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C-N BOND FORMATION VIA ENE REACTIONS AND CYCLOADDITIONS OF ENZYMATICALLY GENERATED NITROSO COMPOUNDS

Christina Jäger

DOCTORAL DISSERTATION

To be presented for public discussion with the permission of the Faculty of Science, of the University of Helsinki, in Auditorium 116, Unioninkatu 35, on the 27th of October, 2023 at 12 o'clock.

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Doch Forschung strebt und ringt, ermüdend nie

Nach dem Gesetz, dem Grund, Warum und Wie.

Johann Wolfgang von Goethe

ABSTRACT

To satisfy the demand of more environmentally friendly and sustainable methodologies in chemistry, biocatalysis offers a particularly mild and efficient alternative to traditional chemical catalysts by employing enzymes. Expanding the enzyme's scope will lead to new-to-nature transformations and can serve as promiscuous catalysts in organic synthesis.

A majority of highly relevant and valuable molecules contain carbon-nitrogen bonds which need to be constructed within the synthesis process. Nitroso chemistry offers a direct incorporation strategy of nitrogen to afford functionalized organic building blocks via a range of reaction types.

In the endeavour to discover new enzymatic tools that are inspired by traditional organic chemistry, a new synthetic methodology for the formation of C-N bonds using nitroso transformations was developed. Via laccases or an oxidase/peroxidase interplay, selective oxidation of N-hydroxycarbamates and other hydroxamic acids utilizing air as terminal oxidant was realized. The thus formed nitroso compounds are generated *in situ* under very mild aqueous and aerobic conditions. They further engage in inter- or intramolecular nitroso-ene or nitroso-Diels-Alder reactions forming new C-N bonds with high efficiency.

In addition, the observation of secondary kinetic isotope effects contributes to the still ongoing debate of the mechanism and sheds light into the reaction pathway of the intramolecular nitroso-ene reaction in aqueous environment.

The methodology employing the oxidase/peroxidase couple was proven to be very robust. Therefore, the catalyst containing aqueous phase can be utilized for several reaction cycles while preserving activity.

Finally, manufacture of the hydroxamic acid substrates from their easily avaible ester derivatives through lipase-catalyzed hydroxylaminolysis prompted the creation of a triple enzymatic methodology to afford *N*-hydroxy-γ-lactams. Utilizing this multi-enzyme protocol, a spirocyclic lactam was produced which serves as a key building block in the synthesis of alkaloids from the *Cephalotaxus* family.

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CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications:

I **C. Jäger**, M. Haase, K. Koschorreck, V. B. Urlacher, J. Deska, Aerobic C-N Bond Formation through Enzymatic Nitroso-Ene-Type Reactions. *Angew. Chem. Int. Ed.* **2023**, *62*, e202213671.

II **C. Jäger**, B. J. Gregori, J. A. S. Aho, M. Hallamaa, J. Deska, Peroxidase-induced C-N Bond Formation via Nitroso Ene and Diels-Alder Reactions. *Green Chem.* **2023**, *25*, 3166 – 3174.

III **C. Jäger**, M. Nieger, K. Rissanen, J. Deska, Multi-enzymatic Synthesis of gamma-Lactame Building Blocks from Unsaturated Esters and Hydroxylamine. *Eur. J. Org. Chem.* **2023**, e202300288.

The publications are referred to in the text by their roman numerals.

AUTHOR'S CONTRIBUTION

Publication I
CJ conceived the project, performed most of the experimental work, interpreted analytical results and wrote the manuscript. Supporting experimental work was carried out by MH as part of her Master thesis project. KS prepared and purified the laccases. VBU supervised the laccase production. JD supervised the experimental work, reviewed and edited the manuscript. All authors critically reviewed the manuscript.

Publication II
CJ conceived the project, performed most of the experimental work, interpreted analytical results and wrote the manuscript. Supporting experimental work was carried out by BJG and JASA. Supporting experimental work was carried out by MH as part of her Master thesis project. JD supervised the experimental work, reviewed and edited the manuscript. All authors critically reviewed the manuscript.

Publication III
CJ conceived the project, performed the experimental work, interpreted analytical results and wrote the manuscript. MN carried out the crystallographic analysis. KR was involved with the crystallographic analysis. JD supervised the experimental work, reviewed and edited the manuscript. All authors critically reviewed the manuscript.

LIST OF OTHER PUBLICATIONS

IV A. F. Kiefer, Y.-C. Liu, R. Gummerer, **C. Jäger**, J. Deska, An Artificial In Vitro Metabolism to Angiopterlactone B Inspired by Traditional Retrosynthesis. *Angew. Chem. Int. Ed.* **2023**, e202301178.

V **C. Jäger**, C. Bruneau, P. K. Wagner, M. H. G. Prechtl J. Deska, Methanol-Driven Oxidative Rearrangement of Biogenic Furans – Enzyme Cacsade vs. Photobiocatalysis. *Front. Chem.* **2021**, *9*, article 635883.

VI D. Thiel, F. Blume, **C. Jäger**, J. Deska, Chloroperoxidase-catalyzed Achmatowicz Rearrangements, *Eur. J. Org. Chem.* **2018**, *20*, 2717 – 2725.

VII A. G. Griesbeck, B. Goldfuss, **C. Jäger**, E. Brüllingen, T. Lippold, M. Kleczka, Strong Asymmetry in the Perepoxide Bifurcation Mechanism: The Large-Group Effect in the Singlet Oxygen Ene Reaction with Allylic Alcohols, *ChemPhotoChem* **2017**, *1*, 213 – 221.

ABBREVIATIONS

Ac	acetyl
AcK	acetyl kinase
ADH	alcohol dehydrogenase
ADP	adenosine diphosphate
ANO	aziridine *N*-oxide
API	active pharmaceutical ingredient
ATP	adenosine triphosphate
Ar	aryl
ArNO	nitrosoarene
BINAP	Bis(diphenylphosphino)-binaphatalene
Boc	*tert*-butyloxycarbonyl
Bn	benzyl
Bu	butyl
CALB	*Candida anarctica* lipase b
Cbz	benzyloxycarbonyl
CPO	chloroperoxidase
CRL	*Candida rugoasa* lipase
DCM	dichloromethane
DEAD	diethyl azodicarboxylate
DERA	deoxyribose-phosphate aldolase
DFT	density functional theory
DIP	diisopinocampheylborane
DMA	dimethylanthracene
DMAP	4-Dimethylaminopyridine
DMF	dimethylformamide
DMSO	dimethylsulfoxide
d.r.	diastereomeric ratio
EDA	ethyl diazoacetate
ee	enantiomeric excess
e.g.	exempli gratia
eq.	equivalent
ESR	electron spin resonance
Et	ethyl
etc.	et cetera
EtOAc	ethyl acteate
EWG	electron-withdrawing group
FMO	frontier molecular orbital
FT-IR	Fourier-transformed infrared spectroscopy
GDH	glucose dehydrogenase
GOx	glucose oxidase
GOase	galactose oxidase

Hex	hexane
HFIP	hexafluoroisopropanol
HOMO	highest occupied molecular orbital
HRP	horseradish peroxidase
$h\nu$	light
i	*iso*
IMHB	intramolecular hydrogen bond
IPA	isopropanol
IRED	imine reducatase
KIE	kinetic isotope effect
KRED	ketoreductase
L	ligand
L-DOPA	L-3,4-dihydroxyphenylalanine
LPO	lactoperoxidase
LUMO	lowest unoccupied orbital
M	moles per liter
Me	methyl
MeCN	acetonitrile
MML	*Mucor miehei* lipase
n	*normal*
NaBArF	Sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate
NAD^+	nictotineamide adenine dinucleotide
$NADP^+$	nicotineamide adenine dinucleotide phosphate
NDA	nitroso Diels-Alder
NMO	*N*-methylmorpholine *N*-oxide
NO	nitroxide
NMR	nuclear magnetic resonance
Nu	nucleophile
p	*para*
PA	phosphoric acid
PanK	pantpthenate kinase
PD	polarized diradical
Ph	phenyl
PNP	purine nucleoside phorphorylase
ppm	parts per million
PPM	phopshopentomutase
Pr	propyl
PyBOX	bis(oxazolinyl)pyridine
R	any group
rac	racemate
$Rad^{\cdot}$	radical
rt	room temperature
RONO	*O*-nitroso compound
SAM	*S*-adenosyl methionine
SET	single electron transfer

t	*tert*
TAD	triazolinedione
TBS	*tert*-butyldimethylsilyl
TDP	thiamine diphosphate
TEA	triethylamine
TIPS	triisopropylsilyl
TME	tetramethylethylene
TPL	thiamine-phenol lyase
Ts	tosyl
TS	transition state
UV	ultraviolet
X	halogen

1 INTRODUCTION

The nitroso group can be found in a large variety of compounds and takes critical roles in numerous synthetic strategies targeting bioactive compounds. Due to its unique reactivity, it is a quite versatile group, which can undergo various reactions important for chemists. Four major types of organic nitroso species R–X–N=O exist: *N*-nitroso, *O*-nitroso, *S*-nitroso, and *C*-nitroso compounds (Figure 1). The latter one was demonstrated to be an important synthetic tool in organic chemistry and will play a very central role in the research presented in this thesis. The intriguing number of chemical methods developed over time to produce such compounds and to understand their behaviour emphasizes the extent of interest in application of nitroso compounds by the chemical community. Due to the vast amount of research, only selected examples are highlighted in this thesis to describe the diverse field shaped by nitroso chemistry.

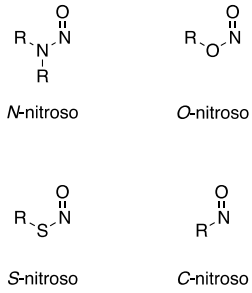


Figure 1 Different types of nitroso compounds.

Drastic changes in the world, such as melting glaciers and pole caps, enormous bush fires and polluted oceans request a need for more sustainable, waste-limiting and generally more environmentally friendly chemical transformations.

Modern biocatalysis enables the highly effective manufacture as well as optimization of enzymatic tools and allows the design of greener and in some cases even more productive synthetic strategies. Enzymes as Nature's catalysts make most biosynthetic chemical reactions possible, and we already use enzymes in a range of reactions based on their intrinsic natural reactivity. Especially baking or brewing would not be possible without the use of enzymes expressed by yeast or other microorganisms in fermentation processes to produce and preserve foods.

For chemists, Nature has always been a role model to mimic the most powerful processes. However, today many transformations are based on toxic or rare metal-based catalysts, hazardous solvents and harsh reaction

conditions, such as high pressure and elevated temperature levels, and not to mention the chemical waste, which is evolving as a consequence of atom inefficient or low yielding reaction designs. Some of the traditional chemical transformations are inspired by biological processes. Finding ways of mimicking enzymatic catalyzed reactions in Nature or imitation of biosynthetic intermediates aims at the development of new chemical reactions. Using the reverse approach to this by gathering inspiration from synthetic chemistry to develop biocatalytic methods, the extended use of enzymes beyond their natural task would give access to a wider portfolio of new-to-nature transformations. By exploiting these biocatalysts for traditional chemoinspired functions, reactions can be realized, which are not rooted in Nature and for which enzymes did not originally evolve (Figure 2).

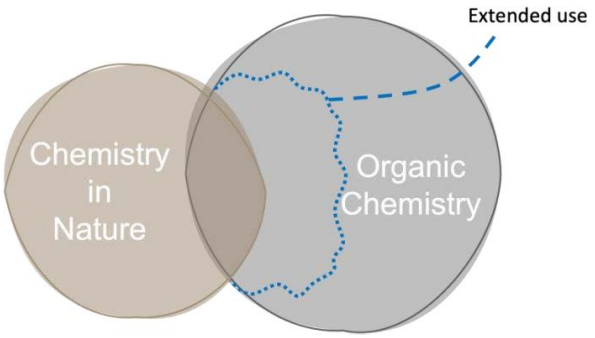


Figure 2 Reactions existing in Nature and organic chemistry. A little overlap of both spheres illustrates the mutual reactions, which can be catalyzed by enzymes as well as by chemical approaches. Extended use of Natures catalysts in organic chemistry would increase the extent of this overlap.

Abiotic biocatalytic tools would not just uncover green and more environmentally friendly alternatives, but their usually high selectivities and overall efficiency can lead to more attractive routes in synthetic chemistry. The aim is not to replace traditional chemistry, but to enhance the toolbox with a powerful reaction portfolio of an enzymatic approach towards non-natural transformations. While this field of research still represents a niche in the chemical society, it was growing rapidly in the last 20 years underlining its great potential.

By constructing bio-based production platforms, the chemical industry would significantly change to a more sustainable manufacture of all kinds of chemical products, from pharmaceuticals to fine and bulk chemicals. Currently, it appears essential to broaden our biological reaction portfolio for chemically relevant transformations to build a proper foundation for better implementation on enzymatic catalysis and meaningful applications in chemical production.

2 REVIEW OF THE LITERATURE

The nitroso group has gained increasing interest over time in the history of synthetic chemistry. This is mostly due to its unique and high reactivity. Interest in this compound class especially rose when the poisonous effect of nitrosobenzene reacting with hemoglobin was discovered, which results in a violet complex.[1,2] Subsequently, toxic nitroso compounds were also observed as metabolites of amine-containing medications.[3–5] Especially when these unexpected but important roles of nitrosoalkanes and nitrosoarenes were discovered in physiological processes, chemists started to investigate more about these reactions to reach a better understanding of the biological metabolic transformations but also to apply the gained knowledge for purely chemical aspects with the desire of utilization in synthesis.

The presence of nitroso compounds in Alder-ene and Diels-Alder transformations gave rise to multi-functionalized intermediates important for the synthesis of heterocycles or amination reactions. Consequently, nitroso chemistry gained popularity and found wider synthetic applications in processes such as modification of natural rubber[6] and numerous total syntheses of natural products and bioactive compounds.[7–9]

With the rising field of biocatalysis a mentionable number of synthetic tools was added to organic chemistry over the last decades. Seeking for a practical, selective, and environmentally friendly alternative to metal- and organocatalysts, enzymes gain increasing attention and are regularly found in today's laboratorial and industrial processes. Starting from observations of microorganisms consuming certain chemicals and transforming them selectively into specific products, the field of modern enzyme engineering developed, leading to adjustment of the biocatalysts for desired reactions or substrates.

2.1 *C*-NITROSO COMPOUNDS AND THEIR ROLE IN ORGANIC SYNTHESIS

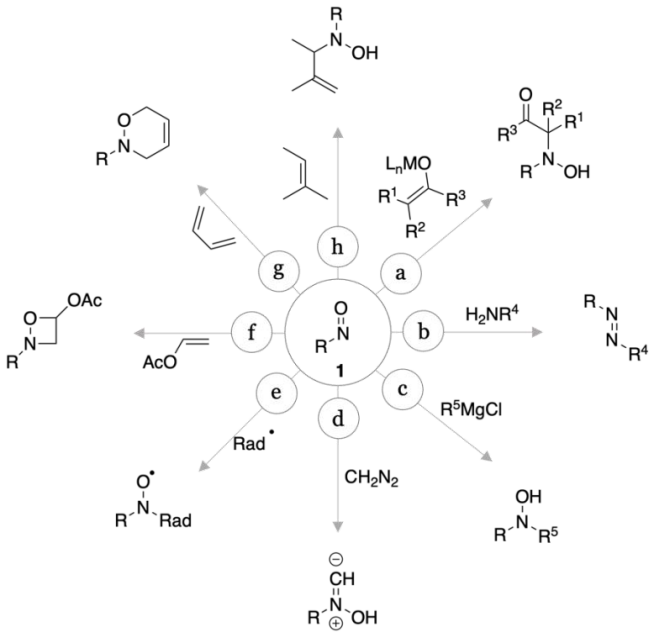
C-nitroso compounds (in the following referred to as nitroso compounds) can be used as versatile reactants in a variety of reaction classes such as additions, isomerizations, oxidations and reductions. This diversity is mostly due to their high reactivity, which makes them attractive intermediates in the development of efficient transformations in organic synthesis.

2.1.1 REACTIONS OF NITROSO COMPOUNDS

To give an overview of reactions that nitroso compound **1** can be involved in, a number of very useful and broadly applied procedures are presented in this

chapter. Proceeding in addition reactions they can act as ideal electrophiles and react with enolates in nitroso-Aldol reactions (Scheme 1, path a)[10,11], as well as with amines (path b)[12] and Grignard-reagents (path c)[13]. The reactivity can theoretically feature three different reaction modes: Nucleophilic attack on the carbonyl function (in carbonylnitroso compounds), attack at the nitrogen or attack at the oxygen atom of the nitroso group.

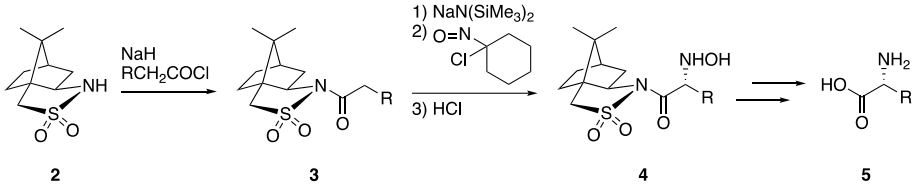
Nitrones can be obtained by reaction with diazomethane (path d)[14] and radicals are easily catched by nitroso species (path e)[15].
Cycloadditions are well studied, even though the [2+2] addition (path f) is rare and just observed with electron-rich alkenes and trichloronitroso-methane.[16] In contrast, [4+2] cycloadditions (path g) seem well established and usually proceed exclusively via this reaction pathway with dienes.[17,18] Lastly, the Alder-ene reaction is the favored option with alkenes (path h), especially by applying olefins with allylic hydrogens.[19,20]



Scheme 1 Reaction portfolio of nitroso compounds in organic synthesis.[20]

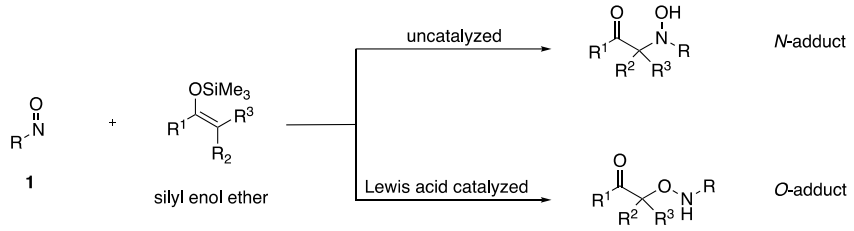
Among several representative reactions of nitroso compounds, the nitroso-Aldol reaction seems to be essential since it represents an easy method to functionalize carbonyls by introducing a nitrogen atom. Here, the nitroso species reacts with enolates to α-*N*-hydroxyamino carbonyl compounds from which a broad spectrum of α-amino acids and related compounds are accessible (Scheme 2). Following the route of Oppolzer et al. using sultam **2** as auxiliary to produce *N*-acyl derivatives **3** leads to highly selective reaction with blue 1-chloro-1-nitrosocyclohexane producing hydroxylamines **4** in excellent

diastereoselectivity.[10] Here, the enolate generated from **3** and the electrophilic nitroso compound are transformed into a nitrone which is hydrolyzed to the corresponding compound **4** in good yields and as a single diastereomer. *N*/*O*-hydrogenolysis of **4** followed by saponification gave rise to the enantiopure amino acids **5** and recovered the auxiliary **2**.



Scheme 2 Auxiliary-controlled diastereoselective nitroso-Aldol reaction utilizing Oppolzer's sultam **2**.[10]

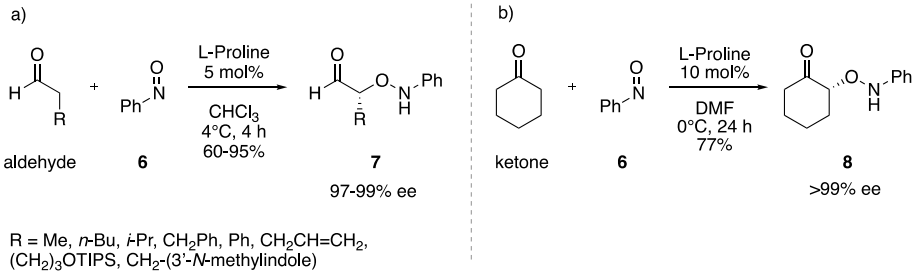
As stated before, the addition of the enolate to the nitroso group **1** can follow different modes of regioselectivity (Scheme 3). Like in the latter example of Oppolzer et al. formation of *N*-adduct is preferred in the uncatalyzed reaction of silyl enol ether and the nitroso compounds. In contrast, when employing a Lewis acid such as trimethylsilyl triflate, exclusively the *O*-adduct was obtained. It is known that the nitroso species favorably dimerizes in the presence of Lewis acid. Therefore, the pathway of the *O*-alkylation may not proceed in the classic Aldol-reaction. It is assumed that the Lewis acid further coordinates to the dimer and promotes the reaction towards the *O*-adduct.[21] Furthermore, metal enolates (M = Li, $SnBu_3$, ZnCl) follow most likely a pathway via the nitroso monomer and therefore afford the *N*-adduct. However, tin enolates were found to also produce the *O*-adduct in presence of Lewis acid.[22,23]



Scheme 3 Regiocontrol in the nitroso-Aldol reaction utilizing silyl enol ethers.[21]

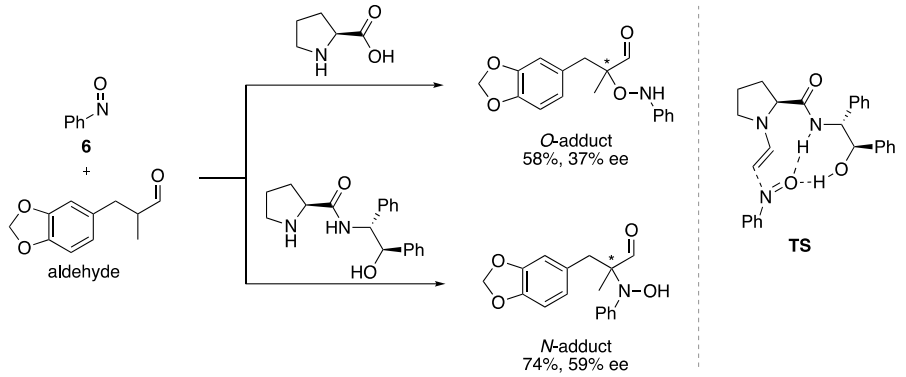
Overall, the selectivity depends on the type of enolate and presence of Lewis acid. The first enantioselective example of the *O*-nitroso-Aldol reaction was realized using Ag(I)-(*R*)-BINAP complexes together with tin enolates producing the *O*-adduct enantioselectively with 91% ee in very good yields, which could be maintained using various tin enolates. In addition, depending

on the nature of the Ag-catalyst the regioselectivity can be switched between the *N*- or the *O*-adduct reaching good to excellent enantioselectivity, respectively.[24–26] Organocatalyzed nitroso-Aldol reactions of aldehydes and nitroso compounds were observed using L-proline, producing enantiopure α-oxyaldehydes **7** in very good yields exclusively forming the *O*-adduct. The reaction follows the same mechanistic pathway which was considered already in traditional Aldol reactions.[27] Here, not just simple aldehydes (Scheme 4a) can be reacted with nitrosobenzene **6**, but also cyclic ketones could be converted into the O-functionalized products (Scheme 4b). However, the yields suffered from the lower reactivity of the latter one but could be improved by a slow addition of nitrosobenzene **6**.[28] The method was further exploited to yield *N*-*O*-heterocycles using nitrosobenzene **6** and distal dialdehydes.[29] Utilizing acyclic ketones also a minor formation of the *N*-adduct was observed, but still clearly favoring the formation of the aminooxylated ketone **8**.



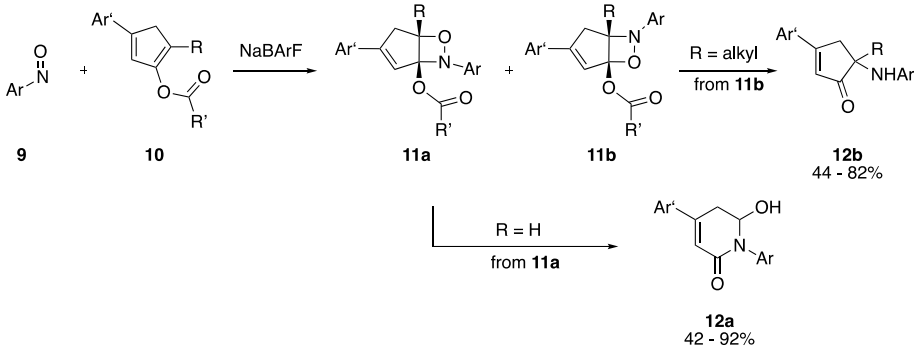
Scheme 4 Enantioselective α-aminoxylation of a) aldehydes[27] and b) ketones[28] with catalytic L-proline.

