TECHNISCHE UNIVERSITÄT WIEN
Vienna University of Technology

Dissertation

CHEMOENZYMATIC ONE-POT REACTION SEQUENCE FOR THE PRODUCTION OF CHIRAL ALCOHOLS AND AMINES

ausgeführt zum Zwecke der Erlangung des akademischen Grades eines Doktors der Naturwissenschaften unter der Leitung von

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eingereicht an der Technischen Universität Wien

Fakultät für Technische Chemie

von

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Wien, 01.05.2017

Only the one who walks his own way

can´t be overtaken.

MARLON BRANDO

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Acknowledgements

Ich möchte mich bei vielen Leuten bedanken und ich hoffe sehr hier niemanden zu vergessen. Falls es doch passiert - ich nehme euch ohnehin mit.

Zuerst möchte ich mich bei Prof. Marko D. Mihovilovic bedanken für seine engagierte Betreuung und für die Möglichkeit an diesem anspruchsvollen und interessanten Projekt zu arbeiten. Lieber Marko, danke dass ich meine Dissertation in deiner Forschungsgruppe durchführen durfte und so viel Neues dabei lernen konnte.

Außerdem gilt mein Dank Associate Prof. Dr. Michael Schnürch. Michl, bei dir möchte ich mich für dein „gerader Michel" sein bedanken, für das Vertrauen in meine Arbeit, für deine immer umsetzbaren Vorschläge. Vielen Dank für deine Hilfe!

Des Weiteren möchte ich mich bei Dr. Florian Rudroff bedanken für seine gute Betreuung insbesondere bei der experimentellen Arbeit. Danke Flo, dass du immer Ansprechpartner für alles warst und für deine 1000 neuen Ideen, wenn etwas nicht so funktioniert hat, wie wir es uns vorgestellt haben. Danke auch für deine Messungen auf der UPC^2.

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Jetzt möchte ich mich bei meinem „+1" Thomasch bedanken. Würde ich genau beschreiben wofür, würde die Gewichtung von Acknowledgements und restlicher Dissertation nicht zu meinen Gunsten ausfallen, somit halte ich mich zurück. Es bleibt mir aber zu sagen: ohne dich wär es nicht gegangen oder nur ganz schwer.

Danke an Wiesi für so viele Lacher und alles sonst. „Bluadige Gams" - wirst du mir fehlen. Außerdem Danke an dich Sofia, für viel mehr als so einige schöne Essen beim Temaya und deine Hilfe mit sämtlichen computernahen Geräten! Danke an David („erfolgreich, knackig, jung"), das beste Bench-Gegenüber, das man sich wünschen kann mit dem tollsten Musikgeschmack. Du hättest schon viel früher da sein müssen. Danke auch an die liebste Anna- schön war's mit dir und niemals wortkarg :)

Vielen Dank an Vlad, der während der finalen Zeit richtig viel gearbeitet hat und mir wirklich sehr geholfen hat noch alles unterzubringen, was ich mir vorgestellt habe. Außerdem an den lieben Heiko, der in kürzester Zeit auch viel beigetragen hat. Danke an Laszlo für die Messungen auf der chiralen HPLC. Außerdem danke für die tolle Zeit mit euch: Gerti, Niko, Daniela, Maria, Dominik, Drasi, Christian, Laurin, Schöni- der gesamten MDM Gruppe.

Thanks to Alessandro, who helped me with his hydrolysis studies and the synthesis of reference compounds.

Lieber Stefano, danke für deine unerschütterliche Zuversicht was meine Dissertation anbelangt und dafür, dass du mich ausgehalten hast, in Zeiten in denen es wahrscheinlich nicht ganz einfach war. Wenn ich mal nicht mehr dran geglaubt hab, hast du es für mich gemacht. Unersetzlich. Danke dir!

Danke an meinen Papa, meinen Opa, meine Mama und meine ganze (große) Familie, nicht nur für die finanzielle Unterstützung während meines Studiums, sondern auch dafür, mir die Voraussetzungen gegeben zu haben, um nicht nur diese Arbeit, sondern insgesamt das was ich mir vorstelle bewältigen zu können. Danke, Papa und Ive für alles!

