h, *s* > 100. The enantiomeric excess (*ee* = 40%) was determined by HPLC (IC chiralpak: heptane/propan-2-ol (60:40); 1 mL/min; 25 °C; 300 nm; t_r= 20.4 and 33.3 min).

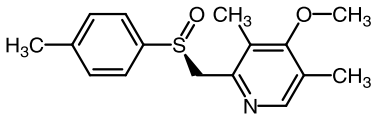


(*S*)-5-Methoxy-2-(propylsulfinyl)-1H-benzo[d]imidazole ((*S*)-123). $C_{11}H_{14}N_2O_2S$ (238.31 g/mol), 14.5 µmol scale, final OD_{600} = 40 (2.0 ppm of DmsABC), conversion 42% in 2 h, *s* = 3. The enantiomeric

excess (*ee* = 28%) was determined by HPLC (IC chiralpak: heptane/propan-2-ol (60:40); 1 mL/min; 25 °C; 300 nm; t_r= 6.3 and 7.7 min).



(*S*)-2-((p-Tolylsulfinyl)methyl)pyridine ((*S*)-124).[292] $C_{13}H_{13}NOS$ (231.31 g/mol), 14.5 µmol scale, final OD_{600} = 40 (2.0 ppm of DmsABC), conversion 20% in 2 h, *s* = 3. The enantiomeric excess (*ee* = 11%) was determined by HPLC (IC chiralpak: heptane/propan-2-ol (60:40); 1 mL/min; 25 °C; 254 nm; t_r= 21.5 and 29.4 min).



(*S*)-4-Methoxy-3,5-dimethyl-2-((p-tolylsulfinyl)methyl)pyridine ((*S*)-125). $C_{16}H_{19}NO_2S$ (289.39 g/mol), 14.5 µmol scale, final OD_{600} = 40 (2.0 ppm of DmsABC), conversion 5% in 2 h , *s* = n.d. After 18 hours, the conversion rate reached 16%, with s = 7. The enantiomeric excess (*ee* = 14%) was determined by HPLC (IC chiralpak: heptane/propan-2-ol (60:40); 1 mL/min; 25 °C; 254 nm; t_r= 27.6 and 36.9 min).

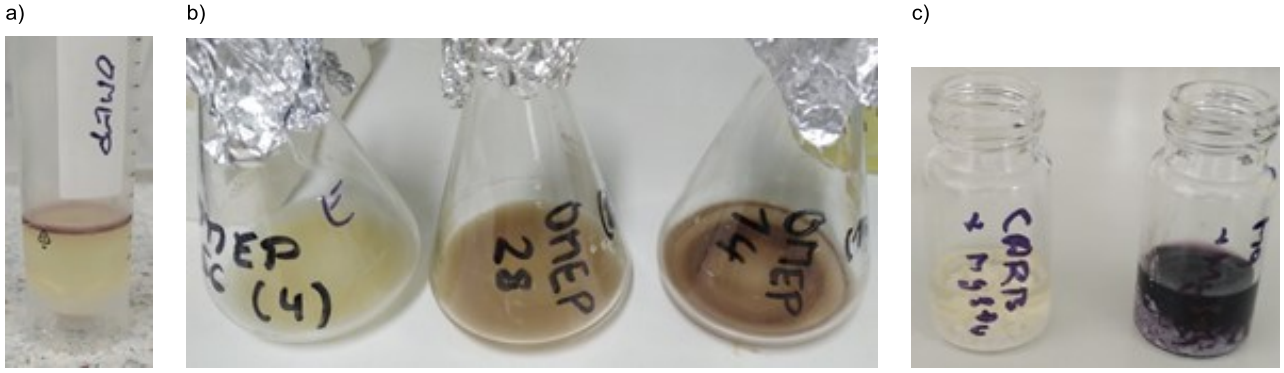
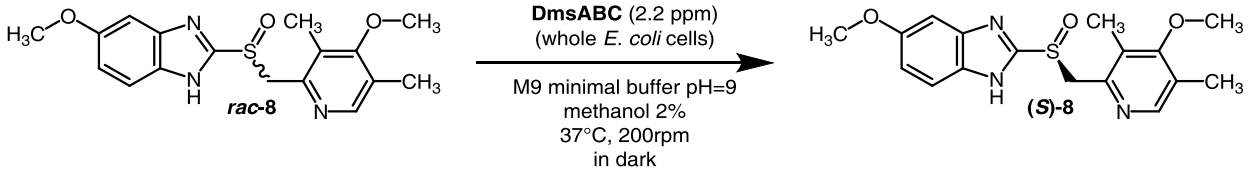