The first direct enantioselective procedure favoring the *N*-adduct was described with L-prolinamide and α-branched aldehydes (Scheme 5). Using L-proline in comparison is yielding the *O*-adduct as the only product. The nitroso-Aldol reaction follows a different transition state with L-prolinamide enabling the *N*-selectivity. With L-proline the oxygen is activated via the protonation of the basic nitrogen by the carboxylic acid. In contrast, using L-prolinamide the nitrogen is activated as electrophile through multiple hydrogen bonds between the oxygen and the β-hydroxyamide side chain in the transition state **TS** (Scheme 5, right).[30] Enantioselective *N*-adducts were also achieved by applying Takemoto's thiourea catalyst in the nitroso-Aldol reaction of nitrosobenzene and α-methylmalonamates. Here, the enantio-selectivity is believed to evolve from hydrogen bonding between the nitrosobenzene and the thiourea moiety. In addition, the enamine is generated by the pyrrolidine moiety of the catalyst. Therefore, the nucleophilic attack of the enolate proceeds in a face-selective manner giving enantioselectivities of up to 91% ee.[31]



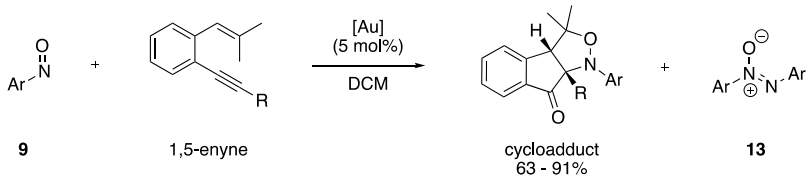
Scheme 5 Direct enantioselective *N*-nitroso-Aldol reaction using catalytic L-prolinamide.[30]

[2+2]-Cycloadditions with nitroso compounds yielding 4-membered heterocycles are scarcely explored, but recently regained attention. Chen et al. used NaBArF as catalyst for the reaction of nitrosoarene (ArNO) **9** and substituted cyclopentadiene **10** as alkene donor (Scheme 6).[32] However, the formed regioisomers **11a** and **11b** undergo, depending on their substitution pattern, either rearrangement to product **12b** or ring expansion to piperidone derivatives **12a** in good yields, respectively.



Scheme 6 [2+2] cycloaddition of nitrosoarenes **9** and cyclopentadienes **10** using NaBArF.[32]

Furthermore, via [3+2] cycloaddition of 1,5-enynes with aromatic nitroso compound **9,** an oxygenated cycloadduct was recently observed utilizing a gold-catalyst (Scheme 7).[33] Here, the nitrosoarene **9** not only reacts with the gold activated intermediates but also oxidizes the intermediates after the addition. The cycloadduct is formed alongside minor amounts of the gold-typical cycloisomerization products and due to the necessary excess of the nitroso compound also the azoxy species **13** is observed.



Scheme 7 Oxidative [3+2] cycloaddition of nitrosoarene **9** and 1,5-enynes.[33]

[4+2] cycloadditions and nitroso-ene reactions form another very important part of nitroso chemistry as they produce valuable synthetic building blocks for natural product synthesis and will be discussed in more detail later in this thesis.

2.1.2 REACTIVITY OF NITROSO COMPOUNDS

The reactivity towards many reaction partners is evoked by the enophilic character of the nitroso species. Being one of the most reactive enophiles, nitroso compounds feature a low LUMO energy compared to other reactive enophiles (Figure 3) or such as triazolinedione and singlet oxygen.[34]

Decorating the nitroso group with electron withdrawing groups leads to an even higher enophilic character. An example for this type is the nitrosocarbonyl species, which is extremely reactive and can be therefore exclusively formed in situ[20,34], has a particular short lifetime[35] and plays a central role in this thesis. The nitrosocarbonyl species propably forms the most reactive, but also most versatile, nitroso enophile. This highly enophilic character is associated with the especially low LUMO energy and makes them valuable intermediates in a variety of synthetic strategies.[34] Besides the characteristic as an electrophile, the nitroso group exhibits an ambiphilic behaviour. The difference in reactivity lies in the lone pairs of the nitrogen and oxygen atom, which is linked to a highly energetic HOMO granting these ambiphilic properties to the nitroso group and makes them also good nucleophiles. In particular, the high reactivity of nitrosocarbonyls originates from a small HOMO-LUMO gap.[36,37]

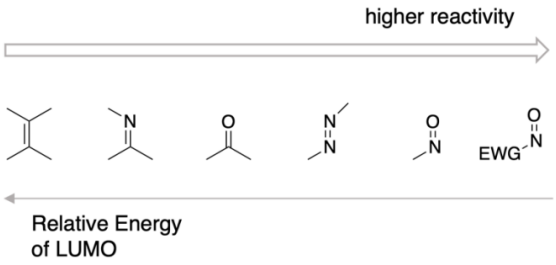
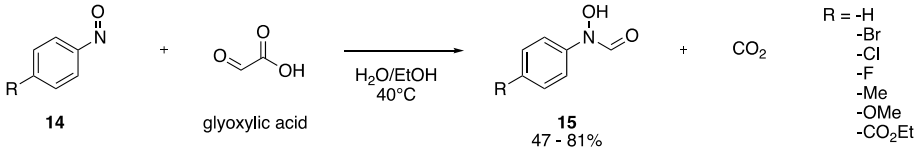


Figure 3 Relative LUMO energies of different enophiles.[38]

Nucleophilic attack by the nitrogen atom of the nitroso moiety is in fact possible, although rare and usually not preferred over other pathways. In the reaction of ArNO **14** and glyoxylic acid, the nitroso moiety behaves as a nucleophile by attacking the aldehyde moiety of the glyoxylic acid to give access to *N*-hydroxyformanilides **15** and equimolar amount of CO_2 (Scheme 8). The assumption that the nitroso arene acted as the nucleophile was supported by the observation that substitution of the aromatic ring with electron-donating groups increased the reaction rate, which was observed to be the opposite in reactions where the nitroso group serves as electrophile.[39] Later, also nucleophilic attack of nitrosobenzene and aliphatic methyl nitrosopropane to a variety of electrophilic carbonyls such as formaldehyde, acetaldehyde, pyruvic acid, glyoxylate and glyoxylic acid were observed resulting in the formation of hydroxamic acids.[40,41]

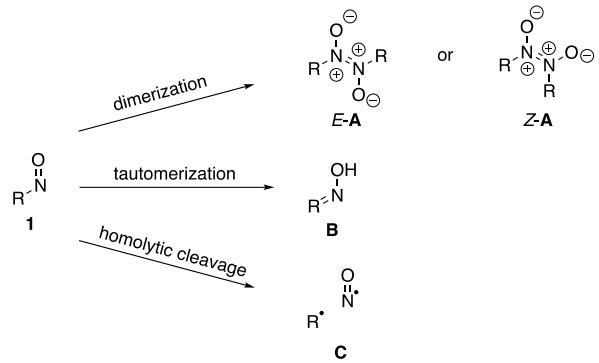


Scheme 8 Nucleophilic addition of various nitrosoarenes **14** to glyoxylic acid.[39]

However, the positive side of the versatile reactivity resulting in many reactions possible, comes with the downside of side product formation leading to a limited attention in the synthetic-organic arena until the 21st century. Most commonly, side products are formed via competitive pathways such as dehydration and undesired oxidation, which can cause formation of radicals[42], polymerization[20,43] or solvolysis.[44] In addition, potential problematic formation of dimers, tautomers or cleavage reactions can hamper the desired outcome originating from the reactive character of the nitroso group (Scheme 9). Therefore, most nitroso species, especially the nitrosocarbonyls, are generated exclusively in situ, and the moderately stable nitrosoarenes can be isolated. Nitrosoarenes mostly prefer to exist in a dimerized form and thanks to the reversibility, they can act as a nitroso reservoir.[45] Nevertheless, several monomeric aromatic nitroso compounds could be isolated when they contain electron-donating substituents in *para*-position at the ring structure or when stabilizing effects via intramolecular hydrogen bonds (IMHBs) of amino substituents in neighboring positions occur.[45–47]

The dimerization process is problematic for organic reactions since it deactivates the nitroso reactant from participating in the desired reaction pathways and can be easily spotted by fading of the characteristic blue or green colour of **1**.[48] The bias towards the formation of nitroso dimers arises from their potential nucleophilic character and results from the interaction of the lone pair located on the nitrogen atom with another nitroso moiety, which

serves as electrophile.[39] In a stable dimerized form arylnitroso species remain as an inactive reservoir. In addition, the dimer can form bidentate complexes with metals[4] and especially reactive nitroso species that proceed in dimerization decompose fast via Kirby's mechanism forming its anhydride along with liberation of nitrous oxide gas.[49] Depending on the nature of the substitution pattern R in compound **1** as well as solvation effects, the equilibrium between the monomer and the azodioxy dimers can yield *Z*- or *E*-isomers *E*-**A** and *Z*-**A**.[34,50]



Scheme 9 Common deactivation pathways of nitroso compounds.

Another crucial factor which influences the reaction outcome is tautomerization, that can occur via intramolecular hydrogen bond formation when the nitroso moiety is located near a hydroxy group via 1,5-H shift, or the molecule bears a hydrogen in α-position to the nitroso group. In that way oximes **B** are generated, which can easily produce several isomers via rotation of the O-H or C-N bond. Therefore, also selectivity can be heavily affected in this case. Since the oxime is usually preferred over the nitroso form, resulting from stronger IMHBs, the overall reactivity is also influenced due to this intramolecular deactivation of the nitroso moiety.[34]

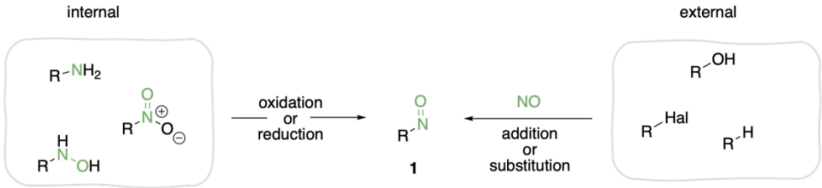
In addition, nitroso compounds suffer from radical decomposition pathways. Via homolytic cleavage, nitric oxide (NO) is produced alongside radical **C**. This is particularly interesting due to the physiological task of neutral NO in mammals. Via single redox processes, NO is highly selectively accessible through *C*-nitroso compounds.[51] Experimental studies of the unimolecular decomposition of nitrosobenzene were performed by Park et al. including FT-IR studies for C-N bond dissociation energies, which were supported by computational results.[42] Later, it was possible to deliberately produce neutral NO which is directly accessible via α-cyanonitroso species and clearly uncovers them as *C*-nitroso donors of nitric oxide. The same study proved that electron withdrawing groups stabilizing the radical through delocalization decrease the C-N bond dissociation energy.[52] However, nitroso intermediates are also known to act as radical traps, which makes them useful

for the synthesis of polymers and other coupling reactions.[53,54] In addition, single electron reduction or oxidation reactions were observed, which play a certain role in the synthesis of sterically hindered secondary amines and high-value heterocycles.[54]

The three described special reactivities obviously cause undesired pathways for most synthetic organic reactions with nitroso compounds, especially in ene-type and Aldol reactions.

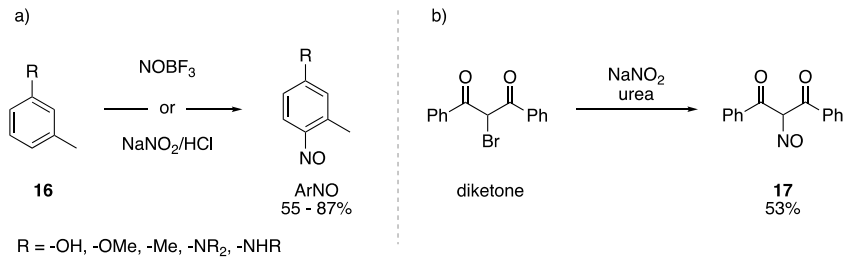
2.1.3 SYNTHESIS OF NITROSO COMPOUNDS

Several ways of forming a nitroso group exist that rely on the formation of a new carbon-nitrogen bond with external NO sources or exploitation of internal NO sources via oxidation of amines or hydroxylamines or via reduction of nitro moieties (Scheme 10).



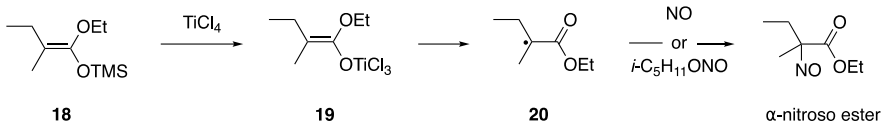
Scheme 10 Formation of nitroso compounds **1** using internal and external NO sources.

External NO sources for the synthesis of nitroso compounds typically comprise commercial reagents such as $NaNO_2$/HCl, $NOBF_4$, NOCl, $NOSbF_6$, or *O*-nitroso compounds RONO.[48] Thus, the synthesis of nitrosoarenes ArNO can be as simple as aromatic substitution (Scheme 11a). Additional functional groups at the ring structure in compound **16** usually force the nitrosation into *para*-position.[55] Halogen-NO exchange reaction allow nitrosation in selective positions. The bromine in the diketone can be exchanged with NO to give rise to nitroso compound **17** (Scheme 11b).[56]



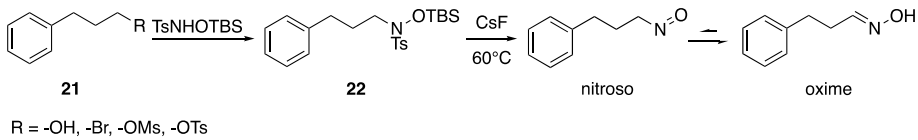
Scheme 11 Nitrosation via a) aromatic substitution[55] and b) halogen-NO exchange[56] using external NO sources.

Nitrosation of enolates is another possibility to introduce an external nitroso moiety.[48,57,58] Ketene *O*-alkyl-*O*'-silyl acetals **18** give rise to α-nitroso esters through addition of direct NO or nitric oxide from isoamyl nitrite to the double bond by employing $TiCl_4$ (Scheme 12). The activated intermediate **19** is formed by treating acetal **18** with titanium tetrachloride. Subsequent addition of the NO or RONO to radical **20** yields the α-nitroso ester.[59]



Scheme 12 Nitrosation sequence yielding α-nitroso esters using external NO sources.

In addition, an elegant way to introduce nitroso groups externally is the substitution reaction with TsNHOTBS followed by a subsequent elimination step (Scheme 13). This method works with versatile starting materials **21** through nucleophilic substitution of alkyl bromides, alkyl sulfonates or Mitsunobu reaction of primary or secondary alcohols. The substitution by TsNHOTBS provides an intermediate **22**, from which through subsequent treatment with CsF the nitroso compound can be obtained. On the downside, since the nitroso species bears a hydrogen in α-position it may readily tautomerize to its oxime.[60] The reaction was not executed with tertiary substituted starting materials or other compounds which would be able to prevent tautomerization but would be in fact an interesting feature of this method.

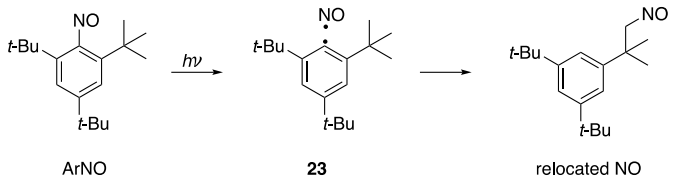


Scheme 13 Substitution at starting material **21** with subsequent elimination towards the nitroso compound and tautomerization to the oxime using external NO sources.

Besides the introduction of nitroso groups from an external NO source, it can be formed also internally in various ways.

Direct internal NO sources are rare but could be accomplished using photochemistry on nitroso arenes. Here, relocation of a nitroso within a molecular framework was realized via irradiation with UV-light (Scheme 14). Through homolytic cleavage of the nitroso group in ArNO in the first step, the radical **23** alongside with the nitrosyl radical is generated. The phenyl radical **23** was observed to abstract a hydrogen atom originating from one methyl group of the *t*-butyl group in *ortho*-position. The actual relocation takes place

under trapping the emerging primary radical by the generated NO-radical leading to a relocated NO-species.[61]

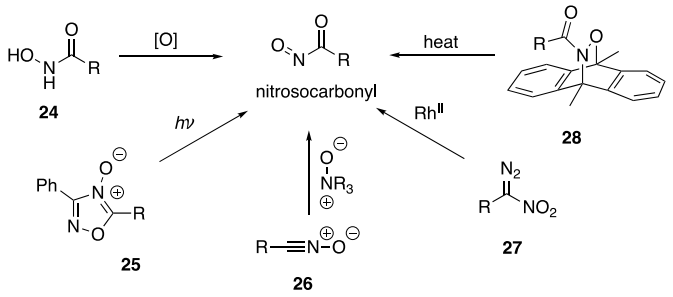


Scheme 14 Direct internal NO source in form of ArNO.

Furthermore, also amines, hydroxylamines or nitro groups can be considered internal NO sources, which are converted into the nitroso moiety via oxidation or reduction.[48,62,63] This is especially interesting for the formation of nitrosocarbonyls, which play a central role in this thesis.

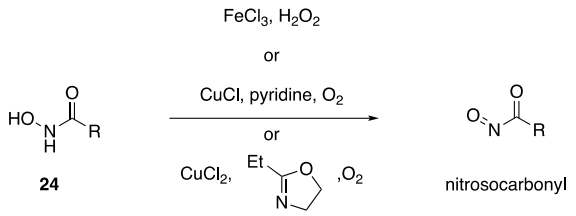
2.1.3.1 Synthetic Methods yielding Nitrosocarbonyls

A substantial number of versatile methods for the formation of nitrosocarbonyls have been developed over the decades, demonstrating that their chemistry seems to be an interesting issue for synthetic chemists. Most frequently used methods are thermal dissociation from anthracene adducts (**28**)[64,65], photolysis of 1,2,4-oxadiazole-4-oxides (**25**)[66,67], application of hypoiodites[68], iodosobenzene[69] or NMO[66] as oxidizing agents in the transformation of hydroxamic acids (**24**) or transition metal catalyzed dehyrogenation of **24** as well as reduction of α-diazonitroalkanes (**27**) to name a few (Scheme 15). Naturally, catalytic methodologies became the most desirable way , and a plethora of metal-mediated processes using Fe[70], Cu[53,71,72], Ir[73], Ru[74–76], Rh[72], Mo[77] and Pd[78,79] as catalysts for the oxidation of hydroxylamines and hydroxamic acids or reduction of nitro groups have been reported. Reaching from simple metal salts to more complex catalysts bearing enantiopure or multi-dentate ligands, a broad variety of methods and catalysts were studied.



Scheme 15 Overview of in situ nitrosocarbonyl generation.[80]

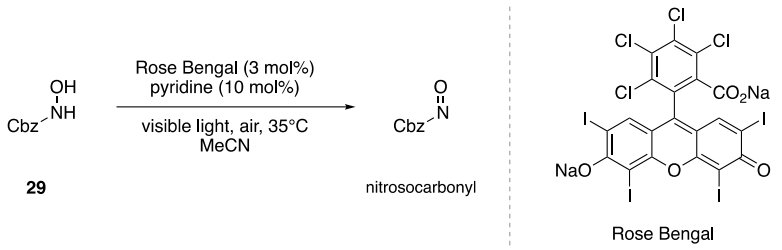
Especially Fe- and Cu-catalysts attracted particular interest due to their ability to oxidize *N*-hydroxycarbamates under very mild conditions, which also allowed the oxidation in an aerobic environment (Scheme 16). Simple iron(III) chloride in combination with hydrogen peroxide leads to the reactive nitrosocarbonyls via oxidation of hydroxamic acids **24**.[70] Alternatively, using copper(II) chloride in combination with an oxazoline ligand, aerobic dehydrogenations were accomplished where the oxidant is simply aerial oxygen.[81] This method was further adapted to employ copper(I) chloride as catalyst alongside with pyridine as ligand. Here, the rate of ene product decomposition was decreased, hence reducing the ability of over oxidation.[71]



Scheme 16 Overview of in situ nitrosocarbonyl generation using Fe or Cu salts.

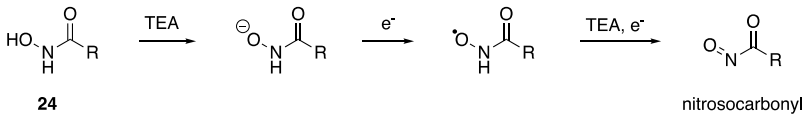
Over the time, many transition metal catalysts were compared with each other and their differences in the mechanism of substrate oxidation raised interest. It is potentially possible to yield enantioselectivity in nitroso-ene transformations, therefore it could be assumed that chiral catalysts can influence on an asymmetric outcome.[82,83] However, first studies by Flower et al. utilizing a chiral Ru(II)-salen catalyst did not induce enantioselectivity in the subsequent nitroso trapping. Therefore, this observation gave evidence that the nitroso intermediate is released from the metal directly after the oxidation.[76] A similar observation was made utilizing Cu(I) and Cu(II) catalysts, using enantiopure aminoalcohols as ligands, yielding a racemic outcome of the trapping product.[84] Nevertheless, it was possible to create selectivity by employing chiral catalysts after all. Several ways of enantioselective nitroso trapping will be discussed in the following chapters.

Even though iron and copper can be considered less problematic than other transition metals from a sustainability perspective, metal free protocols have been also studied as attractive alternatives. Photocatalyzed oxidations using the organic dye Rose Bengal under irradiation with visible light and oxygen as terminal oxidant were succesfully applied in the generation of nitrosocarbonyls from the corresponding hydroxycarbamate **29** (Scheme 17). The reaction is believed to take place via direct electron transfer between the excited state of the photocatalyst and the acylated hydroxylamine.[85]



Scheme 17 Photocatalyzed formation of the nitrosocarbonyl using Rose Bengal.

A recent approach for the manufacture of acylnitroso compounds is the direct electrochemical oxidation of hydroxamic acids using platinum electrodes and alternating current (Scheme 18). In the proposed mechanism of the electrolysis, the hydroxamic acid **24** is first deprotonated by triethylamine and then oxidized to a radical intermediate, which is thought to be stabilized by a HFIP-base electrolyte. In a last step, the radical might be deprotonated again followed by a second oxidation to form the nitrosocarbonyl.[86]



Scheme 18 Electrochemical oxidation of hydroxamic acid **24**.

2.2 THE NITROSO-ENE REACTION

To place the nitroso-ene reaction into the picture of ene chemistry, it is important to start this chapter with a brief introduction to the general ene reaction. The principle was first recognized by Alder in the 1940s and therefore the reaction is also referred to as the Alder-ene reaction.[87]

The nitroso-based methodology to conduct ene-type reactions was discovered in 1965 by Banks, Barlow and Hazeldine and constitutes a scientifically interesting option for the formation of new carbon-nitrogen bonds.[88] Even though it features a valuable approach to mild allylic nitrogen functionalisation of alkenes, it was initially not studied to its full extent.

2.2.1 THE ENE REACTION

The ene reaction itself is a pericyclic reaction in which an alkene bearing an allylic hydrogen forms a bond with a compound containing a double or triple bond. In this scenario the olefin is called the 'ene' while the latter one is described as the 'enophile'. As a result, new bonds are created between the two reactants via hydrogen shift and migration of the double bond of the ene counterpart (Figure 4, left). The reaction pathway follows a thermal addition of the olefin bearing the allylic C-H bond to the enophile via suprafacial orbital orientation including a six electron transition state (Figure 4, right).[89] Interaction between the HOMO of the olefin, the LUMO of the allylic C-H bond of the ene and the LUMO of the enophile leads to a concerted pathway resembling aspects of Diels-Alder reactions and [1,5]-sigmatropic-H shifts.[90]

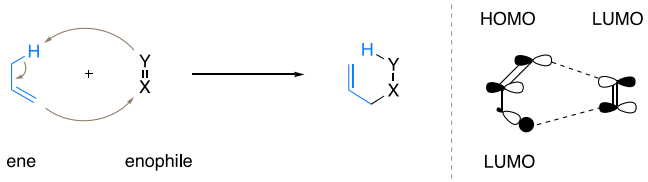
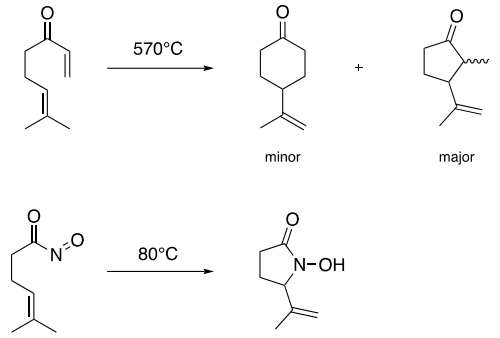


Figure 4 The general ene reaction, illustrated as a pericyclic concerted reaction pathway.

As described in the first part of the literature review, enophiles are π-bonded molecules possessing a characteristic low-lying LUMO. Included are carbon-carbon multiple bonds such as olefins, acetylenes or benzyne, but also carbon-heteroatom multiple bonds such as C=O, C=N, C=S and C=P. In addition, hetero-hetero multiple bonds such as N=N, singlet O=O, N=O and S=O are highly reactive in the ene reaction.[90] These and even more options make the ene reaction an extremely versatile tool for synthetic chemists.

However, the thermal ene reaction using simple olefins as enophiles requires high temperatures and makes it less applicable for synthesis. Yet, significantly less thermal energy is necessary when nitroso enophiles are used,

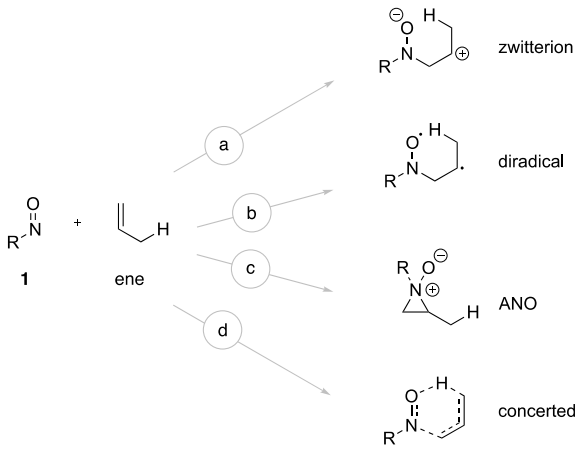
instead of the only moderately activated alkene (Scheme 19).[7] As stated before, the lower the LUMO of the enophile, the higher is its reactivity. Therefore, decoration either side of an olefin with electron withdrawing groups or embedding electronegative heteroatoms into the double bond, such as the nitroso moiety, will increase the reactivity further.