Lieber Opa, jetzt erlebst du es noch („das möcht ich noch erleben"). Zeitweise war ich mir da auch nicht so sicher- aber nicht, weil ich nicht dran geglaubt hab, dass du noch 100 wirst.

Abstract

Chemoenzymatic multistep one pot processes offer a highly attractive concept in synthetic chemistry. Due to the minimized number of workup and purification steps, the combination of bio- and chemocatalytic reactions in a one pot fashion gives rise to significantly improved overall process efficiency. In this thesis sequential and simultaneous chemoenzymatic one pot cascade reactions are presented by combining a metal (Pd/Cu) catalyzed coupling reaction or a gold catalyzed hydration reaction, both generating a ketone, with a subsequent enzymatic transformation of the newly created functional group. This is investigated by applying enantiodivergent alcohol dehydrogenases for the production of enantiopure alcohols, or ω-transaminases for the production of enatiopure amines, respectively. The simultaneous cascade was performed in an aqueous system using two different compartments seperated by a polymer membrane, which enabled an overall reaction concentration of up to 100 mM substrate without dilution or any other intermediate step before the biocatalytic transformation. To demonstrate the versatility of the developed method, both enatiomeric forms of the respective chiral alcohols were produced in moderate to high yields and excellent enantiomeric purities. Further, an extremely straightforward method for the chemoenzymatic production of enantiopure alcohols in a sequential mode was developed by combining a gold catalyzed hydration of alkynes with an enzymatic reduction. Within this process, the solvent of the metal catalyzed transformation serves as cosubstrate for the subsequent biotransformation. In this case no spatial separation of the metal catalyst from the enzymatic part of the reaction was required and an economical attractive strategy was developed, in which the overall reaction mixture of the first step can be employed in the second step of the desired chemoenzymatic one-pot cascade reaction.

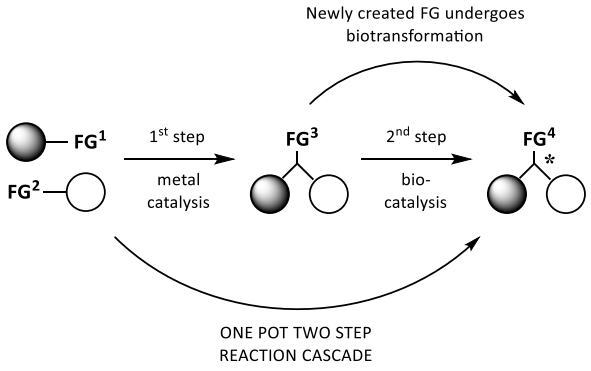


Figure A-1 Creation of a new functionality which subsequently undergoes a biotransformation

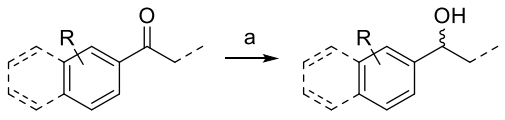
Kurzfassung

Chemoenzymatische mehrstufige “Eintopf“ Prozesse stellen ein attraktives Konzept in der Synthesechemie dar. Durch die reduzierte Anzahl an Aufarbeitungs- und Reinigungsschritten führt die Kombination von Bio- und Chemokatalyse zu einer deutlich verbesserten Prozesseffizienz. In dieser Arbeit werden sowohl sequentielle, als auch simultane chemoenzymatische Eintopf-Kaskaden-Reaktionen vorgestellt, die die Kombination von metallkatalysierten (Pd/Cu) Kupplungseaktionen bzw. goldkatalysierten Hydratisierungsreaktionen mit einer nachfolgenden enzymatischen Transformation der neu geschaffenen funktionellen Gruppe beinhalten. Dieser Prozess wird untersucht durch die Verwendung enantiodivergenter Alkoholdehydrogenasen zur Hertsellung von enantiomerenreinen Alkoholen bzw ω-Transaminasen zur Herstellung von Aminen. Die simultane Kaskadenreaktion wurde in wässrigem Medium durch Verwendung zweier durch eine Polymer-membran getrennten Reaktionskammern durchgeführt. Dieser Reaktionsaufbau erlaubt Substratkonzentrationen von 100 mM ohne jegliche Verdünnung oder andere Zwischenschritte vor der nachfolgenden enzymatischen Reaktion. Um die Vielseitigkeit dieser Methode zu demonstrieren, wurden diverse chirale Alkohole in moderaten bis hohen Ausbeuten und exzellenter optischer Reinheit hergestellt. Des Weiteren wurde eine äußerst einfache, sequentielle Methode durch die Kombination von goldkatalysierten Hydratisierungsreaktionen mit enzymatischen Reduktionsreaktionen für die chemoenzymatische Herstellung von enantiomerenreinen Alkoholen entwickelt. Hierbei dient das Lösungsmittel des metallkatalysierten Schrittes als Cosubstrat für die nachfolgende Biotransformation. In diesem Fall bedarf es keiner räumlichen Trennung zwischen Metalkatalysator und der enzymatischen Teilreaktion. Somit wurde eine ökonomisch attraktive Strategie entwickelt, in der die gesamte Reaktionsmischung des ersten Schrittes im zweiten Schritt der chemoenzymatischen Kaskadenreaktion verwendet werden kann.