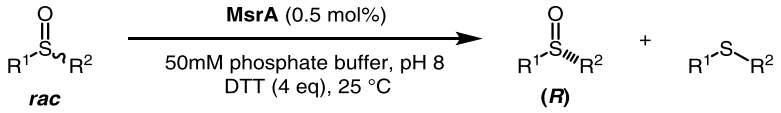
*Figure 19. Several examples of Omeprazole condition screening: a) Kinetic resolution with centrifuged whole cells showing a dark decane layer, b) Kinetic resolution varying E. coli cell concentrations (OD_{600}) without decane in M9 minimal buffer (*pH *= 7), c) Stability screening of Omeprazole with E.coli in basic buffer (*pH *= 10) and M9 minimal buffer (*pH *= 7) - organic phase extracts.*

5.7.3. Scale-up Procedure for the Kinetic Resolution of Omeprazole



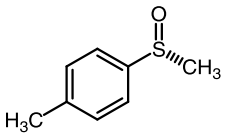
2.5 mL preculture of *E. coli* (*msrA* knock out - KEIO collection ID JW4178)[258] was used to inoculate each of three 500 mL LB media with kanamycin (50 mg/L) in three 2 L Erlenmeyer flasks. The bacterial cultures were incubated at 37 °C, 200 rpm until OD_{600} of 3 was reached (6 hours). Then, cells were harvested by centrifugation (4200 × g, 30 min, 4 °C). Resuspended cells in M9 minimal medium (10 mL, pH 9) were added to a solution of racemic omeprazole (50 mg, 0.145 mmol, final concentration 0.5 mg/mL) in M9 minimal medium (88 mL, pH 9) and methanol (2 mL) in 1 L Erlenmeyer flask. The reaction mixture was incubated at 37 °C, 200 rpm in the dark. The conversion of the reaction was determined by analytical HPLC. After 3 h (conversion = 56 %), the reaction mixture was centrifuged (4200 × g, 20 min, 4 °C) and the supernatant was extracted with diethyl ether (4 × 200 mL), the organic phase was washed with H_2O (40 mL) and brine (40 mL), dried over anhydrous $MgSO_4/Na_2SO_4$ and concentrated by rotary evaporation under reduced pressure. Purification of the crude product by HPLC(method e) afforded **(*S*)-8** in 36% yield (18 mg) as an off-white solid; $[\alpha]_D^{25}$= –147° (*c* = 0.2, $CHCl_3$); lit: $[\alpha]_D^{25}$ = –157° (*c* = 0.5, $CHCl_3$) for (*S*)-omeprazole sodium salt, *ee* > 99%).[308] **^{1}H NMR** (400 MHz, CD_3OD) δ 8.13 (s, 1H), 7.54 (d, *J* = 8.9 Hz, 1H), 7.12 (d, *J* = 2.4 Hz, 1H), 7.00 (dd, *J* = 8.9, 2.4 Hz, 1H), 4.80 (d, *J* = 13.2 Hz, 1H), 4.73 (d, *J* = 13.2 Hz, 1H), 3.87 (s, 3H), 3.72 (s, 3H), 2.26 (s, 3H), 2.18 (s, 3H). The spectrum is in agreement with the reported data.[167] Enantiomeric excess (*ee* = 98%) was determined by HPLC (IC chiralpak: heptane/propan-2-ol (60:40); 1 mL/min; 25 °C; 300 nm; t_r= 18.1 and 29.4 min).

5.8. General Procedure for the Kinetic Resolution of Racemic Sulfoxides with Isolated Enzymes *E. coli* MsrA (WT) and MsrA (F52L) mutant

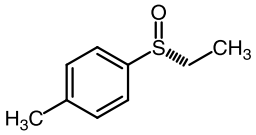


A 1.5 mL Eppendorf tube was charged with individual solutions of phosphate buffer (214 µL, 50mM Na_2HPO_4/NaH_2PO_4, 50mM NaCl, pH = 8) and DTT (250 µL of 52 mM, 4 equivs., 26 mM final concentration) in phosphate buffer and a racemic sulfoxide (10 µL of 324 mM, 3.2 µmol,

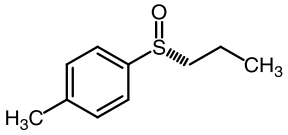
6.5 mM final concentration) in CH_3CN. The reaction was initiated by the addition of MsrA (wild type, WT) or F52L mutant, 25.6 µL of 635 µM, 0.5 mol%, 32.5 µM final concentration) in a storage buffer (Tris buffer, pH = 8). The reaction mixture was flushed with argon and incubated at 25 °C without agitation. The course of the reaction was monitored by analytical HPLC. After 24 h, the reaction mixture was extracted with EtOAc (4 mL), washed with water (0.5 mL), and brine (0.5 mL), dried over $MgSO_4$, and concentrated by rotary evaporation under reduced pressure. The enantiomeric excess of the crude product was determined by HPLC with the chiral stationary phase.