Scheme 19 Thermal ene reactions of enophiles bearing different reactivity.[7,91]

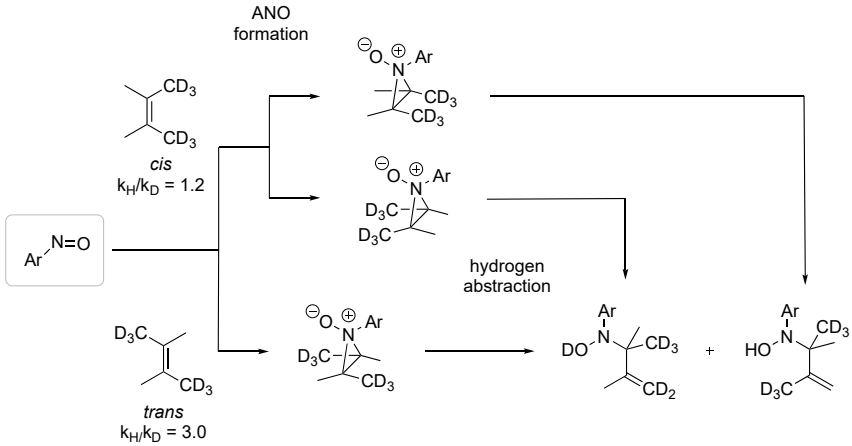
2.2.2 THE MECHANISM OF THE NITROSO-ENE REACTION

The different reactivity found in nitroso compounds does not just lead to faster reactions but also change the mechanistic aspects of the ene reaction. While the typical Alder-ene reaction follows a concerted pericyclic pathway, the ene reaction of nitroso compounds **1** does not proceed in a concerted manner and is believed to follow a stepwise pathway. Over the years a variety of plausible mechanisms were proposed especially for the intermolecular nitroso-ene reaction. Yet none could be unerringly proven and therefore leaving different mechanistic proposals still under debate. Several experimental and computational studies were performed but could rarely unambigiously confirm each other's result. Most pathways are believed to include zwitterionic (Scheme 20, pathway a) or diradical intermediates (Scheme 20, pathway b) as well as intermediary aziridine *N*-oxides (ANO; Scheme 20, pathway c). However, a concerted mechanism is not fully excluded (Scheme 20, pathway d). In addition, even more possible intermediates were investigated, but believed to be very unlikely. They include highly strained four membered cyclic species[92] or two separate radical species[93]. The four most plausible pathways will be discussed in the following section.



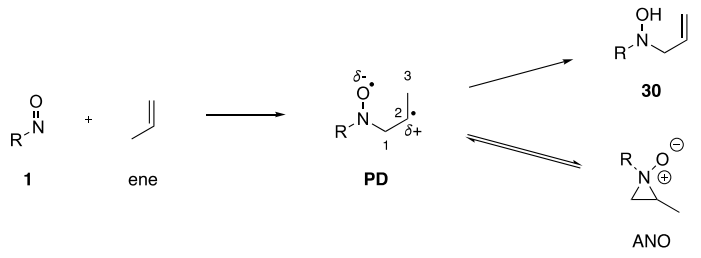
Scheme 20 Plausible intermediates in the mechanism of the nitroso-ene reaction.

A first approach towards mechanism elucidation was attempted by Seymour et al. in the early 1980's by using the Stephenson's isotope test (Scheme 21).[93] Here, they employed deuterium-labeled *trans*- and *cis*-tetramethylethylene (TME) and observed different kinetic isotope effects (KIE) for the two isomers. The *cis*-TME gave an insignificant KIE (k_H/k_D ~ 1), whereas a large KIE ($k_H/k_D \gg 1$) was found for the *trans*-isomer. These KIE's for the intramolecular isotope competition were interpreted as an ANO as plausible intermediary structure.[94] It was postulated, that the ANO is formed irreversibly and as the rate limiting step. For the *cis*-isomer the oxygen atom can either face the deuterated or undeuterated side of the olefin. Therefore no competition between hydrogen and deuterium takes place, which leads to a KIE of ~1. This is different in the reaction with *trans*-TME since a clear competition between hydrogen and deuterium is present in the abstraction step. Both CH_3 and CD_3 are intrinsically accessible and compete, leading to the observed large KIE. Later, an experimental study by Adam et al. confirmed these findings of an intermediary ANO as the rate-limiting but reversible step in the reaction pathway. They found that *cis*-TME and fully deuterated TME expressed smaller, but still significant KIE values ($k_H/k_D > 1$), compared to *gem*- or *trans*-TME ($k_H/k_D \gg 1$) with 4-nitronitrosobenzene, which means that a reversible ANO should be involved.[20,95,96] The activation energies of the two transition states in this reaction pathway are in the same range and since the abstraction of deuterium is less favoured compared to hydrogen, partial reversal of the intermediate from the oxygen facing the deuterated side of the ene to the undeuterated side of the olefin can be expected. Therefore, more of the ene reaction follows a normal H-abstraction pathway which can be observed in the KIE of $k_H/k_D > 1$.[96]



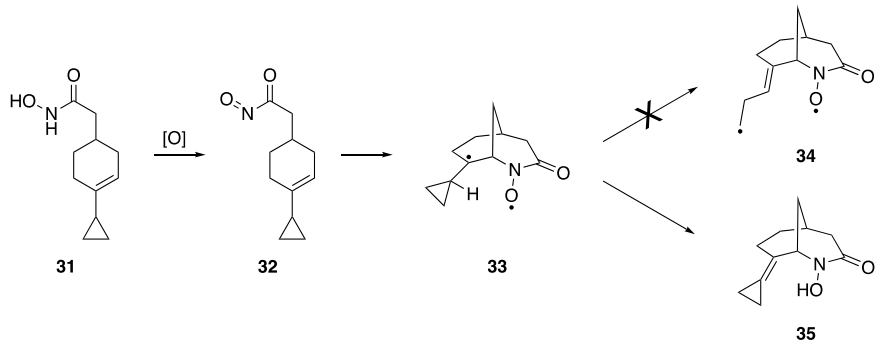
Scheme 21 Stephenson's isotope effect for the ene reaction of nitrosoarene.[20]

Simultaneously, Leach and Houk questioned the pathway via the ANO intermediate through computational results, which identified a polarized diradical (**PD**) as compulsory intermediates in the reaction of nitroso compounds **1** and an olefin (Scheme 22).[37,97] The acyclic **PD** as intermediate is still able to form the cyclic ANO, but the **PD** was found to be energetically favoured over the ANO intermediate. It would be expected that such a species would experience rotation around the C-C and C-N bond, however, the energy barriers of these rotations were higher in energy than the ones leading to the product **30** or the ANO. Therefore, any rotation around these bonds was excluded. These highly energetic barriers are explained by hydrogen bond stabilization between carbon atom C2 and the negatively polarized oxygen atom as well as a potential interaction between the 2p-orbital of C2 and the π^* orbital of the nitroso bond. Another reason is that rotation around these bonds is slow and therefore the nitroso moiety can change the location from one end to the other of the olefin, which can happen via the ANO. However, the final transfer of the hydrogen atom (or proton) does not proceed via the ANO towards the product and therefore the ANO intermediate is termed as 'innocent bystander'.



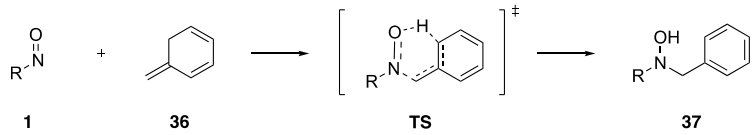
Scheme 22 Proposed mechanism of the nitroso-ene reaction involving a diradical intermediate.[97]

By employing a cyclopropyl substituent as radical clock in their substrate **31**, Zhai et al. wanted to identify whether diradical **33** or a zwitterionic analogue is involved in the intramolecular ene reaction of nitroso compound **32** (Scheme 23).[98] However, the cyclopropyl stayed intact yielding the product **35**. Followed by DFT calculations, the researchers suggest that the reaction proceeds via zwitterionic or diradical intermediates. They found that the hydrogen transfer in **33** is faster than a competing cyclopropane ring opening to **34**, leading to the assumption of diradical **33** as the obligatory intermediate in this nitroso-ene reaction. The zwitterionic pathway expressed similar energies as the diradical one and experimental evidence of the reaction proceeding via both pathways in a parallel manner was found in the *E*/*Z*-selectivity in the olefin in derivatives of **35**. However, they completely omitted the possibility of an involved ANO intermediate, which might be inferred based on the structural geometry of the compound.



Scheme 23 Nitroso-ene reaction of **32** following a diradical pathway without opening of the cyclopropyl ring.[98]

Still, a concerted pathway was investigated by Lu et al. using computational models (Scheme 24).[99] They could show that aromatic stabilization in **TS** leads to a one-step concerted reaction of nitroso compound **1** and aromatic olefin **36** to afford the product **37**, which is energetically favoured over the stepwise diradical or zwitterionic pathways. In addition, the reaction would be highly exothermic due to this stabilization and proceeds, similar to nitroso-Diels-Alder reactions, preferably in *endo*-approach.



Scheme 24 Proposed concerted mechanism of nitroso compound **1** and *o*-isotoluene **36**.[99]

To conclude, the mechanism of the nitroso-ene reaction was studied via various experimental and computational methods and was found to be very likely following a stepwise pathway including a polarized diradical intermediate. Via further in-depth studies a bridging ANO intermediate was identified as 'innocent bystander' in a possibly parallel but reversible pathway connected to the polarized diradical. Therefore, product formation is assumed to take place via the PD. However, experimental evidence, which is supported by computational studies and considers all possibilities, is still unavailable.

2.2.3 SELECTIVITY IN THE NITROSO-ENE REACTION

Depending on the nature of the olefin counterpart different selectivity aspects can be observed in nitroso-ene reactions. These effects concern the regioselectivity as well as the stereoselectivity and can be influenced through several different factors. Therefore, it is possible to decorate the olefin for control of the selectivity to yield the desired reaction outcome.

2.2.3.1 Regioselectivity

There are numerous possibilities on the olefin counterpart for hydrogen abstraction and some are more preferred over others, but the regioselectivity strongly depends on the substitution pattern. While other reactive enophiles such as $^1O_2$ and triazolinediones (TAD) express their own unique abstraction behaviour[100,101], it is not surprising that nitroso compounds differ in that way as well. Nitroso species follow a so-called *skew* trajectory with trisubstituted acyclic and cyclic alkenes **38a** – **38d**. This effect results from steric hindrance between the substituents of the olefin and the ArNO. The hydrogen abstraction occurs highly regioselective in the *twix* position, which resembles a hybrid of the selectivity found with $^1O_2$ and TAD, meaning the nitroso compound abstracts from the more crowded side (*cis*) and the more crowded end (*gem*) (Figure 5). Therefore, the nitroso-ene reaction results in higher extent of regioselectivity than its related counterparts.[20]

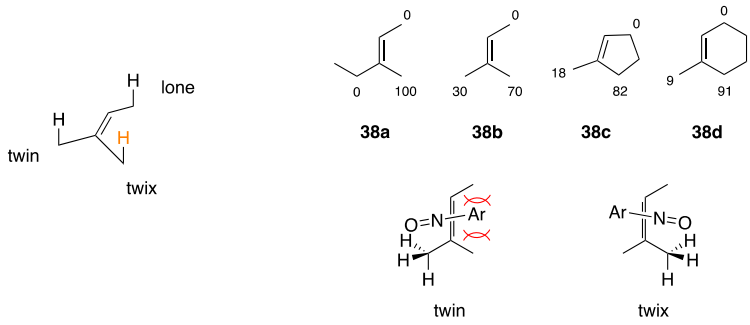


Figure 5 *Skew*-selectivity in the ene reaction of nitrosoarenes ArNO.

The steric effect occurs when the nitroso moiety points to the alkyl substituent in *twin* position. Here, the aryl group of the nitroso compound is situated between the alkyl substituents in *twix* and *lone* position, that is the more crowded side of the olefin. If the nitroso moiety points to the *twix*-alkyl substituent, the aryl group only interacts with the alkyl group in *twin* position at the less crowded side of the double bond, thus resulting in less steric repulsion (Figure 5).[20] However, for trisubstituted *Z*-olefins a slight bias towards *twin*-selectivity was observed in addition to the major *twix* abstraction. In this case conformational effects partly obliterate the steric hindrance, but the latter one still stays prominent.[95,102]

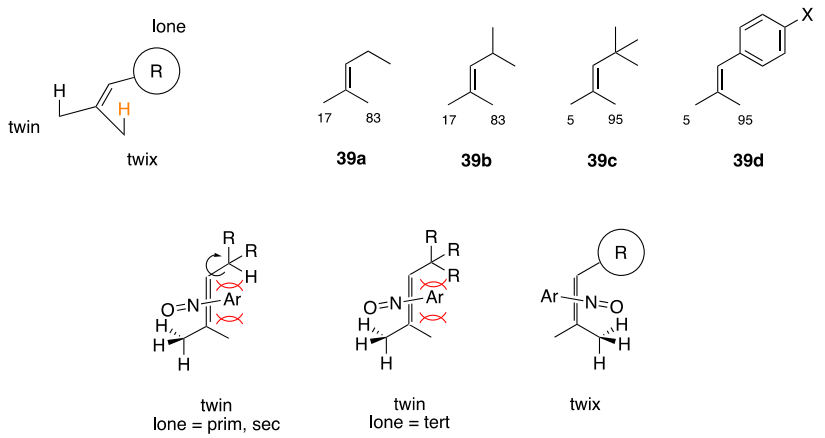
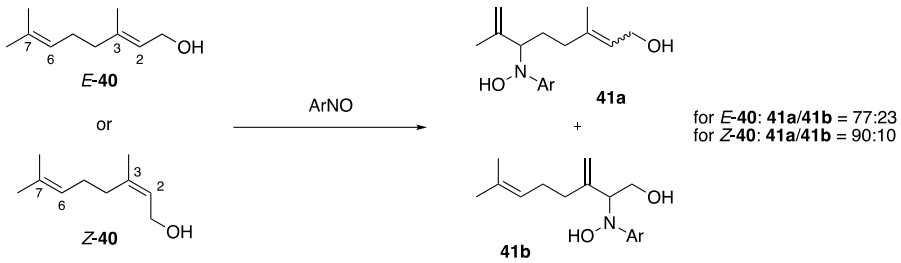


Figure 6 Regioselectivity control by the lone substituent due to steric interactions with ArNO.

Changes to the *lone* substituent gave another major influence on the regioselectivity. While primary and secondary *lone* substituents in olefins **39a** and **39b** yielded mostly a constant *twix*/*twin* ratio of 83:17 for most substrates bearing this substitution pattern, tertiary substituents such as the *t*-butyl group in olefin **39c** gave almost exlcusively *twix* selectivity (Figure 6).[103] Here, the same steric effects occur as previously described for trisubstituted alkenes (*skew* effect). However, primary and secondary *lone* substituents allow a favorable 'inside' conformation of the hydrogen atom, which diminish steric hindrance in the *twin* approach of the nitroso arene and the olefin. Such a conformational arrangement is sterically not favoured with tertiary *lone* substituents, which therefore only allow hydrogen abstraction in the *twix* position. For *lone* aryl substituents in olefin **39d** another effect has a stake on the selectivity. As with tertiary substituents, the *twix* selectivity is highly preferred. This arises from a partial positive charge during the attack of the enophile to the conjugated double bond. The resulting positively charged aryl ring coordinates to the negatively charged oxygen of the ArNO and therefore favours *twix* selectivity.[103]

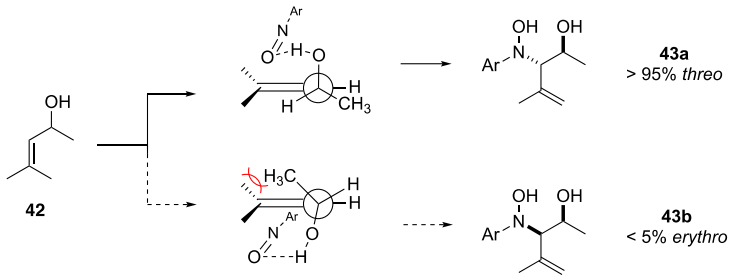
If a molecule contains more than one double bond, the regioselectivity depends on their initial reactivity (locoselectivity). In the case of geraniol *E*-**40** and nerol *Z*-**40**, the 6,7-double bond is favoured for the nitroso-ene reaction to **41a** due to electronical deactivation of the 2,3-double bond of the allylic alcohol (Scheme 25). However, coordination of the nitroso compound and the hydroxy group also allows formation of the locoisomer **41b**. Such hydrogen bonding has a stronger influence for geraniol *E*-**40** because it allows a less hindered hydrogen abstraction in the *twix* position. In case of nerol less adduct **41b** is formed since *twin* hydrogen abstraction would take place and is therefore less favoured.[95] In addition, hydrogen abstraction from the alkyl backbone is not observed in both cases due to build up of allylic strain.[20]



Scheme 25 Double-bond selectivity of geraniol *E*-**40** and nerol *Z*-**40** in the ene reaction with ArNO.[95]

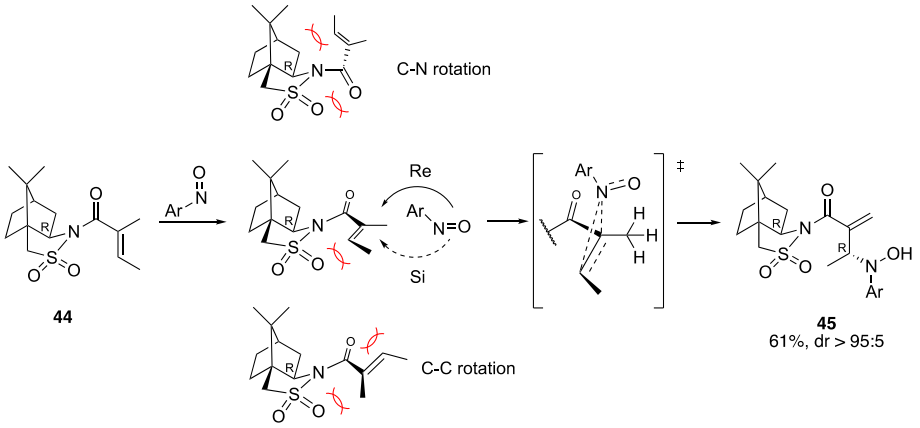
2.2.3.2 Stereoselectivity

Nitroso-ene reactions were also performed in a stereoselective manner. Utilizing trisubstituted chiral allylic alcohol **42**, the *threo*-nitroso adduct **43a** can be obtained in high diastereoselectivity (Scheme 26). Responsible for this outcome is the hydroxy-directive effect. Here, the oxygen atom of the nitroso moiety interacts with the hydroxy group via hydrogen bonding. The *erythro* configuration **43b** requires a transition state which is sterically unfavoured over the *threo*, due to allylic strain. In addition, for these substrates the hydrogen abstraction takes place exclusively in *twix* position. Therefore, the hydroxy group does not just influence the diastereselectivity, but also enhances regioselectivity.[104]



Scheme 26 Hydroxy-directive effect in the ene reaction with ArNO.[104]

Naturally, induced diastereoselectivity as controlling element in the construction of new asymmetry centers was also employed in auxialary-controlled strategies. In analogy to the enantioselective nitroso-Aldol reaction, Oppolzer's sultam **2** was used as directing group to construct a sterically loaded tiglic amide **44** (Scheme 27).

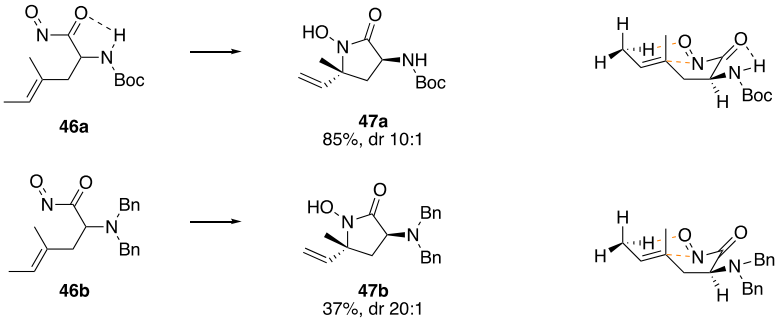


Scheme 27 Auxiliary-controlled nitroso-ene reaction of nitrosoarenes.[105]

Consequently, the alignment of ArNO takes place from the less hindered *Re*-face of the double bond yielding **45** in high diastereoselectivity. The *Si*-side is not available due to the sulfonyl oxygen atoms of the sultam moiety efficiently blocking space for any attack. In addition, rotation around the C-N and C-C bond in **44** is prohibited due to additional strain arising in the molecule (Scheme 27). Furthermore, subsequent release of the sultam group in **45** using silica gel gave rise to enantiopure clyclisized intermediates, which were reduced to the corresponding *β*-amino acids.[105]

Recently, Liu et al. also observed an induced diastereoselectivity in the intramolecular ene reaction of amino acid-derived acylnitroso compounds **46a** and **46b** (Scheme 28). First, the reaction was designed utilizing additional hydrogen bonding within the molecule by decorating the compound

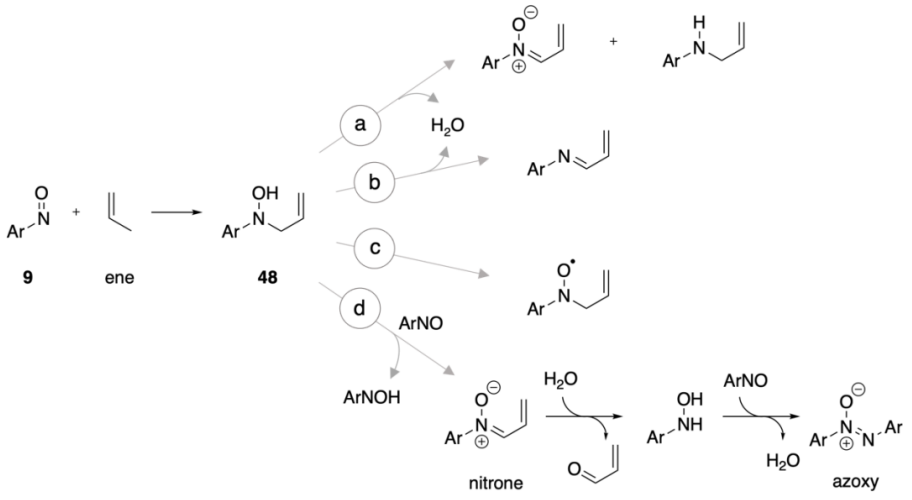
with a monoprotected amine moiety in α-position to the carbonyl (compound **46a**). However, compound **46b** lacks this hydrogen bond but still results in excellent diastereoselectivity which is rationalized with the assumption that the bulky -NBn_2 group is forced into a pseudoequatorial position in the transition state.[106]



Scheme 28 Proposed stereocontrol in the intramolecular nitroso-ene cyclization.[106]

2.2.4 SIDE REACTIONS

Besides the already discussed broad reaction portfolio of nitroso compounds, also degradation of the ene adducts can proceed in subsequent in situ reactions. The initially formed hydroxylamines can follow various reaction pathways leading to undesired side products, which however in some cases can express certain synthetic value themselves. Most common is the formation of nitrones, nitroxides, imines, amines and azoxy species (Scheme 29).[38,107]



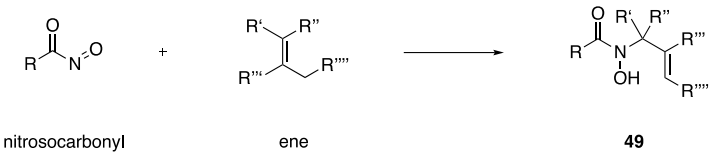
Scheme 29 Degradation pathways of the ene adduct **48** derived from the nitroso-ene reaction of **9** with an olefin as ene.[20]

Disproportionation is very abundant among ene adducts derived from electron-rich nitroso species, e.g. nitroso arenes **9**, hence leading to the corresponding nitrones and amines (Scheme 29, path a).[20,108] Imines can be obtained via treatment of ene adduct **48** with acid, base or heating which all can cause dehydration (Scheme 29, path b). This process can be also found in the Ehrlich-Sachs reaction.[20,109] Further oxidation of **48** caused by excess nitroso species or adventitious oxygen gives rise to radical species (Scheme 29, path c). The presence of these nitrosyl radicals was confirmed via ESR-spectroscopy for the reaction of nitrosoarenes and TME.[110,111] However, the formation of such nitroxyls is mostly inevitable since they are formed in almost all nitroso-ene reactions with exception of electron-poor substrates. Due to signal broadening this generated a challenge for NMR-spectroscopy, but the issue can be circumvented by treatment with a radical scavenger before analysis.[103]
Ene adducts **48** which bear a hydrogen atom proximate to the nitrogen are prone to further oxidation resulting in nitrones (Scheme 29, path d).[38] These highly reactive species are used in polymerizations and 1,3-cycloadditions.[20] Solvolysis of nitrones results in the corresponding hydroxylamine and enone.[44] Thus, the hydroxylamine can react further with a nitroso compound to give rise to the corresponding azoxy species. The resulting water can cause even more accelerated formation of the hydroxylamine. This pathway is especially prefered with less reactive olefins and can lead to azoxyarenes as the only products.

Employing electron-withdrawing groups at the nitroso species increase on one hand their desired enophility and result in relatively stable hydroxylamines **48** on the other hand.[38] However, also here the azoxy species were observed in minor quantities.[20]

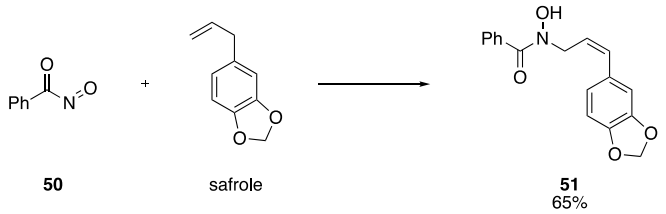
2.2.5 THE ENE REACTION OF NITROSOCARBONYLS

To outline the broad use of nitrosocarbonyls, selected examples will be discussed in the following section. Many different moieties can be accommodated in vicinity to the nitroso group (arylnitroso-, alkylnitroso-, halonitroso-, α-nitroso- and acylnitroso compounds) and the resulting reactive species can be employed in ene reactions. However, the chemistry of nitrosocarbonyls plays the major role in this thesis and their application is therefore pivotal to be discussed. These nitroso derivatives are highly reactive and can react spontaneously with almost any reaction partner after their generation, but also tend to decompose in the absence of trapping agents. They undergo clean ene reactions with a large variety of acyclic and cyclic olefins as the ene partner, thus forming a new C-N bond leading to adduct **49** (Scheme 30). Due to their high reactivity, they are also referred to as 'super-enophiles' in the literature.[80,112]



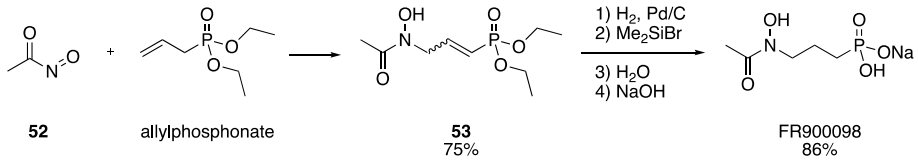
Scheme 30 General scheme of the nitroso-ene reaction of a nitrosocarbonyl and acyclic olefins.

One of the first literature examples dealing with the applications of nitroso-ene chemistry described the synthesis of hydroxamic acid **51**. Nitrosocarbonyl benzene **50** can react with safrole to yield product **51** (Scheme 31). The reaction was favoured due to the double bond conjugation to afford **51** with 65% yield.[49,107]



Scheme 31 Nitroso-ene reaction of nitrosocarbonyl benzene **50** and safrole.

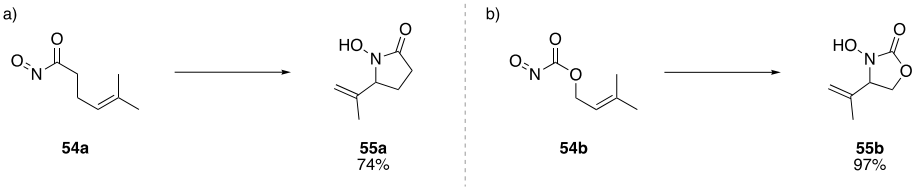
An interesting example is the ene reaction of nitrosocarbonyl methane **52** and an allylphosphonate as a key step in the synthesis of the antimalarial drug FR900098 (Scheme 32).[113] Due to its high reactivity, compound **52** was previously just used in ene reactions with unfunctionalized alkenes. However, also with the functionalized allylphosphonate a smooth reaction was observed with complete integrity of the phosphonyl group. The adduct **53** was obtained as a 1:1 mixture of olefin isomers in a good yield. Subsequent reduction of the C-C double bond, phosphonate hydrolysis and neutralization with NaOH yielded the antimalarial agent in high overall yield.



Scheme 32 Synthesis of antimalarial drug FR900098 via the nitroso-ene reaction of nitrosocarbonyl methane **52** and diethyl allylphosphonate.[113]

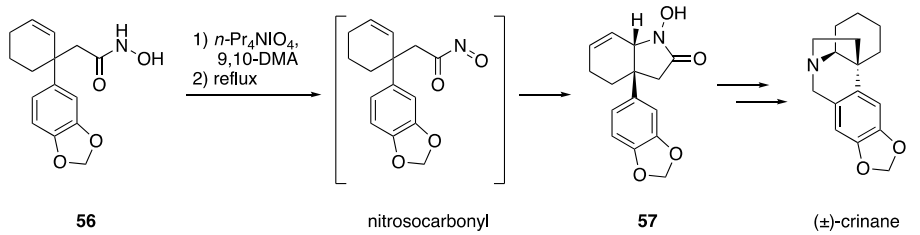
The first intramolecular nitroso-ene reaction was reported with nitroso compound **54a** leading to the 5-membered nitrogen-containing heterocycle **55a** in a good yield (Scheme 33a).[64,114] Similarly, the cyclisation was carried

out with nitrosoformate ester **54b** (Scheme 33b).[115] These nitroso-ene reactions built a particularly interesting foundation for various application in total syntheses, which eventually followed this finding and underline the great practicability and utilization in organic synthesis.



Scheme 33 Intramolecular nitroso-ene cyclisation of acylnitroso compound **54a** and nitrosoformate ester **54b**.[64,115]

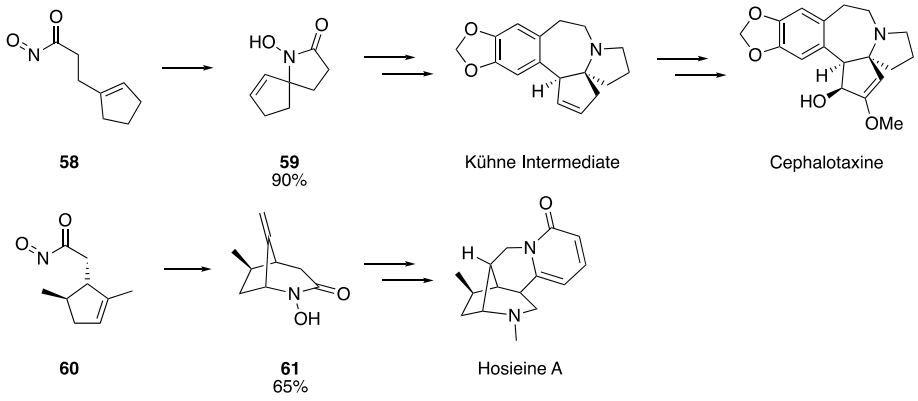
In the first successful total synthetic application, the intramolecular nitroso-ene reaction was employed as key step en route to the alkaloid (±)-crinane (Scheme 34). In the study by Keck et al. simple oxidation of **56** to the intermediate nitrosocarbonyl was not desirable since the ene adduct **57** proved to be labile towards oxidative conditions. Therefore, they applied their technique of 'intramolecular enophile transfer', in which hydroxamic acid **56** is oxidized in the presence of 9,10-dimethylantracene (9,10-DMA) leading to the corresponding Diels-Alder cycloadduct **28**, which was described in chapter 2.1.3.1. In a subsequent thermal release of the nitrosocarbonyl species via retro-Diels-Alder reaction, the ene reaction can take place under non-oxidative conditions and thus led to **57** in a quantitative yield. Reduction of the N-O and double bond afforded an amine, which was transformed into (±)-crinane via Pictet-Spengler cyclization or Prins reaction, in which the latter gave rise to the product in a high yield.[7]



Scheme 34 Synthesis of (±)-crinane via intramolecular nitroso-ene reaction.[7]

In a variety of more recent total syntheses, acylnitroso compounds **58** and **60** containing a cyclopentene moiety have gained interest. The first one found application in the formal total synthesis of cephalotaxine (Scheme 35). However, Hong et al. reported the application of the ene reaction to afford the Kühne intermediate – a key precursor leading to cephalotaxus alkaloids.[8,116]

Nitrosocarbonyl **58** was prepared via oxidation with n-Pr_4NIO_4 and in situ ene reaction, without the previously required 9,10-DMA trapping, led to the spirolactame **59** as one key module of the synthesis in an excellent yield. Similar spirolactames as core structure were produced via intramolecular nitroso-ene reaction and applied in the formal synthesis of (±)-halichlorine and (±)-pinnaic acid.[117,118]



Scheme 35 Synthesis of antimalarial cephalotaxine and hosieine A via the intramolecular nitroso-ene reaction of acylnitroso compounds **58** and **60**.

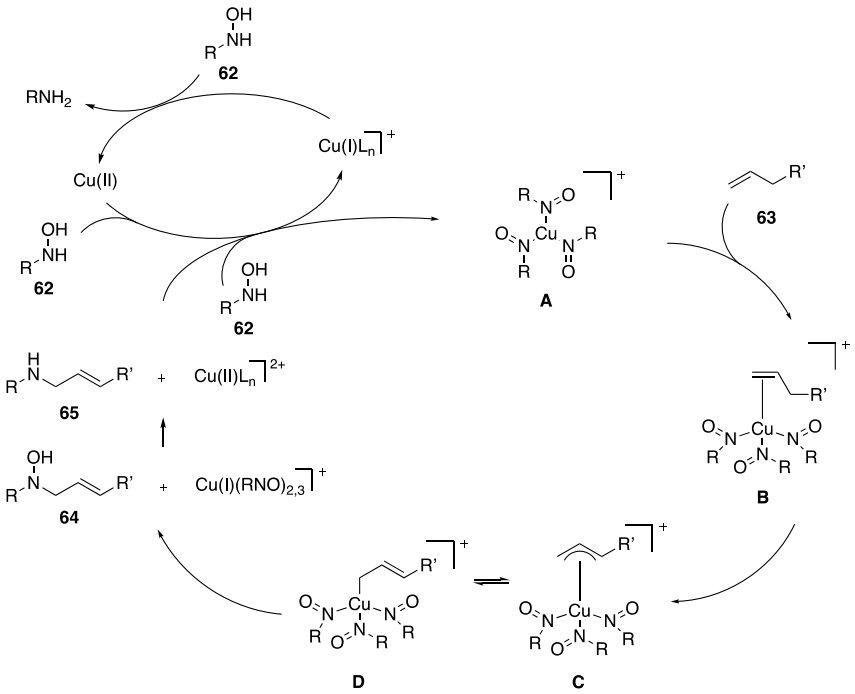
Another elegant application is the total synthesis of hosieine A, in which the core structure **61** is constructed via intramolecular ene reaction of nitroso compound **60** (Figure 39). Here the nitroso species **60** was generated from the corresponding hydroxamic acid via the Diels-Alder cycloadduct **28** (Figure 18) since direct oxidation led to severe decomposition. From the resulting key synthetic intermediate **61**, the natural product hosieine A was further constructed.[8,119] Furthermore, several other total syntheses of natural products such as mesembrine, dihydromaritidine and asmarines to name a few, are based on nitroso-ene chemistry.[120–122]

2.2.5.1 Metal-catalyzed Nitroso-Ene Reaction of Nitrosocarbonyls

Naturally, in addition to the stoichiometric use of oxidants, numerous metal-catalyzed procedures were developed. After various metal complexes or metal salts were already used for the oxidative in situ generation of unfunctionalized nitroso compounds[123–125], the application of catalytic oxidation towards nitrosoarbonyls became desirable.

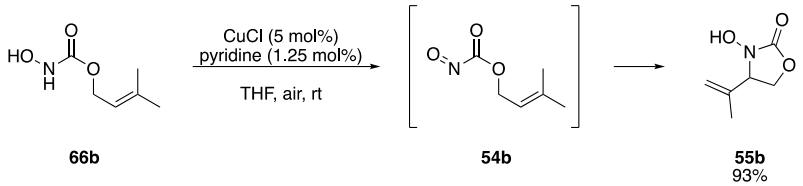
Copper-catalysis is efficiently used for the oxidation of hydroxamic acids and the oxidation of hydroxylamine **62** led to utilization in nitrosocarbonyl chemistry (Scheme 36).[126,127] Disproportionation of **62** forms the active Cu(II)-catalyst alongside with the corresponding amine. The active catalyst is able to oxidize hydroxylamine **62** into the nitroso species upon formation of a

copper nitroso complex **A**. Olefin association of **63** generates species **B**, which results in the allyl-copper complex **D** via allyl-H-transfer and Cu-haptotropic shift. Reductive elimination results in hydroxamic acid **64**. Depending on the ligand of the catalyst, **64** is not the final product of this reaction cycle. Further catalytic reaction yields the allyl amine **65** and feeds another activated Cu(II) into the cycle. To give an insight to the mechanism of the catalysis, a complex between the copper and the nitroso compound as an intermediate of the nitroso-ene reaction was isolated, proofing that the copper catalyst is not just oxidizing the substrate, but also takes a part in the ene reaction pathway.[127–129]



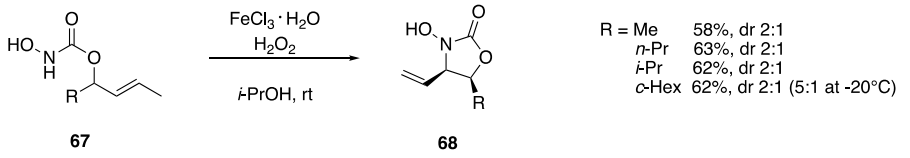
Scheme 36 Cu-catalyzed oxidation of **62** and nitroso-ene reaction of the resulting amine **65**.[127]

Later, Whiting et. al and Read de Alaniz et al. made further progress by utilizing catalytic amounts of Cu(I) and Cu(II) salts in aerobic conditions.[71,80,81] The latter research group was able to employ their method to inter- and intramolecular nitroso-ene reactions. As an example, the intramolecular-ene reaction of **54b** resulted in a comparable yield of **55b** as the non-catalytic thermal procedure (Scheme 37). Importance of the molecular geometry of **66b** was observed as substrates bearing *Z*-olefins merely decomposed.



Scheme 37 Cu-catalyzed oxidation of **66b** and intramolecular nitroso-ene reaction of **54b**.[71]

A mild oxidation of *N*-hydroxycarbamate **67** was discovered by Atkinson et al. employing simple iron(III) chloride as catalyst together with hydrogen peroxide as terminal oxidant (Scheme 38).[70] In addition, diastereoselectivity of the subsequent ene reaction was observed. Product **68** was obtained as *syn*/*anti* isomers with a ratio of 2:1 regardless the size of the substituent. Different solvents and catalysts were tested with no effect on that ratio. However, when the oxidation of **67** bearing *c*-hexyl as substituent was cooled to -20°C an improved ratio of 5:1 *syn*/*anti* in heterocycle **68** was observed.



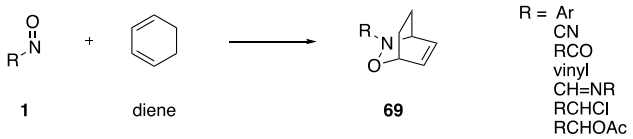
Scheme 38 Fe-catalyzed oxidation of **67** and in situ intramolecular nitroso-ene reaction leading to diastereselectivity in the formation of **68**.[70]

In addition, a few examples of Ir(I)- and Ru(II)-catalyzed nitroso-ene reactions for the oxidation of hydroxamic acid **24** are extensively reported for nitroso-Diels-Alder reactions, but rarely for the ene reaction. Besides simple metal salts, complexes with hydrogen peroxide as oxidant were employed. Using the iridium or ruthenium catalyst for oxidation of Cbz-protected hydroxylamine **29** and subsequent trapping with olefins low to mediocre yields of the adducts were achieved. However, applying chiral PyBOX ligands for the ruthenium catalysis, did not alter the outcome of the subsequent trapping reaction in an enantioselective way.[82]

In summary, it seems to remain a prevalent challenge to introduce significant selectivity into the nitroso-ene reaction. The application of chiral ligands is limited and not studied to its extend.

2.3 THE NITROSO-DIELS-ALDER REACTION

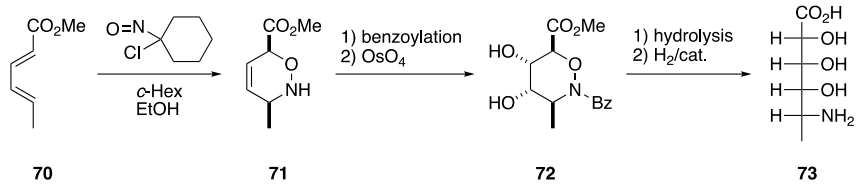
Other than the ene reaction of nitroso compounds, nitroso-Diels-Alder (NDA) reactions play a minor but still significant role in this thesis. These powerful hetero-Diels-Alder transformations participate in efficient and creative strategies en route to biologically relevant molecules and are found in a variety of total syntheses of natural products. Especially asymmetric variants of this reaction type are valuable for building bioactive skeletons containing six-membered heterocycles. Therefore, several methods were developed over time to yield those valuable synthetic intermediates by either chiral auxiliaries, or organo and metal catalysis. Nevertheless, examples for the induction of NDA reactions in a highly stereoselective manner is still today scarce.[130]



Scheme 39 Diels-Alder reaction of nitroso compound **1** and cyclohexadiene.

As with all Diels-Alder reactions also the version with nitroso compounds consists of the reaction of a dienophile, here **1**, with a diene yielding cycloadduct **69** via a [4+2]-cycloaddition (Scheme 39).[130] A large variety of nitroso compounds were utilized as dienophiles. Particularly widely used are quite simple aryl- or cyanonitroso compounds, but also other derivatives such as reactive acyl-, vinyl- or iminonitroso species as well as α-chloronitroso compounds were proven to be useful reactants in NDA transformations.[131] While Diels and Alder established the concept of alkenes reacting with cyclohexadiene already in 1928, a version with nitroso compounds was not reported until 1947.[132–134]

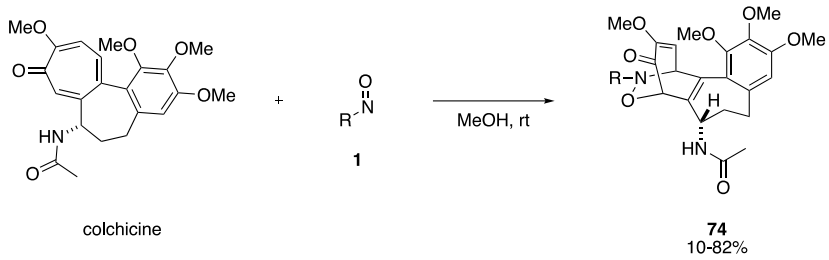
One early application was executed in the synthesis of amino acid **73** with the NDA reaction of diene **70** and α-chloronitroso cyclohexane (Scheme 40). The cycloadduct **71** was first converted into intermediate **72** by benzoylation of the free amine and subsequent dihydroxylation using OsO_4. Via hydrolysis of **72** followed by hydrogenation of the N-O bond, the valuable product **73** was obtained.[135,136]



Scheme 40 Diels-Alder reaction of diene **70** and α-chloronitroso cyclohexane leading to amino acid **73**.

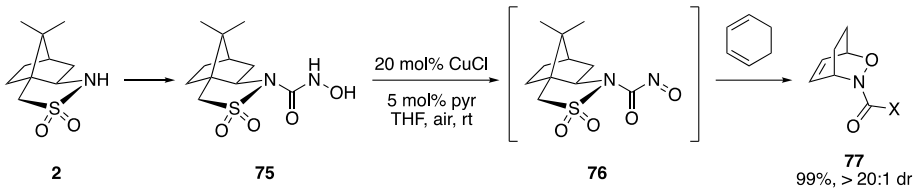
Another elegant example is the application of the NDA reaction as an efficient tool in the functionalization of diene-containing natural products. In order to broaden the library of potent compounds for medicinal purposes such a functionalization gives rise to new useful derivatives or convert toxic natural products with beneficial biological activity into potential pharmacologically valuable compounds. This method was used to modify structural features in a variety of natural products such as thebaine, steroidal dienes, rapamycin, leucomycin, colchicine, isocolchicine and piperine.[136]

Colchicine is a highly active promising anti-cancer lead. The activity arises from its ability to inhibit mitosis through interference with microtubuli. The higher rate of replication makes the cancer cells more vulnerable and ensures a certain selectivity towards tumor cells. Unfortunately, the natural colchicine is still highly toxic and therefore not used in cancer therapy.[137,138] Furnishing the compound with an oxazine ring resulting from NDA reaction with various nitroso compounds **1** yielded the major isomer **74** (Scheme 41).[139] Biological evaluation showed that especially cycloadducts **74** with iminonitroso compounds express a similar effect as colchicine. It was found that retro-Diels-Alder reactions of these compounds take place at 37°C, hence indicating that **74** would serve as a prodrug.[140]



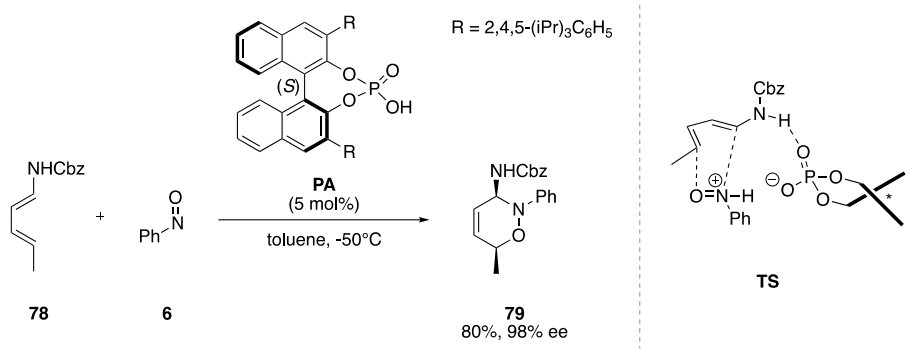
Scheme 41 Structural functionalization of colchicine by NDA reaction with nitroso compound **1**.

As nitroso-Diels-Alder reactions serve as an important step in various total syntheses, asymmetric or stereoinducing variants of the method are of outmost interest. By utilizing chiral auxiliaries, diastereoselective NDA reactions are feasible and are explored more deeply than the nitroso-ene reaction. Several research groups used Oppolzer's auxiliary **2** to produce a sterically biased N-hydroxyurea **75**, which was oxidized *in situ* via copper catalysis to the corresponding nitroso intermediate **76** (Scheme 42).[141,142] Addition of cyclohexadiene leads to diastereomerically pure cycloadduct **77**. Different to other applications in asymmetric nitroso chemistry, sultam **2** was used to synthesize a sterically demanding nitroso compound **76** instead of a sterically hindered reaction partner containing the diene.



Scheme 42 Diastereoselective NDA reaction utilizing sultam **2** (X in **77** = **2**).[142]

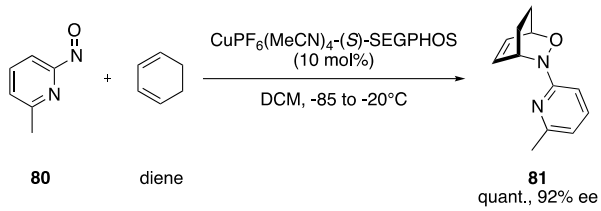
In addition, chiral phosphoric acids were successfully utilized as catalysts to obtain the products in a highly stereoselective manner (Scheme 43). With only 5 mol% of the phosphoric acid **PA** the reaction of nitrosobenzene **6** and diene **78** proceeded exclusively *O*-regioselective, as well as favoring the *cis* diastereomer, and yielded, the almost enantiopure product **79**.[143] Due to the high azaphilicity of **PA**, hydrogen bonds with the nitrogen atoms of the nitroso direct the regioselectivity towards regioisomer **79**. In addition, another hydrogen bond is formed between the phosphoric acid **PA** and the carbamate of the diene leading to transition state **TS**. This dual activation of the substrates by the catalyst allows an efficient enantioselective NDA reaction.



Scheme 43 Regio-, diastereo- and enantioselective NDA reaction utilizing phosphoric acids **PA**.[143]

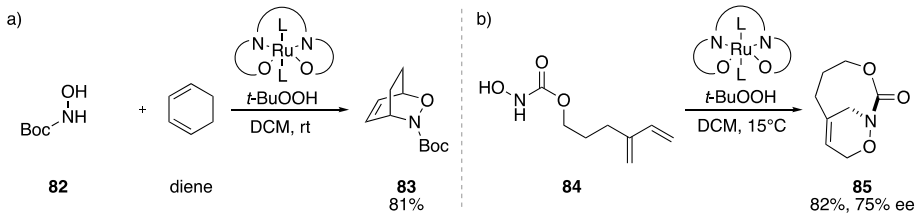
Among the metal-catalyzed NDA reactions particularly catalysts based on copper or ruthenium have been exploited, and some chiral ligands/metal combinations proved to provide significant enantioselectivity.