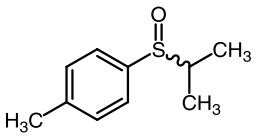
(*R*)-1-Methyl-4-(methylsulfinyl)benzene ((*R*)-1).[307] $C_8H_{10}OS$ (154.23 g/mol), WT: conversion 51% in 24 h, *ee* > 99%, *s* > 100; F52L: conversion 51% in 24 h, *ee* > 99%, *s* > 100. The enantiomeric excess (*ee*) was determined by HPLC (ODH chiralcel: heptane/propan-2-ol (98:2); flow rate 1 mL/min; 25 °C; 254 nm; t_r= 31.3 and 34.1 min).



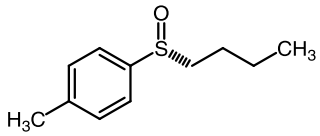
(*R*)-1-(Ethylsulfinyl)-4-methylbenzene ((*R*)-102).[307] $C_9H_{12}OS$ (168.25 g/mol), WT: conversion 50% in 24 h, *ee* > 99%, *s* > 100; F52L: conversion 51% in 24 h, *ee* > 99%, *s* > 100. The enantiomeric excess (*ee*) was determined by HPLC (IA chiralpak: heptane/propan-2-ol (95:5); flow rate 1 mL/min; 25 °C; 254 nm; t_r= 16.4 and 17.9 min).



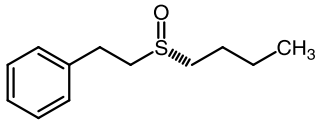
(*R*)-1-(Propylsulfinyl)-4-methylbenzene ((*R*)-107).[307] $C_{10}H_{14}OS$ (182.28 g/mol), WT: conversion <4% in 24 h, *ee* = 3%, *s* = n.d.; F52L: conversion 51% in 24 h, *ee* = 99%, *s* > 100. The enantiomeric excess (*ee*) was determined by HPLC (IA chiralpak: heptane/propan-2-ol (95:5); flow rate 1 mL/min; 25 °C; 254 nm; t_r= 14.7 and 16.0 min).



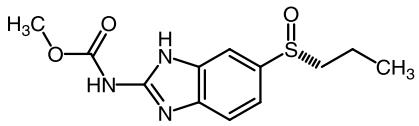
1-(Isopropylsulfinyl)-4-methylbenzene (108).[309] $C_{10}H_{14}OS$ (182.28 g/mol), WT: conversion < 4% in 24 h, *ee* = 0%, *s* = n.d.; F52L: conversion < 4% in 24 h, *ee* = 0%, *s* = n.d. The enantiomeric excess (*ee*) was determined by HPLC (IA chiralpak: heptane/propan-2-ol (95:5); flow rate 1 mL/min; 25 °C; 254 nm; t_r= 13.8 and 15.1 min).



(*R*)-1-(Butylsulfinyl)-4-methylbenzene ((*R*)-129).[278] $C_{11}H_{16}OS$ (196.31 g/mol), WT: conversion 0% in 24 h, *ee* = 0%, *s* = n.d.; F52L: conversion 50% in 24 h, *ee* > 99%, *s* > 100. The enantiomeric excess (*ee*) was determined by HPLC (IA chiralpak: heptane/propan-2-ol (95:5); flow rate 1 mL/min; 25 °C; 254 nm; t_r= 14.2 and 15.5 min).



(*R*)-(2-(Butylsulfinyl)ethyl)benzene ((*R*)-130).[284] $C_{12}H_{18}OS$ (210.34 g/mol), WT: conversion 0% in 24 h, *ee* = 0%, *s* = n.d.; F52L: conversion 52% in 24 h, *ee* > 99%, *s* > 100. The enantiomeric excess (*ee*) was determined by HPLC (IA chiralpak: heptane/propan-2-ol (95:5); flow rate 1 mL/min; 25 °C; 220 nm; t_r= 17.1 and 23.0 min).



(*R*)-Albendazole sulfoxide ((*R*)-131).[298] $C_{12}H_{15}N_3O_3S$ (281.33 g/mol), a stock solution was prepared in *N*-methyl-2-pyrolidone instead of CH_3CN, using 1.5mol% of MsrA. White precipitate (Albendazole) with some coprecipitated albendazole sulfoxide was centrifuged down, and it was continued with the supernatant. MsrA WT : conversion = 6% in 24 h, *ee* = 6%, *s* = n.d.; F52L MsrA: conversion = 49% in 24 h, *ee* = 94%, *s* > 100. The enantiomeric excess was determined by HPLC (IC chiralpak: heptane/propan-2-ol (70:30); flow rate 1 mL/min; 25 °C; 294 nm; t_r= 24.5 and 31.4 min).

6. Use of Artificial Intelligence in This Work

Artificial intelligence, ChatGPT (OpenAI), and Grammarly (Grammarly, Inc.), were employed as a supplementary tool during the preparation of this thesis. It was utilized for providing linguistic corrections, stylistic suggestions, improving clarity in Englishm, and for shortening sections and eliminating redundancy. All AI-generated outputs were carefully reviewed, edited, and adapted by the author to ensure accuracy and compliance with academic standards. The use of AI aligns with ethical practices and does not substitute for the author's critical analysis and independent research.

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