The application of a chiral copper-phosphine catalyst in the NDA reaction of nitrosopyridine **80** and a diene resulted in cycloadduct **81** in quantitative yield and up to 92% ee.[144] This example of a catalytic enantioselective NDA reaction originates from chelation in the catalyst-substrate complex (Scheme 44). Similar high enantioselectivity in this reaction was also found with Walphos-CF_3 ligands.[145] However, it was later observed that Cu(I) is likely oxidized into Cu(II) by nitrosopyridine **80** and forms the active catalyst for this reaction.[83]



Scheme 44 Enantioselective NDA reaction of nitrosopyridines **80** and a diene utilizing chiral copper catalyst.[144]

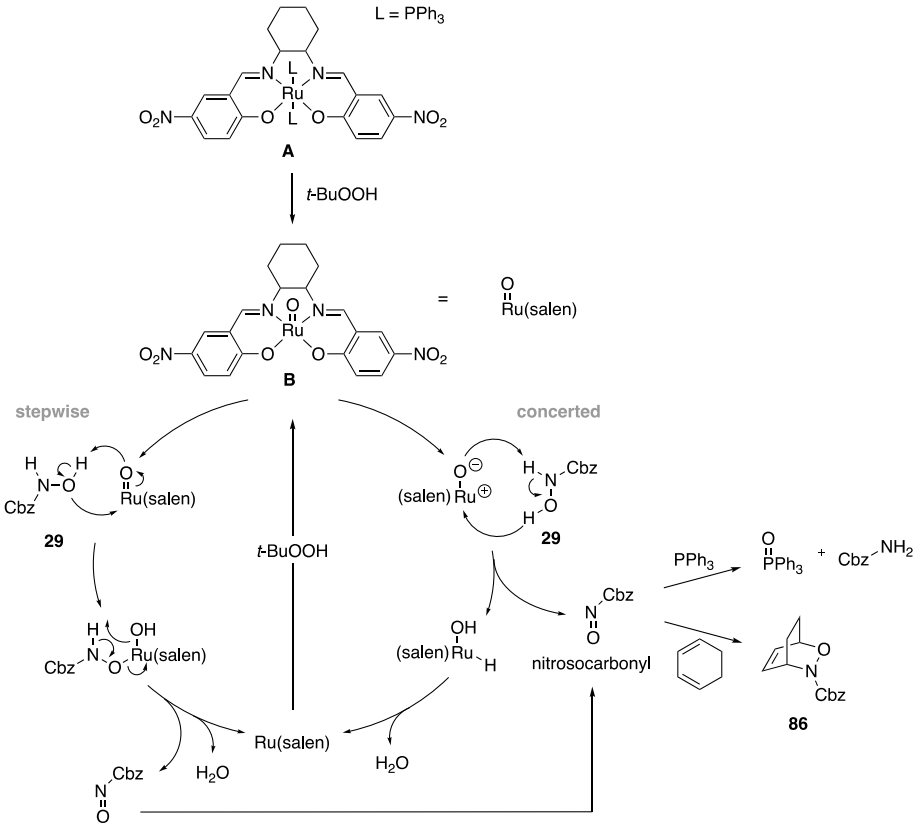
With the hope for an enantioselective outcome, chiral ruthenium-salen catalysts were applied in the oxidation of Boc-protected hydroxylamine **82** and subsequent trapping with cyclohexadiene, but also various other dienes, afforded the adduct **83**. However, in this case no enantioselectivity was reached (Scheme 45a).[76] Later, enantioselectivity was observed in application of a slightly adjusted procedure in the intramolecular NDA reaction of N-hydroxy formate ester **84** (Scheme 45b). The cycloadduct **85** was obtained in a good yield with 43% ee which could be improved to 75% ee by incremental dilution and cooling of the reaction mixture to 15°C. The enantioselctivity is likely introduced due to a possible nitroso-ruthenium complex. The same group suggested that the lower enantioselectivity is possibly caused by the breakdown of this complex prior to cycloaddition by dissociation of the nitroso species competing with the reoxidation of the complex by *t*-BuOOH.[146]



Scheme 45 Diastereo- and enantioselective NDA reaction utilizing chiral ruthenium-salen catalysts.[76,146]

Howard et al. investigated this phenomenon to find the reason for the absence of selectivity in intermolecular NDA reactions with ruthenium-salen catalyst **A**.[83,147] In their very comprehensive and detailed work, they used time-resolved IR-spectroscopy to uncover a Ru(oxo)(salen) species **B** as active catalyst in the racemic reaction of the nitrosocarbonyl resulting from **29** (Scheme 46). In addition, they transformed substrate **82** with the ruthenium-catalyst utilizing chiral ligands but could not observe any asymmetric induction in **83**.[76] The proposed mechanism was summarized into three parts: the oxidation of the ruthenium-salen catalyst into its oxo-species **B**,

which oxidizes **29** and releases the corresponding nitrosocarbonyl, and lastly the NDA-reaction with the diene to give rise to product **86** (Scheme 46).



Scheme 46 Proposed mechanism for the oxidation of hydroxamic acids and in situ NDA reaction with cyclohexadiene.[83]

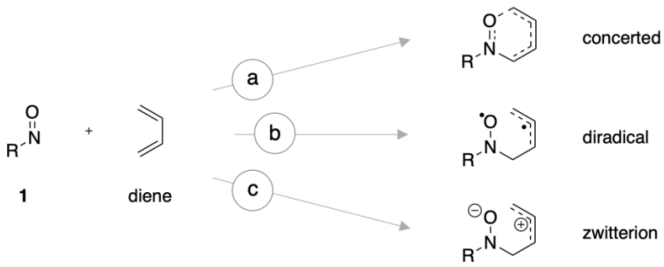
The oxidation of the hydroxamic acid can follow a concerted or a stepwise pathway (Scheme 46). Simultaneous deprotonation of the hydroxamic acid **29** and hydride transfer to the catalyst yields the nitrosocarbonyl in a concerted manner. The catalyst loses water and results in Ru(salen), which is reoxidized by *t*-BuOOH. For the stepwise manner, the deprotonation of hydroxamic acid **29** is followed by coordination of the oxygen to the metal center. Subsequent collapse releases the nitrosocarbonyl and water, resulting in the Ru(salen) species, which is also in this case reoxidized to the active catalyst **B**. The stepwise mechanism is considered more likely due to known coordination of hydroxylamines to metals[4,147] and analog oxidation of alcohols does occur stepwise.[83] However, the last step is either oxidation of PPh_3 by the nitrosocarbonyl or NDA-reaction with the diene (Scheme 46). This seems to take place outside the coordination sphere of the catalyst and therefore no

selective asymmetric outcome is observed. For this reason and the potential lack of involvement observed in the NDA-reaction of nitrosotoluene, as well as observed inactivity of ruthenium catalysts towards other nitroso compounds, indicates that the nitroso species is not bound to the catalyst during the cycloaddition.

Kinetic experiments of the NDA-reaction using more stable nitrosotoluene and cyclohexadiene with a set of catalysts containing copper, iron, ruthenium, and chromium as metal center, revealed that most catalysts did not accelerate the reaction nor increase the yield of the adduct compared to its thermal reaction.[83] Therefore, it was concluded that the nitroso species is only poorly coordinating.

2.3.1 THE MECHANISM OF THE NITROSO-DIELS-ALDER REACTION

The usual Diels-Alder reaction follows a concerted and synchronous [4+2] pathway. While the ene reaction of nitroso compounds expressed significantly different behaviour than its counterpart utilizing simple enophiles, the NDA reaction was thought to proceed via either a concerted, a diradical or a zwitterionic mechanism (Scheme 47). Several selective NDA-procedures with nitroso compounds were assigned to stepwise mechanisms.[148] However, after all, the reactions seem not to differ significantly from the original Diels-Alder process, even though they bring their own characteristics.

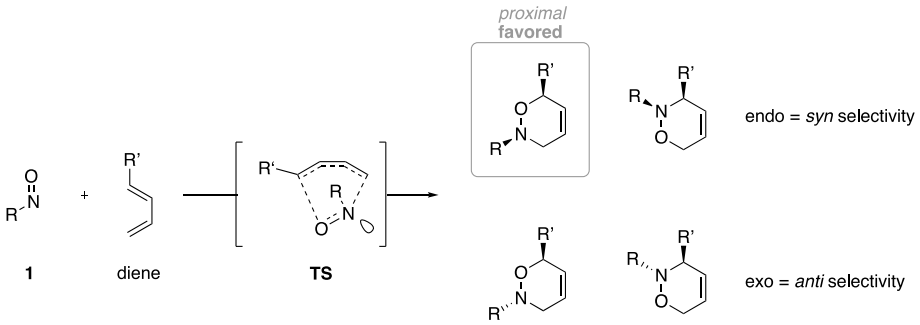


Scheme 47 Proposed mechanism the NDA reaction of nitroso compounds **1** with a diene.[148]

Early studies of Kresze et al. towards the mechanistic elucidation of the thermal NDA reaction exposed sterical and polar effects on the orientation of the reactants to be responsible for several selectivities. The same group considered both a zwitterionic (Scheme 47, pathway c) and an asynchronous concerted mechanistic pathway with a polarized character but underlined that the concerted option goes hand in hand with their observed stereoselectivity (Scheme 47, pathway a).[149] Further Hammett studies could confirm the unsymmetric concerted mechanism but have excluded sterical interactions from playing a major role for the mechanism.[150,151]

Later, calculations by Leach and Houk indeed revealed that the NDA-reaction proceeds via a concerted mechanism with an unsymmetric transition

state **TS** (Scheme 48).[148] Several nitroso compounds **1** were evaluated in the reaction with butadiene as well as other dienes and all gave preference for the concerted mechanism. In addition, the reaction was found to only follow *endo* selectivity, since the *exo* pathway was determined to require higher energy.

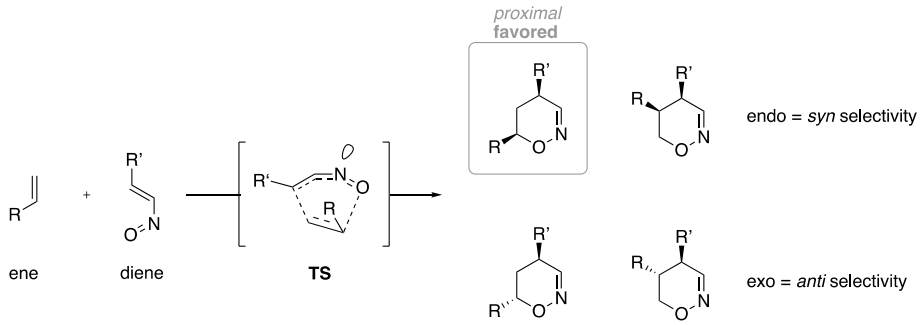


Scheme 48 Proposed mechanism for the NDA reaction with normal electron demand.

The asynchronous arrangement of the transition state originates most likely from the nature of the nitroso moiety. The *endo* selectivity arises from minimization of electrostatic repulsion between the lone pairs of the nitrogen atom and the π-electrons of the diene. The *exo* pathway features highly repulsive interactions, which are also known as the '*exo* lone pair effect'.[148,152] Another reason for the selectivity in favor of the *endo* pathway could be found in FMO interactions. Here, the symmetry-forbidden interaction between the HOMO of both reagents is minimized and therefore the *endo* selectivity is preferred.

A few trends could be observed for the regioselectivity of the NDA reaction supported by experimental and computational studies: 1) 2-substituted dienes express lower selectivity than 1-substituted dienes, 2) the largest orbital coefficient at the fourth position at 1-substituted dienes leads to *proximal* cycloadducts (with EWG a stronger selectivity is observed), 3) *distal* cycloadducts are less preferred with 2-substitued isomers due to the structure of the nitroso compounds and 4) substituent effects are additive. The asynchronous transition state with *endo* preference as well as selectivity towards the *proximal* cycloadduct was confirmed in several subsequent studies of different NDA reactions.[153,154]

However, even though the one-step mechanism is preferred, zwitterionic and diradical pathways are not excluded in the cycloaddition of nitroso compounds with small substituents and substituted dienes, which are able to stabilize radicals.[148]



Scheme 49 Proposed mechanism for the NDA reaction with inverse electron demand.

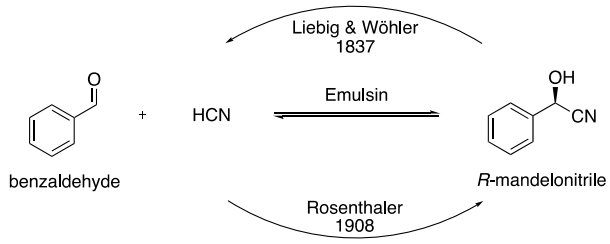
While most nitroso compounds act as dienophiles in the NDA reaction, nitroso dienes mostly react as the electron-poor heterodiene with electron-rich enes in an inverse electron demand NDA reaction (Scheme 49). Studies of Houk et al. revealed a 'comformational switch' of enol ethers as the dienophile from s-*cis* in ground state to s-*trans* in the transtition state due to electrostatic effects stabilizing the orbital interaction for the *endo* selectivity. However, if these dienophiles bear bulky silyl groups, steric hindrance is forcing the reaction pathway into an *exo* transition state, while the s-*trans* of the dienophile is still preferred. This switch towards the s-*trans* conformation was experimentally proven via conformationally frozen enol ethers.[155]

2.4 BIOCATALYSIS

Back in the 1980's, Whitesides et al. highlighted enzymes as having a great potential in synthetic organic chemistry.[156] This assumption turned into a nowadays-obvious truth and enzymes are applied more and more in chemical laboratories. Usually, enzymes play a key role as catalysts in most processes that are found in life. The molecular understanding of these processes led chemists using them for grafting modern organic chemistry, which resulted in completely new possibilities in synthetic strategies. First, many problems related to factors such as accessibility for practical application, substrate specificity and limited solubility of many enzymes in organic solvents were faced. These issues were generally solved during the last 20 years, mainly by means of the rapidly developing field of genetic engineering.[157] However, since we have not reached out to isolate, characterize and test each and every existing enzyme in each genome, there is still a lot of hidden potential in wild-type enzymes and to uncover both their natural purpose and their possible ability to catalyze other reactions beyond their natural role. The following chapters will highlight the state of art concerning biocatalysis in organic chemistry and give a useful overview for the chemist about synthetic possibilities.

2.4.1 HISTORICAL BACKGROUND

On one hand, enzymes are interesting to organic chemists due to their operational space with very mild reaction conditions, which mostly include ambient temperatures, aqueous environments, low toxicity, and easy reaction set ups. On the other hand, they often express high regio- as well as stereoselectivity which is important for various synthetic applications, thus making molecules accessible which would be hard to achieve by traditional organic chemistry. Especially, compounds with multiple functional groups are challenging to classical metal- and organocatalysts, but enzymes work naturally with them. One example for this is the construction of DNA or RNA strands, which are huge molecules containing several functional groups and need an efficient and sound assembly to serve their purpose correctly. Enzymes do this with high accuracy and work everyday in our bodies, yet a chemical equivalent which can keep up with this is non existent.[158]



Scheme 50 First example of the use of emulsin by Liebig & Wöhler and Rosenthaler.[159]

In the middle of the 19th century, Louis Pasteur observed a phenomenon which is considered a cornerstone of modern application in enzymatic catalysis. He treated racemic tartaric acid with a mold called *Penicillium glaucum* and observed an enrichment of a single isomer. The mold only comsumed the (+)-enantiomer.[157,160] Later, this kind of reaction turned out to be a transformation of high importance for enantioselective synthesis, the kinetic resolution. Around that time also Liebig and Wöhler found activity using a crude preparation of an enzyme from almond (*Prunus amygdalus*), which released HCN and benzaldehyde from *R*-mandelonitrile (Scheme 50). This crude biological isolate contained hydroxynitrile lyase and was named emulsin.[161] Later, in the beginning of the 20th century, Rosenthaler was able to reverse the process to synthesize enantiopure *R*-mandelonitrile.[159,162,163] Historically this is classified as the first wave of biocatalysis and is comprised of traditional biotransformations using whole cells or extracts from nature (Figure 7).[163] The enzymes found in this wave were used to catalyze reactions rooted in their natural task to produce natural products. Even though these findings were striking, they did not find a way into broad application among chemists yet.

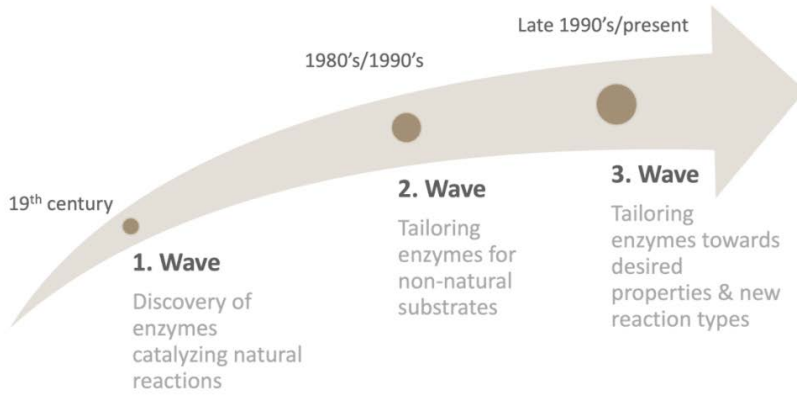


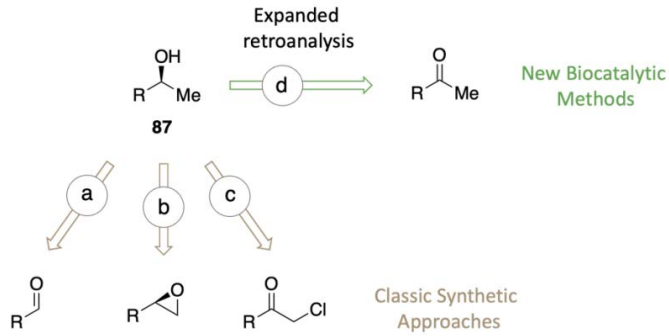
Figure 7 The three waves of biocatalysis.[163]

Later, chemists got more interested in applying the proteins in the native reactions but with various derivatives of their natural substrate. While some enzymes often accept only a very narrow substrate scope, anabolic enzymes such as lipases, proteases and esterases, to name a few, easily accepted a broad range of molecules. These enzymes all have a similar catalytic mechanism, the catalytic triade, to address desired substrates. However, to circumvent the enormous task of finding the right natural enzyme for the optimal transformation of a few suitable subtstrates, the second wave of biocatalysis in the 1980's and 1990's introduced enzyme tailoring by mutations often targeting selectivity (Figure 7). The goal was to optimize the biocatalyst for non-natural substrates.[163]

This was quickly followed by the third and present wave starting from the late 1990's (Figure 7), which is shaped by directed evolution of enzymes to improve their stability, substrate specifity, enantio- and regioselectivity and other desired properties such as adjusting them to different reaction conditions. In addition, the produced enzymes could engage in reactions that they do not catalyze in Nature. Directed evolution and engineering of new-to-nature biocatalysis was eventually awarded with the Nobel prize in Chemistry of the year 2018.[159,163,164] While Nature optimized the enzymes' structure over millions of years, genetic engineering altering their properties via directed evolution or rational design allows to do the work in a fractional amount of that time.

2.4.2 BIOCATALYSIS IN ORGANIC CHEMISTRY

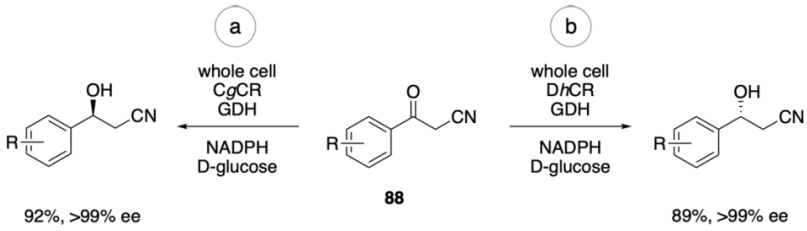
Biocatalysis has significantly extended the portfolio of tools for the synthetic chemist to use in chemical transformations. Instead of replacing traditional chemistry, additional useful possibilities were explored to find most efficient ways in synthesis and catalysis by broadening for example the scope of retrosynthetical strategies (Scheme 51). From a classical synthetic point of view it would be challenging to yield enantiopure alcohol **87** via simple reduction of ketone (path d), though not totally impossible but strongly dependend on the nature of group R and involvement of bulky and chiral catalysts or reagents. However, asymmetric addition to an aldehyde (path a) would for example require an alkylation using dialkylzinc in combination with a chiral amino alcohol promoting the enantioselectivity.[165] Commonly used is also the strategy of opening chiral epoxides (path b), which is already bearing the right selectivity and can be synthesized from the racemic epoxide via hydrolytic kinetic resolution using chiral salen-cobalt complexes.[166] In addition, chloroketones (path c) were useful in enantioselective reductions using chiral metal-catalysts.[167]



Scheme 51 Retrosynthetic analysis of enantiopure alcohol **87** via classic synthetic approaches and new biocatalytic methods.[168]

Biocatalytic options give rise to alcohol **87** by enantioselective reduction of a ketone (path d). Ketoreductases (KRED) and alcohol dehydrogenases (ADH) are nowadays a well studied addition to the classic synthetic approaches. They tolerate a wide scope of susbtrates and accept other functional groups such as free alcohols or further carbonyl moieties in the molecule while maintaining their selectivity. However, they express a common downside of biocatalysis. Namely the limitation towards only one of the two stereoisomers.[168]

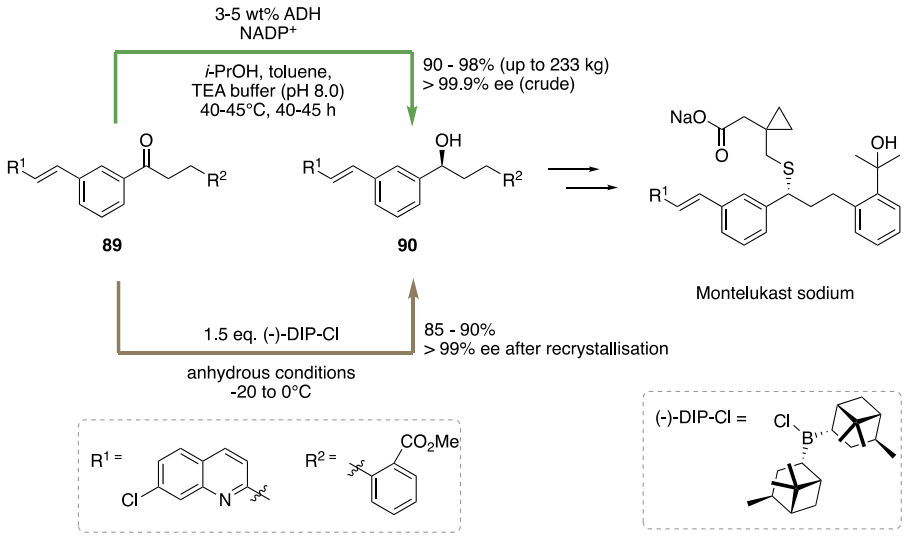
Via genome mining Xu et al. identified two KREDs to gain selective access to both enantiomers in the enzymatic reduction of ketonitrile **88** -an important precursor for the antidepressant Fluoxetin (Scheme 52).[169] They utilized *E. coli* as a recombinant whole cell system for this transformation, which turned out to be very robust and accepted the biphasic environment of toluene and phosphate buffer. *E. coli* at C*g*CR gave exlusively the *S*-enantiomer (path a) and *E. coli* at D*h*CR afforded the *R*-enantiomer (path b) with >99% ee and very good yields, respectively. In addition, the *E. coli* was used to coexpress glucose dehydrogenase (GDH) to yield recycling of the necessary cofactor.



Scheme 52 Stereocomplementary bioreduction of ketonitrile **88**.[169]

A very popular example of industrial application of KREDs and ADHs is the synthesis of the 'billion-dollar' drug Montelukast sodium (Singulair).[170] Merck replaced the chemical reduction of ketone **89** to the enantiopure

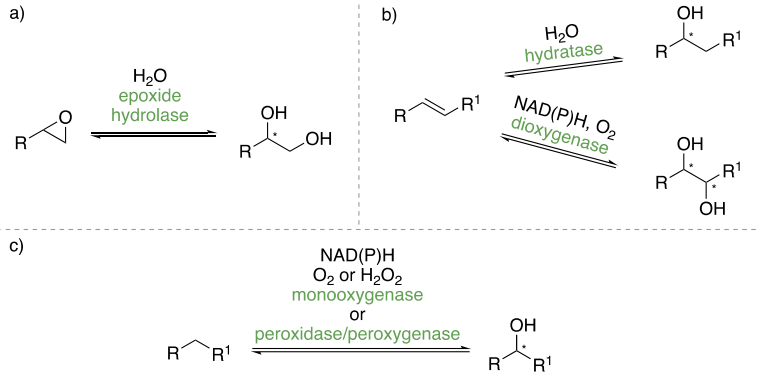
alcohol **90** with a biocatalytic method (Scheme 53, green). For this reason an ADH was engineered to fit the bulky ketone **89** and can withstand high amounts of *i*-PrOH and reaction temperatures of 40 – 45°C as well as homogeneous mixtures of water and organic solvents to dissolve the hydrophobic ketone substrate. Isopropanol is used to recycle the cofactor NADPH in this reaction, which releases acetone as side product. By removal of acetone the reaction could be pushed to full conversion and yielded the alcohol **90** in excellent yields and almost perfect enantioselectivity. The biocatalytic method offers several advantages over the chemical process, which requires very low temperatures and excess of the corrosive and moisture sensitive reducing reagent (-)-DIP-Cl (Scheme 53, beige). In comparison, the biocatalytic version runs on industrial scales of up to 233 kg product formation with 3 – 5 wt% of enzyme in aqueous conditions. In addition, the process utilizing the engineered ADH allows a more efficient manufacture of the asthma and allergy drug Montelukast sodium in terms of atom economy, yield and ee, as well as energy consumption and complexity of the work-up.



Scheme 53 Biocatalytic and chemical enantioselective reduction of ketone **89** as a key step in the synthesis of pharmaceutical Montelukast sodium.

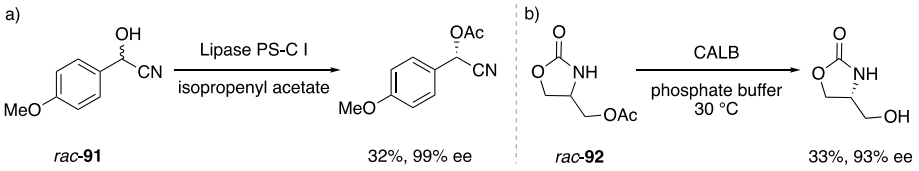
Nowadays, a variety of biocatalytic methods can be applied to yield enantioenriched or, if desired, racemic alcohols. Some are alternative pathways to traditional chemical approaches, but some transformations feature exclusively a biocatalytic process. Besides ketones, also other substrates can be transformed into various derivatives of alcohols (Scheme 54). Aldehydes can be transformed into alcohols via aldol-type C-C couplings, which will be discussed later in this chapter. In addition, epoxide opening utilizing epoxide hydrolase yields vicinal diols (Scheme 54a).[171] The

transformation of epoxides often follows a kinetic resolution.[172] Oxidation of olefins by hydratase or dioxygenase affords an alcohol or diol, depending on the type of enzyme (Scheme 54b).[173,174] While hydratases are cofactor-independent, dioxygenase as well as monooxygenase requires electrons in form of NAD(P)H in order to activate oxygen. In addition, simple carbohydrate moieties are transformed by several oxidative enzymes into an alcohol (Scheme 54c).[175–177] Peroxidases and peroxygenases are hydrogen peroxide dependent, yet have not been applied for larger scale synthesis.[170]



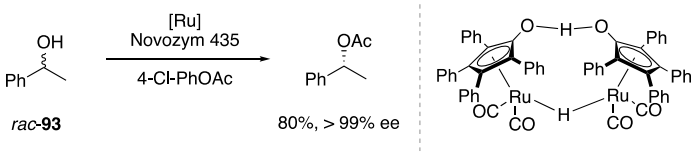
Scheme 54 Biocatalytic transfomations towards alcohols.

Furthermore, kinetic resolutions utilizing hydrolases are another popular feature to produce enantiopure alcohols or esters and therefore they are broadly applied in the synthesis of pharmaceutically relevant compounds. While the chemically catalyzed versions also deliver impressive outcome, they are depending on catalytic in situ formation of chiral acylating agents.[168] Using for example lipases, simple acetate sources such as those originating from solvent (eg. EtOAc) are sometimes sufficient for a highly selective reaction. Depending on the given substrate and the environment, hydrolases will either acetylate or deacetylate. Alcohol *rac*-**91** can be acetylated by an immobilized lipase using isopropenyl acetate and yields the *R*-isomer enantioselectively (Scheme 55a).[178] However, employing the racemic ester *rac*-**92** in aqueous environment, the lipase will catalyze the reverse reaction and affords the enantiopure *R*-alcohol upon ester hydrolysis (Scheme 55b).[179]



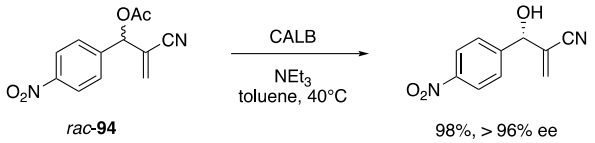
Scheme 55 Kinetic resolution of alcohol *rac*-**91** and acetate *rac*-**92** using lipase in different conditions.[178,179]

Kinetic resolutions unfortunately always yield a maximum of 50% of the desired products. This limitation can be circumvented by an elegant chemoenzymatic method using a combination of an immobilized form of lipase B from *C. antarctica* (Novozym 435) and a dimeric ruthenium catalyst (Scheme 56).[180] Here, the lipase perfoms the kinetic resolution of *rac*-**93** to afford the acetate as pure *S*-isomer and the catalyst epimerizes the remaining enantiomer of the alcohol. In that way the *S*-acetate can be obtained in a higher yield of 80% while maintaining perfect enantioselectivity.



Scheme 56 Dynamic kinetic resolution of alcohol *rac*-**93** using a chemoenzymatic method.[180]

The addition of organic bases was found to racemize chiral acetates. This finding was used in the dynamic kinetic resolution of Morita-Baylis-Hilman (MBH) acetate *rac*-**94** (Scheme 57).[181] As usual, lipase was used for the kinetic resolution and simple NEt_3 as organic base gave rise to enantiopure *S*-alcohol in 98% yield. The epimerization of the unreacted enantiomer is accomplished via reversible addition and elimination of the amine to the MBH acetate.

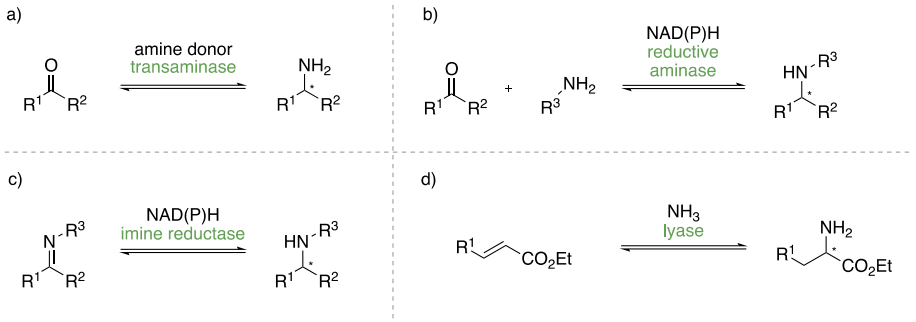


Scheme 57 Dynamic kinetic resolution of acetate *rac*-**94** using lipase and organic base.[181]

Kinetic resolutions of racemic amides or amines are enzymatically available in a similar way by employing hydrolases as well. While cleavage of amides into amines is chemically challenging due to the need of harsh conditions, proteases usually catalyze both reactions -the formation of amines and amides- and the reaction outcome can be adjusted by equilibrium control.[170] Proteases were also combined with basic conditions to result in dynamic kinetic resolutions yielding natural and non-natural amino acids in enantiopure form at Degussa.[182]

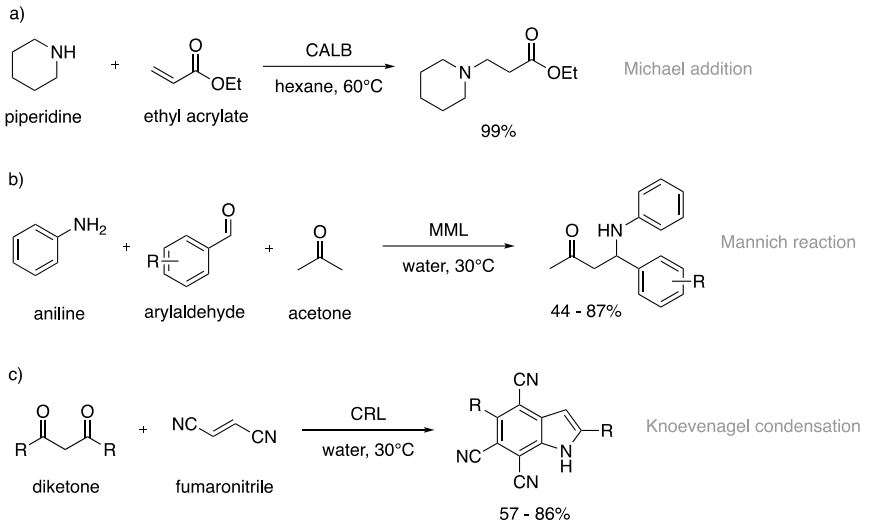
As amines are important building blocks also for synthesis in organisms, several applications were established also for organic chemistry (Scheme 58). A ketone can be transferred by transaminase into a primary amine in the presence of an amine donor (Scheme 58a).[183] In addition, also secondary

amines are accessible from ketones by employing NAD(P)H dependent reductive aminase and a primary amine (Scheme 58b). An alternative biocatalytic pathway towards secondary amines is achieved by reduction of an imine by imine reductase (IRED) (Scheme 58c).[184] However, IRED's are sensitive enzymes and need to be applied in high amounts. Furthermore, the reaction itself is challenging, since two reactions need to be performed (imine formation and subsequent reduction) to afford the secondary amine. Hydroamination of an olefin utilizing lyase and simple ammonia leads regioselectively to amino acids (Scheme 58d).[185] All of these biocatalytic transformations can be nowadays found in industrial large scale applications and are especially crucial in the synthesis of APIs in pharmaceutical industry including companies such as Pfizer, GlaxoSmithKline and Novartis.



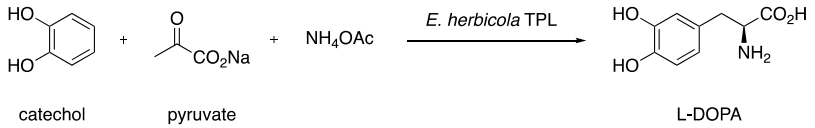
Scheme 58 Biocatalytic transformations towards amines.

Lipases in particular are able to catalyze a variety of synthetically important reactions forming C-N-bonds.[186] In contrast to the amination of double-bonds utilizing lyase (Scheme 58d), lipase CALB catalyzes the Michael-reaction between a piperidine and ethyl acrylate to afford the Michael-adduct in excellent yield (Scheme 59a).[187] Furthermore, lipase MML from *M. miehei* was successfully applied in the multi component Mannich-reaction of aniline, an arylaldehyde and acetone to afford the product in good yields (Scheme 59b).[188] Knoevenagel condensations of diketones and fumaronitrile are another reaction featured by lipases (Scheme 59c).[189] In this case formation of the indole was catalyzed by lipase CRL from *C. rugosa* with yields ranging from 57 – 87%. In addition, Lipase CALB showed activity in both, the Mannich and Knoevennagel reactions, but did not result in satisfactory reaction outcome.



Scheme 59 Example of biocatalytic transformations towards amines utilizing lipases.[187–189]

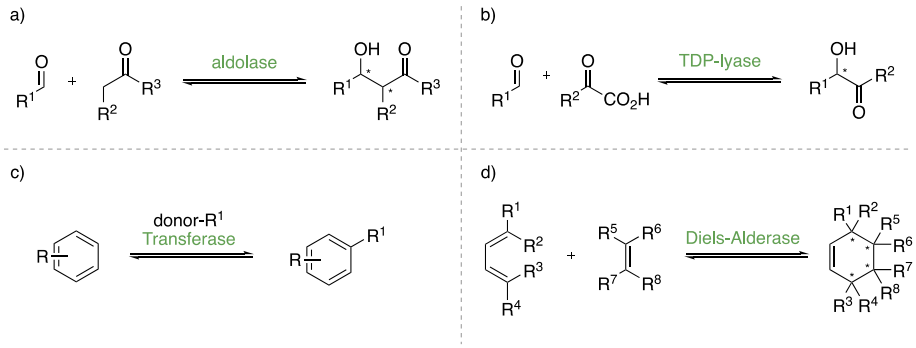
L-DOPA is an important medication in the treatment of Parkinson's disease. Among four developed processes for commercial purposes the most efficient route is represented by the Ajinomoto Process (Scheme 60).[186] Compared to longer routes commercialized by Roche and Monsanto starting from vanillin and employing classic kinetic resolutions or asymmetric catalysts as well as protection group chemistry, the Ajinomoto process only constists of one single step utilizing tyrosine-phenol lyase (TPL).[186–188] This enzyme usually degrades L-tyrosine into phenol, pyruvate and ammonia. Here, the reverse three-component reaction including catechol, sodium pyruvate and ammonium acetate is catalyzed by TPL to produce L-DOPA with around 110 tons per year, which covers approximately half of the yearly demand.[189]



Scheme 60 Ajinomoto process for the biocatalytic synthesis of L-DOPA.[186]

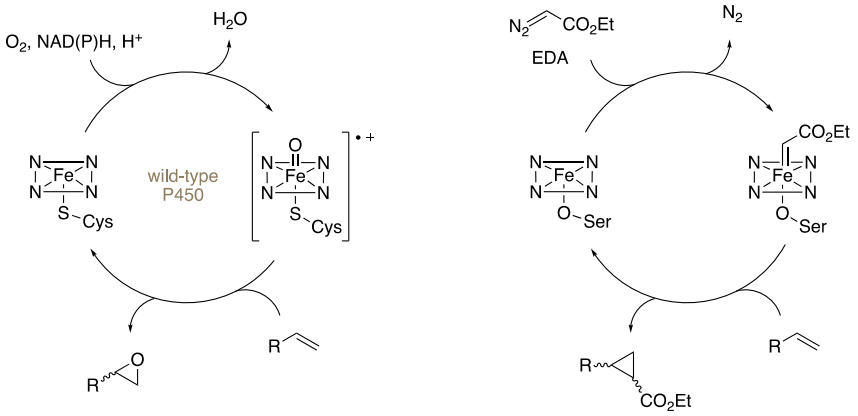
Furthermore, lyases are used in biocatalytic C-C-bond formations (Scheme 61). Especially aldolases are commonly applied lyases to catalyze the reaction of an aldehyde with a ketone in a classic Aldol-transformation to afford the coupled product (Scheme 61a). They have been extensively studied and feature a broad application profile.[190] Utilizing thiamine diphosphate-dependent lyases (TDP-dep.-lyase), an enzyme bound acyl anion equivalent is generated in situ via decarboxylation of α-ketocarboxylates. Nucleophilic

attack to the aldehyde gives rise to α-hydroxy ketone in an enantioselective way (Scheme 61b).[191] Most chemists might know this process under the name benzoin condensation. New C-C bonds can be also achieved via transferases. Alkylations form crucial reactions in nature and in synthetic chemistry. As their name already indicates, transferases transfer groups from certain donor molecules to a substrate. Friedel-Crafts alkylation of an aryl was achieved by using methyltransferase and SAM derivatives as donor molecules to yield the alkylated product (Scheme 61c).[192] Moreover, the transformation of a diene with an olefin can be enzymatically catalyzed to yield Diels-Alder products (Scheme 61d). Enzymes, usually synthases, engaging in that reaction outcome are often called Diels-Alderase, although this description caused a controversy in the chemical community, since the enzymes often express dual action and might mechanistically not follow a classic Diels-Alder mechanism.[190,193]



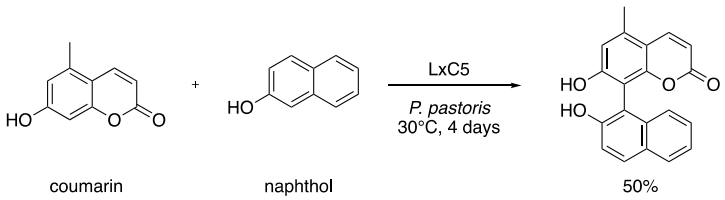
Scheme 61 Biocatalytic transformations towards C-C bond formation.

A prime example for enzymatic C-C-bond formation is the cyclopropanation of double bonds by engineered cytochrome P450, that was pioneered by Frances Arnold.[164] Studies of P411-mutants of the heme-containing enzyme P450 led to a completely non-natural behaviour of the enzyme engaging in carbene-type C-C-bond formation (Scheme 62).[194,195] The usual reaction of P450 and an olefin is the aerobic oxidation to an epoxide (Scheme 62, left). By exchanging the distal cysteine ligand of the heme in the active site of the enzyme with a serine ligand was enough to promote activity towards biocatalytic cyclopropanation of olefins (Scheme 62, right). Ethyl diazoacetate (EDA) serves as carbene donor under release of nitrogen gas. The method was also applied to simple hydrocarbon moieties as substrates resulting in transfer of the alkyl group.[196] The P411 mutants were further engineered by the group to suit even transfer of the carbene moiety for C(sp^2)-H alkylation, cyclopropanation of internal alkynes as well as accepting a variety of diazo esters.[197–199]



Scheme 62 Natural epoxidation of olefins (left). Biocatalytic olefin cyclopropanation via carbene transfer (right).

However, activity mining of mutated P450 for non-natural transformations turned out to be a fruitful and versatile discovery campaign. Selective nitration using nitric oxide as well as amination via nitrene transfer are additional examples of the non-natural application of engineered P450.[200,201] Another feature is the formation of C-Si-bonds via directed evolution of P450 and other cytochromes.[202] The work of Arnold et al. paved the path for future solutions in organic chemistry using engineered enzymes resulting from directed evolution. Recently, a biocatalytic oxidative cross-coupling reaction of coumarin and naphthol was realized utilizing cytochrome P450 expressed in the yeast *P. pastoris*.[203] Wildtype P450 showed only a slight activity towards the cross-coupling. After five rounds of evolution, activity and selectivity was improved for the cross-coupled product (Scheme 63).

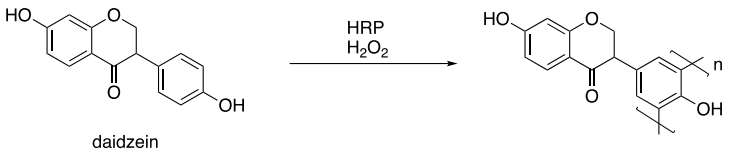


coumarin naphthol

Scheme 63 Biocatalytic cross-coupling reaction of a coumarin and naphtol.[203]

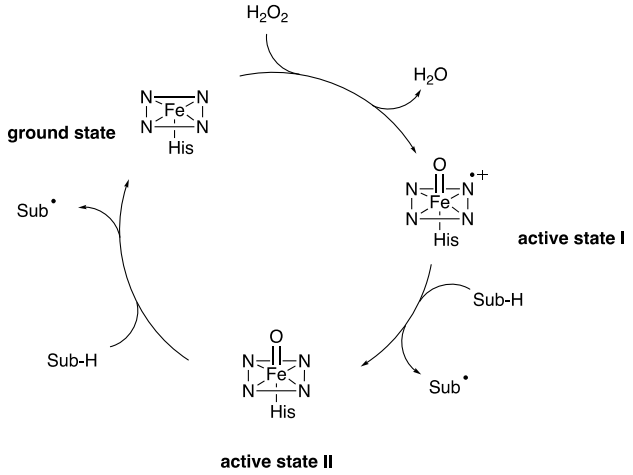
2.4.2.1 Peroxidases

Peroxidases (EC 1.11.1.x) belong to the class of oxidoreductases and are used to catalyze oxidation reactions with consumption of hydrogen peroxide. These abundant enzymes can be found in almost all living systems and are involved in manifold processes in nature. For the chemist, however, these enzymes are highly interesting for application in purely synthetic oxidation reactions. Peroxidases are highly involved in polymerization or other coupling reactions, such as the polymerization of daidzein (Scheme 64).[204]



Scheme 64 Polymerization using horseradish peroxidase (HRP).

The catalytic feature of those enzymes is linked to a heme ligand typically connected with the enzyme by coordination of the heme-iron to a histidine residue of the protein structure. To be able to oxidize substrates, hydrogen peroxide needs to be present to form the active state (Scheme 65).[205–207]



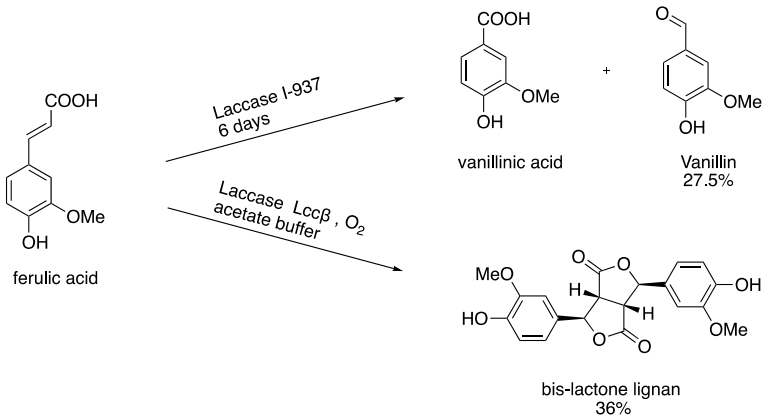
Scheme 65 Suggested catalytic cycle of horseradish peroxidase.

As a first step the catalytically active state I, including an oxoferryl center along with a cation radical in the porphyrin structure is formed from the ground state and hydrogen peroxide via the Poulus-Kraut mechanism.[208] For horseradish peroxidase it was observed that an intermediate iron hydroperoxy complex leads to active state I.[209] This is a similarity to the peroxide shunt pathway in P450s in general and therefore also related activity with

cytochrome P450 can be observed.[210] The peroxidase is able to perform in total two oxidation steps towards a substrate via the first and second active state. The first oxidation takes place under reduction of active state I to the oxoferryl species in active state II. The second oxidation happens via reduction of the oxoferryl center to the ground state of the enzyme. However, the heme prosthetic groups are identical with P450s and therefore a different mechanism can be expressed by HRP resulting in similar products.

2.4.2.2 Laccases

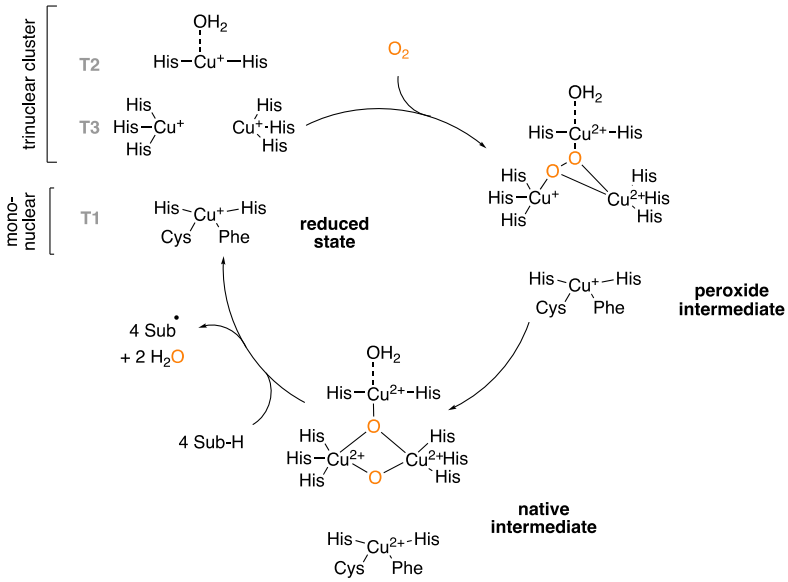
As blue multi-copper oxidases, laccases (benzenediol:oxygen oxidoreductase, EC 1.10.3.2) catalyze oxidation reactions with concurrent reduction of oxygen to water. They can be found in plants, fungi, bacteria and some insects. While plant laccases are mostly involved in lignin synthesis, the lignin degradation in form of wood rotting is found to be the predominant task of fungal laccases. Therefore, they are widely used for the direct oxidation of lignin-like compounds such as phenols or related molecules (Scheme 66). A fungal laccase from *Pycnoporus cinnabarinus* I-937 gave access to vanillinic acid and vanillin from ferulic acid.[211] The same substrate was transformed into a bis-lactone lignan by laccase Lccβ from the fungus *Trametes versicolor*.[212]



Scheme 66 Transformation of ferulic acid by different laccases.[211,212]

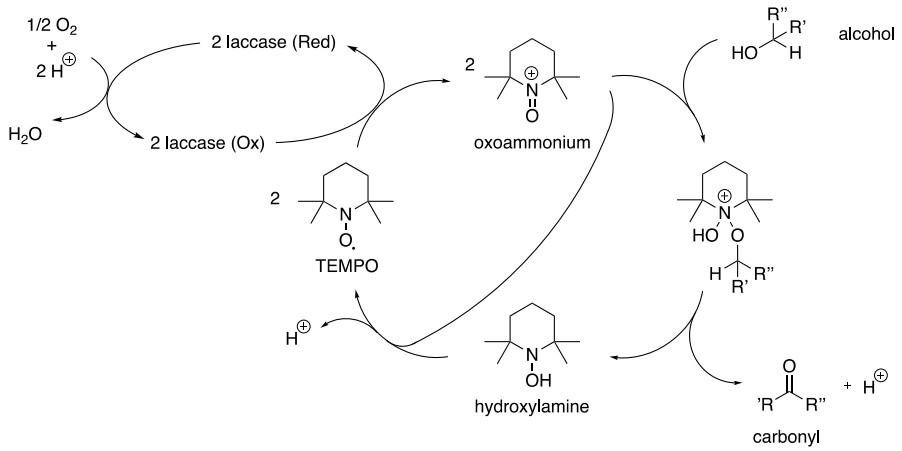
The catalytic sites of laccases contain four copper atoms. They are classified as the monocuclear 'blue' T1 copper and the T2/T3 copper trinuclear cluster, forming the two active sites of the enzyme (Scheme 67). The T1 copper site acts as electron acceptor and engages in single-electron oxidations of the substrates. The electrons are channeled via a conserved His-Cys-His transfer route to the T2/T3 copper site. They originate from four independent mono-oxidation reactions at the T1 copper and are used for the four-electron reduction of oxygen to water. Starting the catalytic cycle from the reduced state

of the laccase, the peroxide intermediate is formed by addition of oxygen (Scheme 67). One of the oxygen atoms is bound to the T2 copper and the other one to the T3 copper. To form the fully oxidized intermediate of the enzyme, the peroxide bond is cleaved and the copper atoms in the T2/T3 site are all connected via an oxo-ligand. This structure is relatively stable and also called the native intermediate. Via direct oxidation of four substrates via single electron transfer (SET) at the T1 copper alongside with the release of water from the trinuclear cluster, the reduced state is recovered.[213–215]



Scheme 67 Suggested catalytic cycle of laccase.[215]

Since laccases express a low redox potential, they usually only catalyze the oxidation of electron-rich aromatic substrates.[216] However, oxidation of non-phenolic compounds with a higher redox potential than the enzyme can be achieved by employing a chemical mediator as 'electron shuttle' between the laccase and the substrate. Especially the system of laccase coupled with TEMPO is a widely applied method to oxidize primary and secondary alcohols or more recently also N-heterocyclic compounds in slightly acidic conditions.[217,218] Here, the activated form of the laccase generates an oxoammonium cation via one-electron oxidation of TEMPO (Scheme 68). The alcohol can now react with the oxoammonium via an ionic oxidation pathway to afford the carbonylic product alongside with hydroxylamine as the reduced state of TEMPO. The catalytic cycle is closed by a non-catalyzed comproportionation of the generated hydroxylamine with the oxoammonium cation to regenerate TEMPO.[219]



Scheme 68 Proposed mechanism of alcohol oxidation catalyzed by laccase/TEMPO.[219]

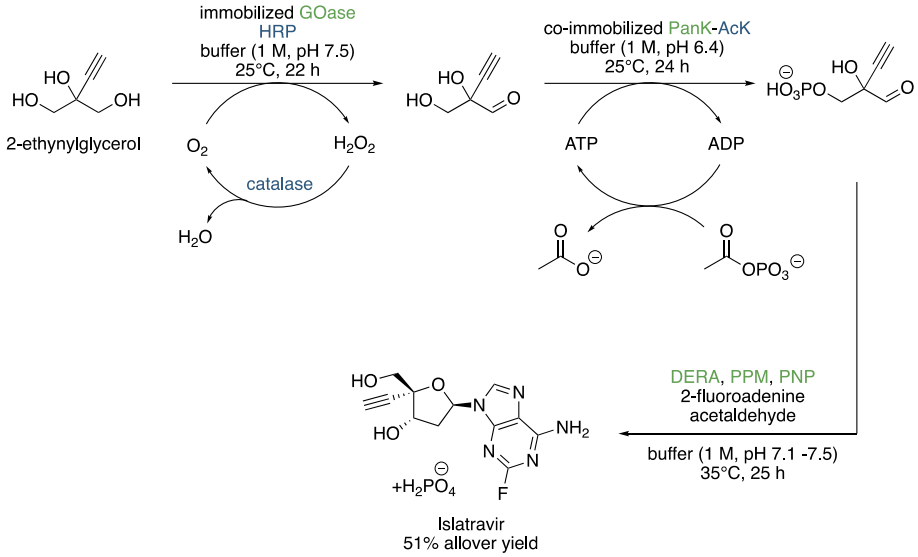
2.4.3 BIOCATALYTIC CASCADES IN ORGANIC SYNTHESIS

While adding new possibilities to the chemists reaction portfolio, biocatalysis can also reduce the length of a synthesis, since oftentimes a simple molecule can be directly transformed into the product by a selection of different enzymes. These enzyme cascades are the 'essence of life' and occur in all metabolic processes of organisms.[159] Biocatalytic cascades give rise to complex molecules from simple starting materials. The concentration of intermediates is kept low, therefore inhibition can be automatically prevented and sensitive intermediates are more likely to be succesfully transformed. In addition, cascade reactions allow easy cofactor recycling, since reduction and oxidation steps are usually included in the sequence. As such, enzyme cascades in organic synthesis relatively closely resemble the natural conditions where consortia of enzymes convert simple metabolites into complex bioproducts. The high mutual tolerance of biological catalysts can be seen as a major advantage compared to traditional organic chemistry, which usually requires work-up or purification of the intermediates since chemical catalysts are often not compatible in a one-pot approach. As a result, a large amount of waste and loss of material is often experienced alongside with other more or less significant disadvantages.[159,170,220]

The biocatalytic cascade for the production of islatravir developed by Merck is a prime example for the application of enzyme sequences (Scheme 69). Here, nine enzymes are applied in three steps.[221] The strategy based on the bacterial nucleoside salvage pathway utilizes 2-ethynylglycerol as starting material. The process contains five engineered enzymes, which were modified via directed evolution to meet the required properties for the process. In addition, four auxiliary enzymes are used for producing reagents or recycling cofactors. The reaction takes place in a single aqueous solution without the need of protecting groups. It is so far the shortest route to

islatravir. Routes applying traditional organic chemistry consist of 12 – 18 steps.[170,222]

In a first step, 2-ethynylglycerol is oxidized by galactose oxidase (GOase) to afford an aldehyde intermediate. The resulting, potentially problematic hydrogen peroxide is immediately consumed by catalase and horseradish peroxidase (HRP). Subsequently, the resulting aldehyde is phosphorylated in a regioselective manner by pantothenate kinase (PanK). This step proceeds under consumption of ATP, which is recycled by acetate kinase (AcK) and acetyl phosphate as phosphate donor and resulting in acetate as side product. The last step consists of three enzymes. The phosphorylated aldehyde is first reacting with acetaldehyde in an aldol condensation catalyzed by aldolase DERA. The phosphate group of the intermediate is transfered internally by phosphopentomutase (PPM). Finally, purine nucleoside phosphorylase (PNP) catalyzes the coupling with 2-fluoroadenine to afford islatravier. As a last step the phosphate is consumed by sucrose phosphorylase SP to keep the equilibrium at the product side.

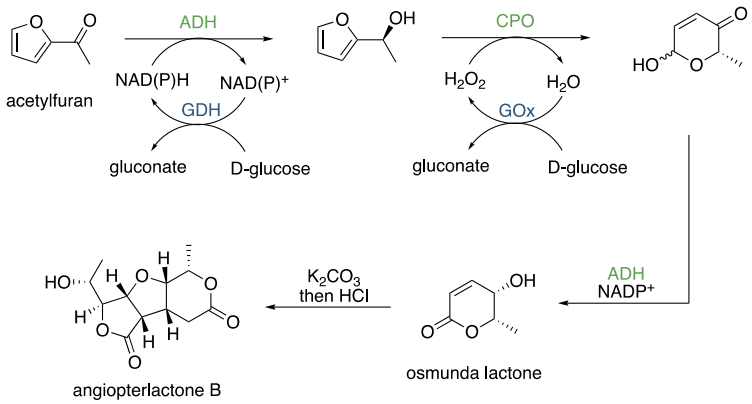


Scheme 69 Biocatalytic cascade yielding islatravir.

Another example of biocatalytic cascades is the synthesis of angiopterlactone B developed in our research group (Scheme 70). The natural product was synthesized by a combination of five enzymes sequentially in one-pot (publication IV). Two other syntheses are published containing 4 – 9 steps via traditional chemical methods.[223,224]

Simple acetylfuran is stereoselectively reduced by alcohol dehydrogenase (ADH) to the enantiopure furfuryl alcohol. This step consumes the cofactor NAD(P)H, which is recycled by the auxiliary glucose dehydrogenase (GDH)

under formation of gluconate. Next, the furfuryl alcohol is oxidized by chloroperoxidase (CPO) and follows an Achmatowicz-type rearrangement to yield the intermediate lactol. For this step hydrogen peroxide is required, which is produced by glucose peroxidase (GOx) from water and D-glucose. A subsequent redox isomerization of the lactol using alcohol dehydrogenase (ADH) and $NADP^+$ in a 'borrowing hydrogen' fashion affords osmunda lactone. Finally, the reaction mixture is treated with potassium carbonate, followed by hot acidification resulting in the formation of angiopterlactone B with an overall yield of 14%.



Scheme 70 Biocatalytic cascade yielding angiopterlactone B (publication IV).

Those examples show why biocatalysis is such a powerful tool for the synthesis of relevant bioactive compounds in organic chemistry. The synthetic approaches are not just shorter routes but also enable highly efficient and economic transformations, which are not in reach of traditional chemistry. So far cascades comprised of a total ten enzymes combined in a one-pot sequence were applied, resembling 'artificial metabolism'.[170] However, the need of traditional chemistry is still highlighted through chemoenzymatic processes representing a powerful interplay of enzymes and metal- or organo-catalysts resulting in most striking cross-over processes.

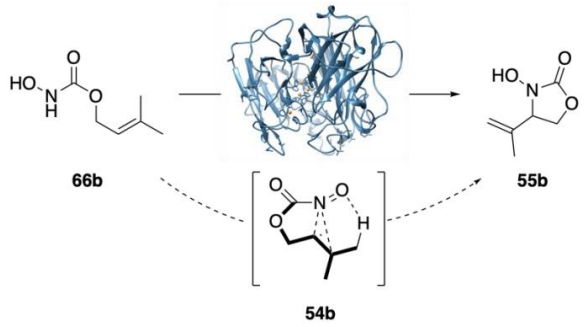
3 RESULTS

Activity mining of enzymes towards performances in transformations beyond their natural reaction portfolio allows the creation of highly efficient transformations or reaction cascades, which lead, in the best case, to the desired product in one pot. This feature of enzymes to catalyze unexpected reactions not belonging to their usual task is called catalytic promiscuity and forms a new frontier in organic chemistry to extend the use of biocatalysts.[186]

A detailed description of the results can be found in the attachments **I**-**III**. This chapter will present only a short summary of the key findings.

3.1 PUBLICATION I

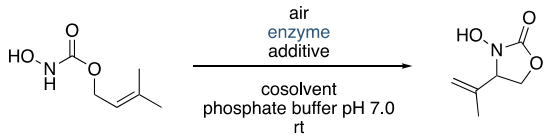
In the curiosity of finding promiscuous enzymes expressing activity towards reactions not rooted in Nature, the nitroso-ene reaction seemed to be an elegant method to investigate for the biocatalytic production of heterocyclic building blocks via C-N bond formation (Scheme 71). The use of TEMPO or related compounds as mediators in enzymatically catalyzed strategies gave a first hint of biocatalytic activity in these compound classes, since hydroxylamines can be observed as intermediates of the catalytic cycle.[219] The nitroso species generated via stoichiometric application of oxidants, iron and copper catalysts combined with hydrogen peroxide or oxygen yields C-N bonds in a mild manner. However, the production of enantiopure compounds is limited to diastereoselective transformations through utilization of auxiliaries.



Scheme 71 Biocatalytic nitroso-ene reaction of model substrate **66b**.

Leaning on previous studies containing iron and copper catalysts[70,71], a broad screening of oxidative metalloenzymes bearing these metals in their active sites revealed oxygen-activating laccases CotA or Lccβ as useful enzymes (Table 1, entry 3 & 5) and specifically horseradish peroxidase (HRP) in combination with glucose oxidase (GOx) as another suitable system (Table 1, entry 10) for the oxidation of model substrate **66b** into its nitroso species **54b**, which is reacting in situ further via nitroso-ene reaction to *N*-heterocycle **55b**. While the HRP/GOx-system affords heterocycle **55b** in excellent yield in a neat reaction within 2 h, laccases performed with good yields in 6 – 7 h.

Table 1 Identification of suitable enzymes for the nitroso-ene reaction of N-hydroxycarbamate **66b**. Isolated yields, conversion in parenthesis.

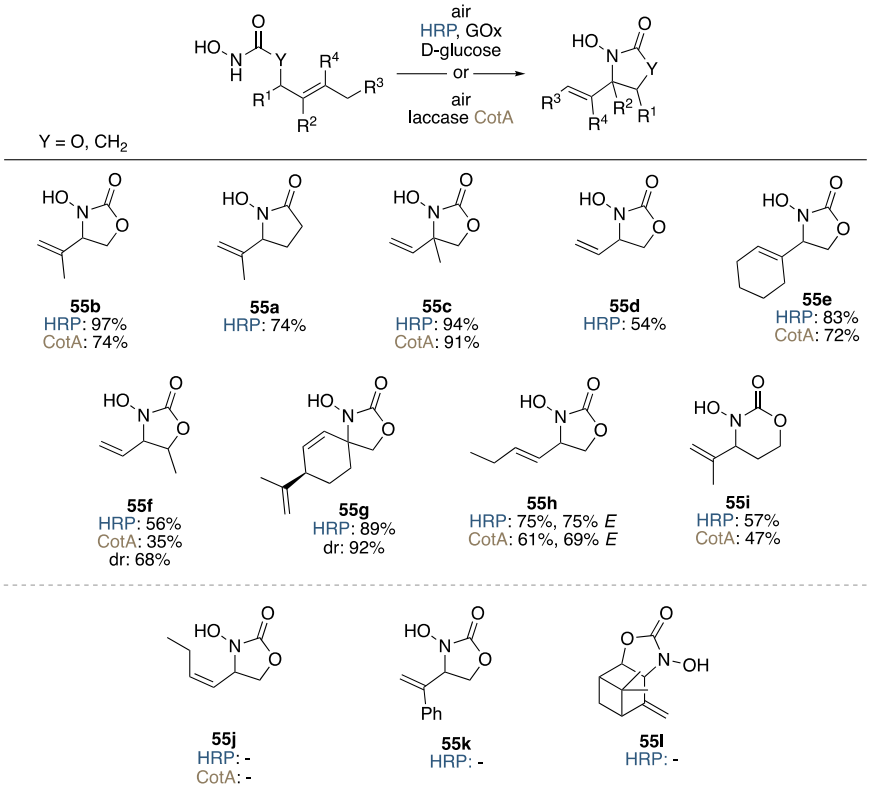


66b **55b**

Entry	Enzyme	Additive	Cosolvent	Time [h]	Yield [%]
1	laccase Mtl	-	-	6	9 (n.a.)
2	laccase Mrl 2	-	-	6	4 (n.a.)
3	laccase Lccβ	-	-	6	78 (100)
4	laccase Ssv1	-	-	6	3 (n.a.)
5	laccase CotA	-	-	7	74 (100)
6	laccase CotA	-	dioxane	7	69 (100)
7	Peroxygenase (*A. Aegerita*)	D-glucose, GOx	-	48	0 (0)
8	LPO	D-glucose, GOx	-	72	5 (n.a.)
9	CPO	D-glucose, GOx	-	29	64 (100)
10	HRP	D-glucose, GOx	-	2	97 (100)
11	HRP	D-glucose, GOx	EtOAc	2	96 (100)
12	HRP	D-glucose, GOx	dioxane	2	94 (100)
13	-	D-glucose, GOx	-	48	0 (0)

As both enzymes were active and enabling the transformation with substrate **66b** at room temperature, it seemed obvious to elucidate the synthetic versatility through a broad set of related substrate derivatives (Scheme 72). Cosolvents needed to be used for most substrates, since they are not dissolving very well in exclusively aqueous media. Fortunately, miscible solvents were well tolerated by laccase CotA (Table 1, entry 6). The system using HRP also allowed transformation in a two-phase system with EtOAc or with water-miscible dioxane without loss of efficiency (Table 1, entry 11 & 12). The method performed well over a range of substrates with different substitution patterns around the double bond, giving high yields for the products **55a-e**

(Scheme 72). Using traditional chemical methods, high temperatures were neccessary to achieve similar yields. In addition, the spirocyclic compound **55g** or six-membered product **55i** were accessible by the HRP/GOx-system. However, as soon as *Z*-configured olefin substrates were employed, full conversion was observed, yet the product **55j** was not formed. This phenomena was also observed previously in related studies.[71] Such a selection aspect strongly indicates, that the substrate is oxidized by the enzymes, but the subsequent nitroso-ene reaction does not take place. The corresponding olefinic alcohol was observed as major side product, hinting at a potential hydrolytic decomposition of the nitroso intermediate. Moreover, substrates **55k** and **55l** bearing a sterically demanding substitution pattern, did not proceed in the reaction utilizing HRP/GOx.

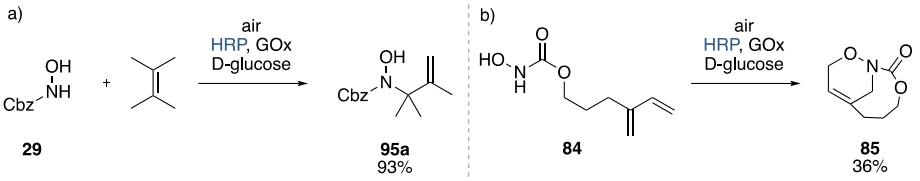


Scheme 72 Substrate scope of enzymatic nitroso-ene reaction of *N*-hydroxycarbamates.

First attempts on intermolecular ene reactions with TME via oxidation of **29** using the HRP/GOx-system, were disappointing and only gave rise to adduct **95a** in mediocre yields. However, when TME was used in excess as a cosolvent, the product yield was significantly increased (Scheme 73a).

Utilizing the copper-dependant biocatalyst CotA, only a modest yield was reached, which can be ascribed to a lower cosolvent tolerance of laccases.

Furthermore, the focus shifted also to the possibility of Diels-Alder transformations and indeed, substrate **84** oxidized by the HRP/GOx-couple underwent the [4+2]-cycloaddition yielding the bicycle **85** (Scheme 73b).



Scheme 73 a) Biocatalytic intermolecular nitroso-ene reaction. b) Biocatalytic intramolecular NDA reaction.

The reaction of model substrate **66b** generates a chiral center. Catalyzed by HRP, the transformation resulted in an excellent yield, but only gave rise to racemic **55b**. However, even though laccases CotA and Lccβ delivered lower yields than the peroxidase, a moderately enantioselective outcome was observed, particularly in a slightly acidic environment (Figure 8). This enantiocontrol is rare for both systems - laccases and the nitroso-ene reaction - and while there are singular reports on stereoinduction by laccases, so far no catalytic method for the nitroso-ene reaction has achieved any degree of enantioselectivity.[71,106] For inducing the stereoselectivity, the active site of the enzyme would likely need a Lewis-acid binding site to coordinate the nitroso species. The oxidative mechanism of HRP with its coordinatively saturated iron-oxo heme species does not allow such binding. However, laccases bearing a mononuclear copper in their active site may allow binding, such it was observed with copper catalysts.[128] In addition, the chiral environment of the enzyme can induce enantioselective reaction outcome by interaction of the nitroso species with amino acid residues in the active site.

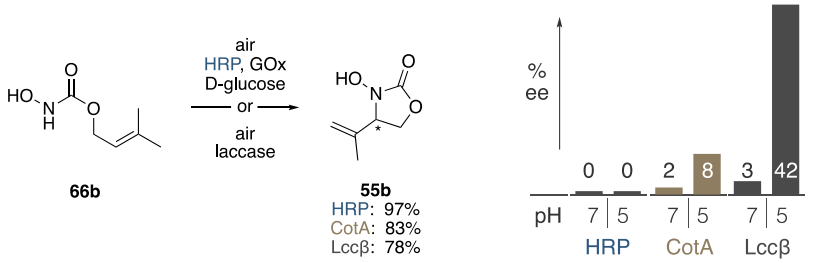
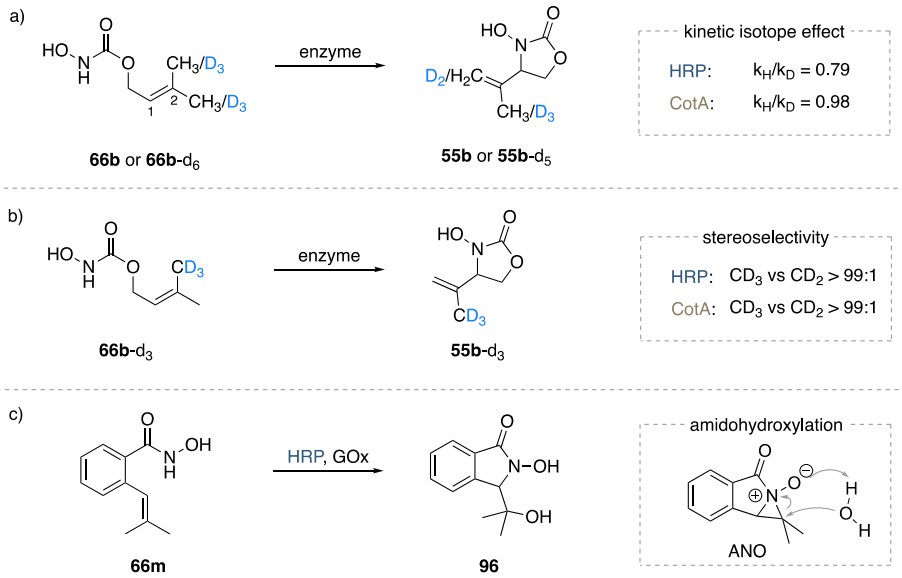


Figure 8 Biocatalytic nitroso-ene reaction of model substrate **66b** and enantioselective outcome of various enzymes at different pH.

This observation led to the assumption that laccases participate in the transformation beyond the oxidation step, while HRP simply induces the reaction through dehydrogenation whereupon cyclization proceeds without protein assistance. To gain insights into the mechanistic features of the nitroso-ene reaction, kinetic isotope effects (KIE's) between unlabeled substrate **66b** and deuterated substrate **66b**-d_6 were measured (Scheme 74a). Since the mechanism of the nitroso-ene reaction is not completely elucidated yet, the HRP/GOx-System was also subjected for the KIE's. The observed inverse β-secondary KIE of 0.79 indicates a stepwise reaction alongside a hybridization change from sp^2 to sp^3 at the C2 position in the rate-limiting step. This is a strong marker for the formation of a three-membered ANO intermediate. Similar KIE's were observed in the ene reaction of triazolinediones, which were associated with a ANO-like intermediate as well.[225,226] The ANO also supports the selectivity of the ene reaction for exclusively *E*-olefins observed in the scope studies of the enzymatic protocol, since it proceeds through a rigid intermediate which prohibits hydrogen abstraction from the *Z*-position (Scheme 74b). In addition, the scope studies revealed another hint on an intermediary ANO. The ene reaction of the hydroxycarbamate **66m** delivered compound **96** as only product resulting from an amidohydroxylation (Scheme 74c). It is known that amido-hydroxylations can occur subsequently to aziridination of double bonds.[227] The three membered ring can be opened via attack of a suitable nucleophile, in this case water.[228] Therefore it can be assumed that the nitroso intermediate might also react to the oxazolidinone via formation of a three membered cycle, namely the ANO-intermediate.



Scheme 74 Mechanistic delights of the intramolecular nitroso-ene reaction.

The kinetic isotope studies of laccase CotA, where no significant KIE ($k_H/k_D \sim 1$) could be found, leads to the assumption that the two biocatalysts perform via different mechanisms and the laccase might be involved in more than only oxidizing the substrate.

3.2 PUBLICATION II

Adding value to chemical procedures, scale-up reactions take a huge part in potentially further application processes of chemical reactions. Especially for industrial synthetic purposes, scalability is an attractive and beneficial attribute. The excellent yield of the nitroso-ene reaction via oxidation of **66b** utilizing the HRP/GOx-System seemed to be a good model for scale-up studies. With the goal to perform gram-scale reactions, smaller scale-ups (up to 25-fold), to evaluate whether less amount of enzyme or D-glucose can be used were therefore tested first. This would, if succesful, give access to an even more economic method. However, while small reactions still performed well with the reduced amount of enzyme, the slightly bigger scales revealed a decrease in yield of **55b**. Therefore, the original procedure was retained. Finally, testing a large gram-scale reaction with almost 3 L reaction volume yielded **55b** in 73% yield within 24 h without formation of side products. The increased reaction time as well as the neat reaction with lower yield, suggests either a problem in the extraction step or a lack of proper aeration of the reaction mixture by ensuring efficient stirring. This could be achieved by using an adequate reactor setup.

To address this problem in a pure academic organic chemistry laboratory, another strategy was contrived. Since the HRP/GOx-couple perfectly tolerates a two-phase system utilizing 10 vol% EtOAc, recycling of the aqueous phase was considered by separating the phases after each cycle (Figure 9, beige). The usual reaction protocol delivered 3.5 cycles in total. The cycle length was thereby the prevailing reaction time of 2 h and the same yields of 96% were achieved for each cycle. To rule out full consumption of the hydrogen peroxide produced by GOx, more D-glucose was fed into the system, but did not result in continuation of the reaction.

To test whether inactivation by solvent or via enzyme bleaching by hydrogen peroxide appears, the reaction was performed in pure buffer (Figure 9, blue). The product **55b** was extracted after each cycle with EtOAc and new **66b** was added for the next round. Here, the reaction achieved 4.5 cycles without loss of efficiency by using the original amount of D-glucose. By adding more D-glucose the reaction continued, clearly ruling out a bleaching process. If smaller doses of D-glucose are added with each cycle, the reaction continues to complete 5 cycles. Using EtOAc just for the extraction seems to be tolerated and only inactivates when the enzymes are exposed for longer periods. Especially HRP seems to suffer when in longer contact with EtOAc, since it is known that hydrogen bonds inside the enzyme can be cleaved

leading to inactivation.[204] However, the recycling studies successfully afforded higher amounts of product **55b** in quantitave yields. Combining this outcome with a slightly bigger scale, which still achieved >90% yield in the previous scale-up studies, would lead to a powerful method for the production of bigger amounts of the synthetically valuable products.

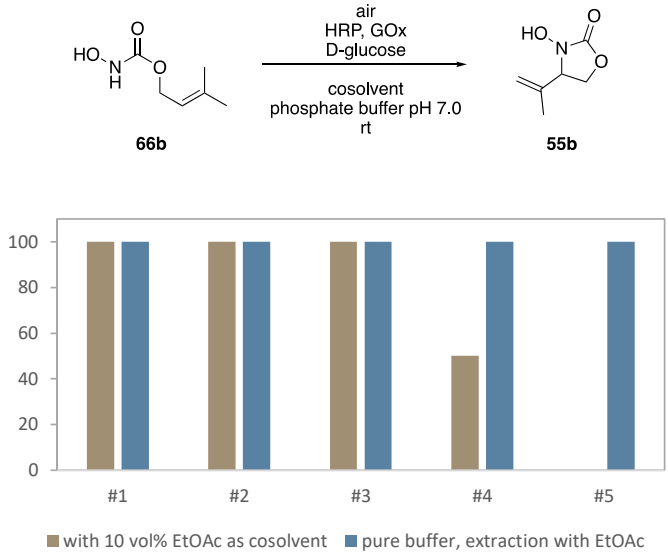
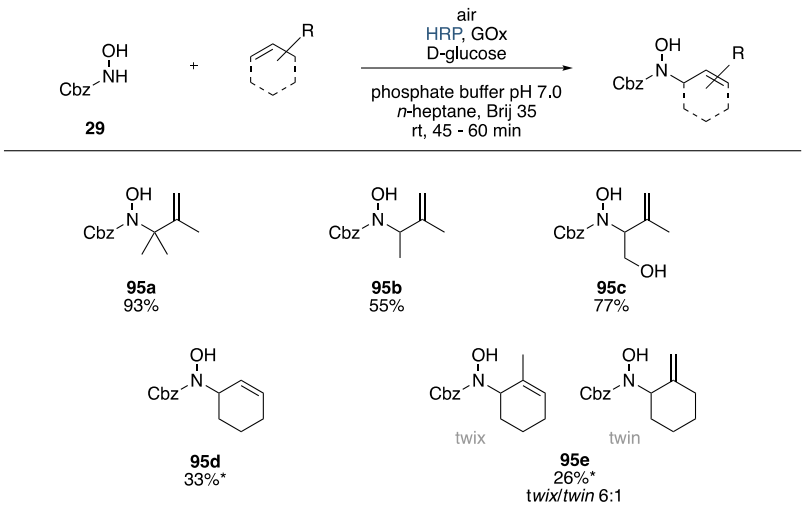


Figure 9 Recycle studies of aqueous media containing the biocatalysts.

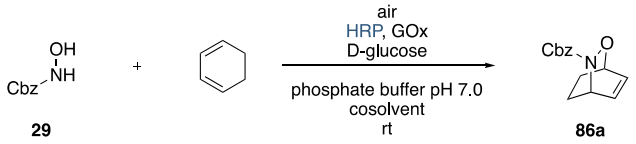
Applying the protocol for the nitroso-ene cyclization from publication I to bimolecular systems, excellent results were obtained for the transformation of hydroxylamine **29** by using an excess of the olefinic reaction partner to afford adduct **95a**. The reaction was subsequently optimized, and using an emulsion system containing *n*-heptane and Brij 35 as emulsifier, allowed to reduce the necessary concentration of the ene to minor excess (5.0 eq.). With TME the reaction gave excellent results for product **95a** in very short reaction time of 0.75 h. The scope studies revealed that other substituted olefins were well accepted and gave rise to adducts **95b** and **95c** in good yields (Scheme 75). However, with cyclic olefins **95d** and **95e** only poor yields were obtained, which could be only improved by utilization as cosolvent.



Scheme 75 Scope of the bimolecular nitroso-ene reaction between **29** and various olefins. *Olefin as cosolvent.

When applying the emulsion system for the intermolecular nitroso-Diels-Alder reaction of the Cbz-protected hydroxylamine **29** and the commonly used cyclohexadiene, the NDA-adduct **86a** was obtained in a good yield (Table 2, entry 1). However, when the original reaction conditions of the intramolecular nitroso-ene reaction from publication I were applied to a bimolecular nitroso-Diels-Alder reaction of **29** and cyclohexadiene, the yields were disappointing (Table 2, entry 2). By adjusting the GOx-loading to 5:1 HRP/GOx to produce lower concentrations of hydrogen peroxide in the reaction mixture, slightly improved yield was obtained. Full conversion in short reaction times revealed that the activity of the enzyme is not a limiting factor in this case. Nevertheless, for a bimolecular reaction both reaction partners need to be properly dissolved. After screening various solvent systems (Table 2, entry 3 – 12), simple increase of cosolvent to 20 vol% EtOAc resulted in an excellent outcome of 90% yield for product **86a** (Table 2, entry 6).

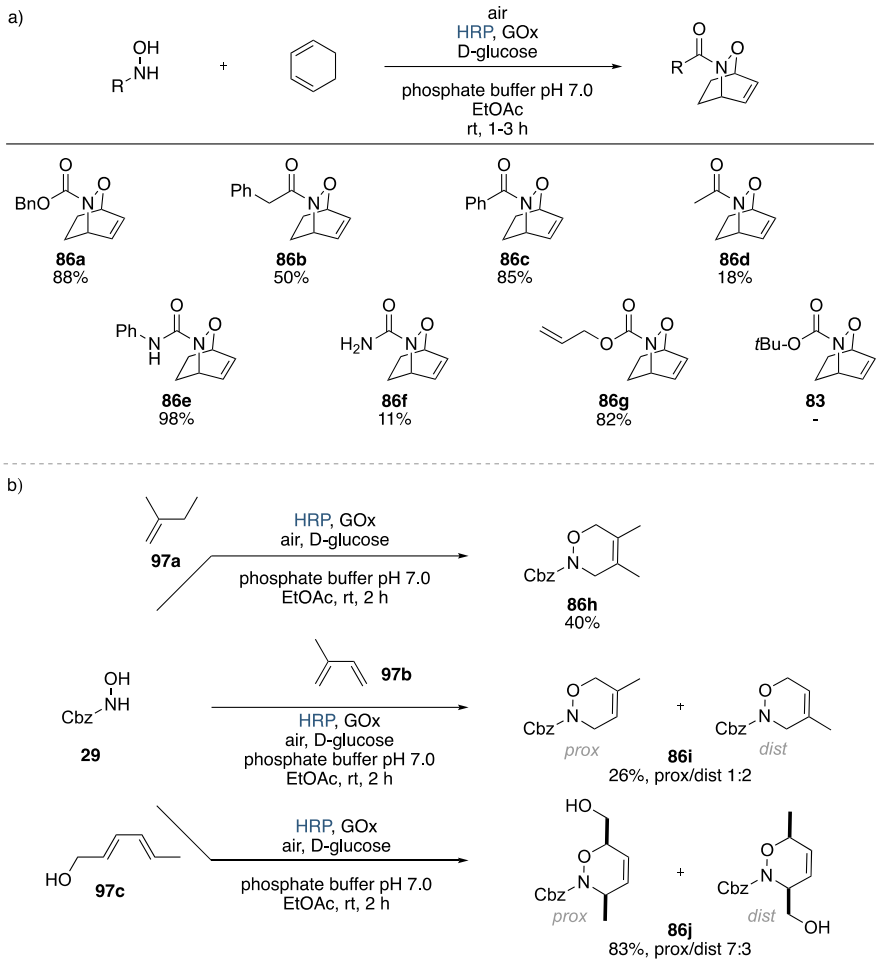
Table 2 Optimization for the HRP/GOx-mediated nitroso-Diels-Alder reaction of **29** and cyclohexadiene. ^{1}H-NMR yields.



Entry	cosolvent	[% v/v]	Time [h]	Yield [%]
1	*n*-heptane/ Brij 35	10	2	63
2	-	-	6	12
3	*n*-heptane	10	2	76
4	*n*-heptane	20	1	82
5	EtOAc	10	2	47
6	EtOAc	20	1.5	90
7	toluene	10	4	38
8	DCM	10	22	<5
9	Dioxane	10	3	13
10	DMSO	10	4	13
11	*t*-BuOH	10	4	10
12	MeCN	10	4	12

With this slightly adjusted method in hand, NDA reactions of various hydroxylamines were carried out (Scheme 76a). Cyclohexadiene was chosen as reaction partner, since it worked well in the mentioned cosolvent studies and is known to react efficiently with nitroso intermediates to afford high yields of NDA-adducts **86a**-**g**. The scope studies revealed significantly higher yields when aromatic groups were located in the vicinal position to the nitroso group, a feature which was not observed in NDA reactions using traditional chemical methods and therefore can be attributed to characteristics of HRP.[229] In addition, slower reacting substrates would naturally result in lower yields since acylnitroso species show low stability in the aqueous environment and undergo decomposition if not reacting fast enough with the diene partner.

Utilizing **29** with various dienes **97a**-**c** (Scheme 76b) showed that dienes containing polar groups, such as alcohol **97c** result in better yields. Hence, improved encounter of the reactants occurs, which is crucial in this reaction to avoid decomposition or side reactions of the nitroso intermediate. In addition, the adducts **86j** were obtained in pure *cis*-configuration. Therefore, it can be assumed that the enzyme-mediated NDA reaction also proceeds as a concerted reaction.



Scheme 76 Bimolecular NDA reaction with a) various hydroxylamines and b) various dienes.

An interesting discussion point in the review process of this publication was the sustainability and greeness of this procedure. Since enzymatic protocols usually go hand in hand with most of the principles of Green Chemistry[230] it is oftentimes criticized for the higher dilution factor compared to traditional chemical procedures. Hence, more waste in form of decontaminated water would be formed leading to elevated *E*-factors - a measure for the actual amount of waste produced in the process.[231] It seemed pivotal for the reviewers to put the biocatalytic procedure into comparison (Figure 10). Important to mention is that none of the illustrated reactions were optimized towards minimal waste production.

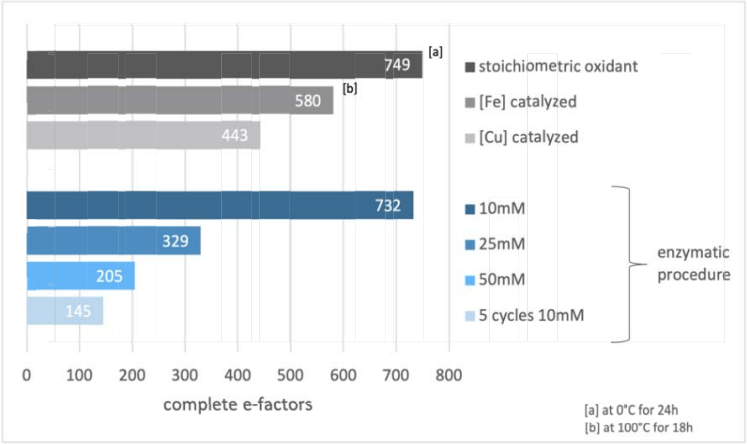
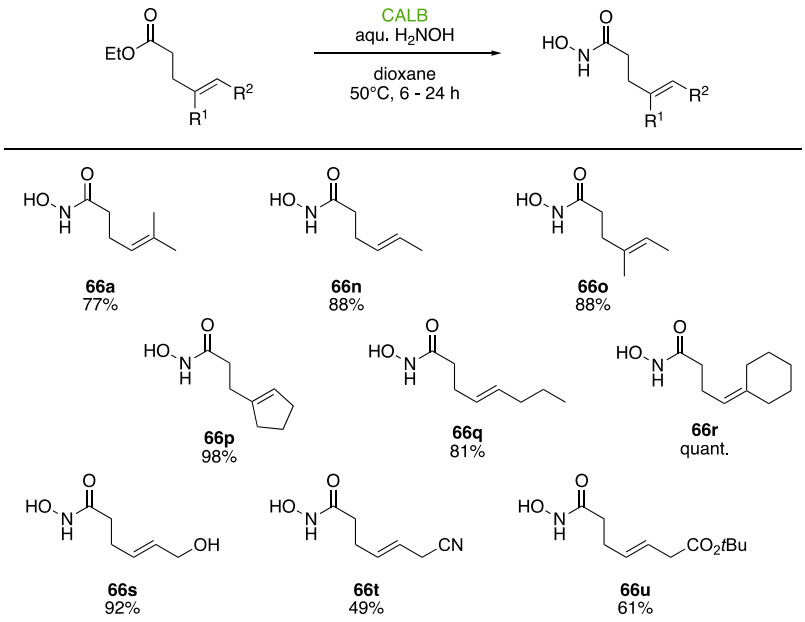


Figure 10 *E*-factor analysis of the nitroso-ene reaction. Extraction solvents were not taken into account.

Evaluating the *E*-factors for the traditional protocol using stoichiometric amounts of oxidant[69] did unsurprisingly result in a high *E*-factor (Figure 10, dark grey). In addition, the procedure does not go along with the principles of Green Chemistry since it uses chlorinated solvents and halogenated reactants. The metal-catalyzed reactions[70,71] perform better according to the *E*-factor and the Green Chemistry principles as well, however, the copper catalyzed system is still using THF as reaction solvent. In comparison, the waste evolving from the enzymatic procedure leads to a poor *E*-factor for a single cycle of substrate **66b**. Turning to higher substrate loadings does not lead to comparable yields of the product, but the *E*-factor improves significantly. However, this is somewhat misleading, since loss of atom economy and efficiency is present at the same time. The waste of the enzymatic protocol is mostly constituted from water (>97%) containing glusose (1.2 eq.) and phosphate (2.0 eq.) at neutral pH and can be therefore considered as an environmentally friendly bias in biocatalysis. Additionally, the recycling of the aqueous phase for up to 5 cycles performs efficiently according to atom economy and outperforms the *E*-factor of the other described procedures (Figure 10, lightblue), indicating a greener and more sustainable process.

3.3 PUBLICATION III

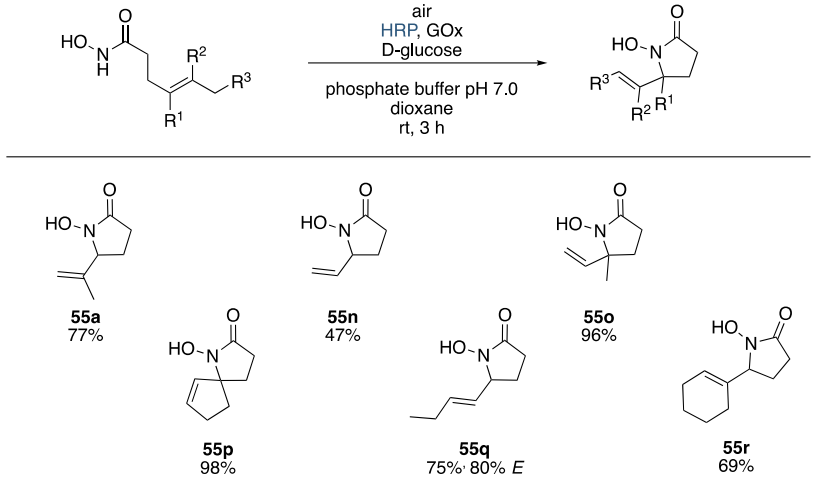
As described in the previous chapter, lipases are mostly used in organic chemistry to produce chiral alcohols or esters via kinetic resolution. However, hydroxylamine was also used to trap fatty acids using lipases to produce hydroxamic acids.[232] To combine a hydroxylaminolysis with the found nitroso-ene protocol, the conditions were optimized to produce a method for unnatural esters and to fit well into a potential cascade design with a subsequent HRP/GOx-catalyzed reaction. In addition, the method could be applied to a range of unsaturated carboxylic acid derivatives in good to excellent yields of the hydroxamic acids using immobilized CALB and hydroxylamine (Scheme 77). No isomerization was observed when olefinic esters were subjected as *trans*-isomers and the hydroxamic acid **66n** and **66q** were exclusively obtained in *trans*-configuration. Cyclic moieties were readily accepted and gave rise to the corresponding hydroxamic acids **66p** and **66r** in quantitative yields. Moreover, the hydroxylaminolysis giving rise to **66p** and **66r** was scaled by 10-fold and even though the reaction time increased to 48 h, the reaction still delivered the products in quantitative yield. Finally, also heterofunctionalized substrates were tested and appear to be readily converted by CALB into the products **66s** – **66u**.



Scheme 77 Scope for the biocatalytic hydroxylaminolysis of esters.

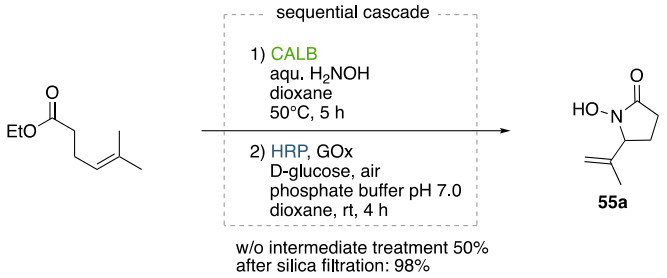
When applying the methodology to carbonates only poor yields could be obtained for the resulting N-hydroxycarbamates by showing no selectivity towards any side of the carbonate and additionally, hydrolysis resulting in the corresponding alcohols was observed.

For the second step of a potential sequence, the hydroxamic acids were subjected to the oxidation protocol using the HRP/GOx couple (Scheme 78). The substitution pattern around the double bond was readily accepted and the γ-lactams **55a**, **55n**, **55o** and **55r** were obtained in good to excellent yields. Additionally, the method gave rise to the spirocyclic product **55p** in nearly quantitative yield when employing substrate **66p**. The nitroso-ene reaction of the nitroso intermediate originating from **66q** as pure *trans*-isomer resulted in product **55q** in a good yield and an *E*/*Z* ratio of 4:1.



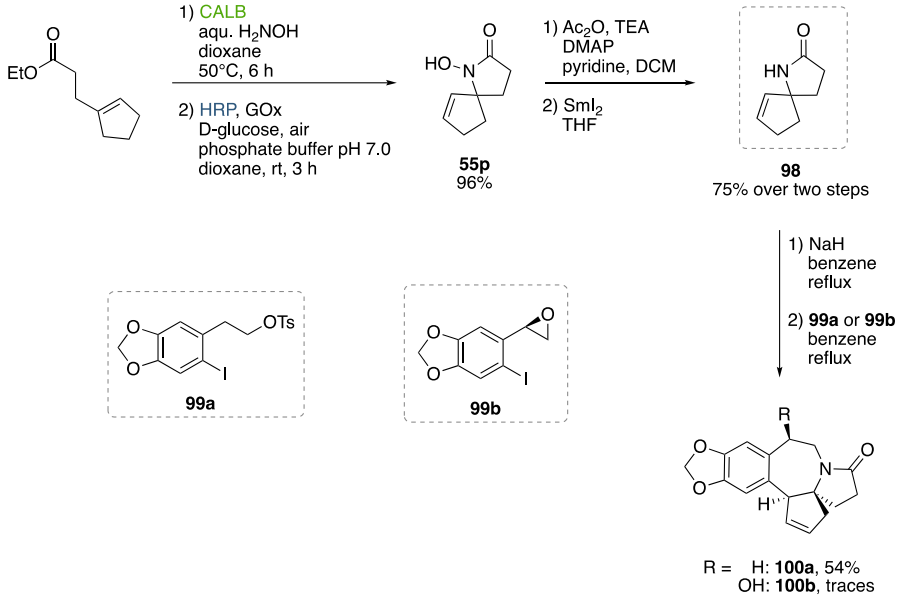
Scheme 78 Substrate scope of the enzymatic nitroso-ene reaction of hydroxamic acids.

Next, the combination of both methods to afford a potential cascade was tested (Scheme 79). The hydroxylaminolysis gave rise to hydroxamic acid **66a** after 5 h. After that the immobilized CALB was simply filtered off and the dioxane solution containing **66a** was added to the HRP/GOx-couple in the aqueous buffer solution. The reaction time of the nitroso-ene reaction slightly increased to 4 h and unfortunately the yield of γ-lactam **55a** dropped to 50%. Remaining hydroxylamine in the dioxane solution from the hydroxylaminolysis was identified to be problematic for the second step. However, on the other hand, equimolar amounts of hydroxylamine were not sufficient to complete the first step of the sequence. Hereafter, the dioxane phase was filtered through a silica pad and then subjected for the second step, eventually resulting in excellent yield of product **55a**.



Scheme 79 Sequential cascade of the hydroxylaminolysis and nitroso-ene protocol.

An interesting structural feature in cephalotaxine-type alkaloids is the spirocyclic backbone of this compound family that has been synthetically addressed by means of the multicyclic Kühne intermediate. Such alkaloids are known to express activity against certain cancers and were proven to be still active when other pharmaceuticals lost effect.[116]



Scheme 80 Chemoenzymatic synthesis yielding precursors of *Cephalotaxus* alkaloids.

The spirolactam **55p** is used as a building block towards the Kühne intermediate **100a**.[8] The scalability of the peroxidase protocol allowed production of compound **55p** in synthetically relevant scale (tenfold increase from the original protocol) in excellent yield (Scheme 80). However, when applying the corresponding ester in the sequential cascade only a mediocre yield of product **55p** was produced. Also here, by applying the two step methodology this shortcoming could be circumvented, giving rise to **55p** in

96% yield after 2 steps. Via acetylation of **55p** and subsequent reduction by SmI_2, spiroamide **98** was obtained in 75% overall yield. Coupling of the generated sodium amide of **98** with epoxide **99b**, an alcohol intermediate was obtained as a mixture of diastereomers along with an unknown impurity. The trapping of the sodium amide with tosylate **99a** worked more cleanly. Subsequent Heck reaction gave rise to the Kühne intermediate **100a** in 54% yield. Unfortunately, the hydroxylated Kühne variation **100b** was only observed in trace amounts, likely due to the impurity of the C-N coupling product.

4 CONCLUSION

In the focus of this thesis stands the development of novel enzyme-mediated protocols to catalyze nitroso-ene reactions. Starting from the discovery of suitable enzymes through optimization of the methods and the quest for selectivity, the path eventually led to a robust reaction protocol, which was also tested in scale-up and recycling studies. In addition, mechanistic investigations revealed interesting insights into the still disputed reaction pathway of the nitroso-ene reaction and also highlighted the involvement of certain laccases in the ene reaction itself. The method was utilized in biocatalytic cascade reactions as a valuable outline for application in total syntheses of cephalotaxine-type alkaloids. Even though not in the spotlight, a broad scope of nitroso-Diels-Alder transformation was also found to be catalyzed via the same protocol. The findings in this thesis reveal a particularly mild and operationally simple biocatalytic method, which is widely applicable as stand-alone tool but can be likewise implemented into cascade reactions producing valuable building blocks.

In publication I, two enzymatic methodologies for the formation of heterocyclic products were designed. Via an HRP/GOx interplay or different laccases N-hydroxycarbamates are oxidized to the corresponding nitroso intermediates, which spontaneously undergo ene-type transformations. Utilizing laccases in a slightly acidic environment even provided moderate enantioselectivity in the nitroso-ene reaction. In addition, various labeling studies gave insights into mechanistic features of the reaction. The peroxidase-induced cyclization seems to follow a zwitterionic pathway via a bicyclic ANO intermediate. With laccases, no such trend could be found, strongly suggesting a different mechanistic pathway and further involvement of the enzyme.

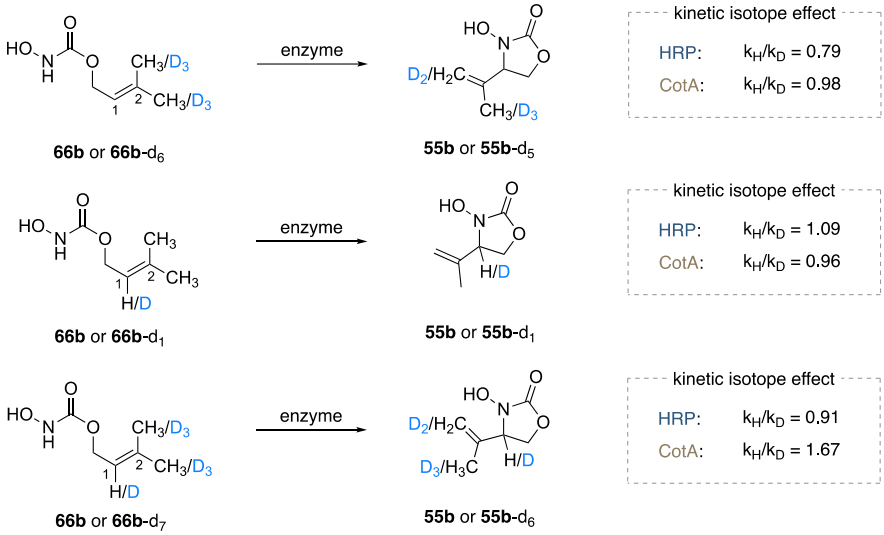
In publication II, the scope was extended towards bimolecular nitroso-ene reactions and nitroso-Diels-Alder cycloadditions. The scalability of the reaction protocol was demonstrated, and the easy recyclability of the enzyme-containing aqueous phase provided a more efficient synthesis of the nitroso-ene products. Through a sustainability assessment and comparison with previously reported protocols, the methodology employing the HRP/GOx couple was identified as a certainly green and sustainable approach.

In publication III, an enzymatic sequence yielding lactams was designed starting from unsaturated esters. A three-enzyme methodology containing lipase CALB, HRP and GOx was established. The lipase transforms ethyl esters into hydroxamic acids, which are subsequently employed as substrates for the HRP/GOx oxidation protocol. A spirocyclic product was succesfully applied in the chemoenzymatic synthesis of the Kühne intermediate, a compound

resembling the backbone of *Cephalotaxus* alkaloids. In addition, scalability and high overall yields via mild and non-toxic reaction conditions demonstrate a convenient synthetic method to afford N-heterocycles.

Since the project started with the work presented in this thesis, its scope is still far from exhaustively explored. There are so many more aspects which will require further scientific work. Especially the nitroso-ene activity of laccases is one part of an already ongoing cooperation. Here, it is particularly interesting from which factors the enantioselectivity arises. First preliminary results of computational studies revealed a pH-dependent effect of the amino acids in the active site of laccase Lccβ, a feature which could be interesting for a potential protein engineering campaign.

Additionally, the KIE's reported in publication I made me wonder if I could reach further meaningful insight to the mechanism when labelling different locations in the molecule (Scheme 81). For the laccase no differences could be seen in the KIE's resembling changes on C1 and C2 of the olefin. However, with HRP the KIE at C1 showed a significant difference to C2. A small normal secondary KIE was observed. While different effects are able to cause this outcome, computational studies in the future would be required to support meaningful interpretations of these experimental results.



Scheme 81 Observed kinetic isotope effects for deuterated substrates **66b**-d_6,**66b**-d_1 and **66b**-d_7.

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