A Synthetic schemes

All compounds prepared or used as starting materials in this thesis are numbered in bold Arabic numerals with prefixes A, B, C, D, E, F, or G. Thioesters are designated with the prefix A. Ketones are labeled with the prefix B and chiral alcohols with prefix C. Amines are labeled with the prefix D and the respective amides with the prefix E. Boronic acids are labeled with the prefix F and alkyne structures are designated with the prefix G. Structures not prepared or used in this thesis are labeled with Arabic numbers without prefix. Other compounds and general structures are labeled with roman numerals.

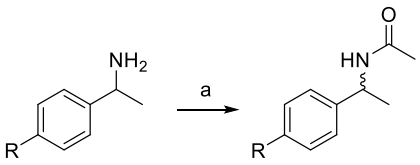
A.I Synthesis of racemic alcohols



Alcohol	Yield [%]	Cpd. No.
1-(4-Methylphenyl)ethan-1-ol	98	**C-1**
1-(3-Methylphenyl)ethan-1-ol	100	**C-2**
1-(4-Fluorophenyl)ethan-1-ol	91	**C-3**
1-(3-Fluorophenyl)ethan-1-ol	92	**C-4**
1-(4-Chlorophenyl)ethan-1-ol	84	**C-5**
1-(3-Chlorophenyl)ethan-1-ol	88	**C-6**
1-(4-Bromophenyl)ethan-1-ol	91	**C-7**
1-(3-Bromophenyl)ethan-1-ol	99	**C-8**
1-(4-Trifluormethylphenyl)ethan-1-ol	69	**C-9**
1-(3-Trifluormethylphenyl)ethan-1-ol	95	**C-10**
1-(4-Methoxylphenyl)ethan-1-ol	100	**C-11**
1-Phenylpropan-1-ol	84	**C-12**
1-(1-Naphtyl)ethan-1-ol	92	**C-13**
1-(2-Naphtyl)ethan-1-ol	94	**C-14**

Scheme A-1 Reagents and conditions: a) $NaBH_4$, EtOH abs.

A.II Synthesis of racemic amides

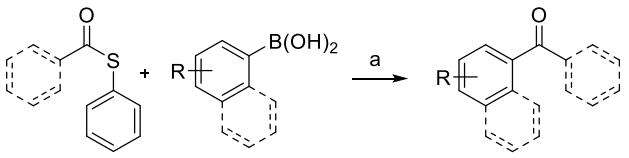


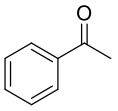
R	Yield [%]	Cpd. No.
H	81	**E-1**
CH_3	82	**E-2**
F	73	**E-3**

Scheme A-2 Reagents and conditions: a) acetic anhydride, DCM

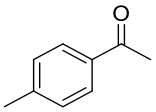
A.III Substrate synthesis by Liebeskind Srogl cross coupling

A.III.1 $Pd(PPh_3)_4$ catalyzed ketone synthesis

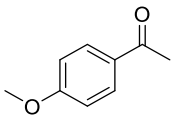




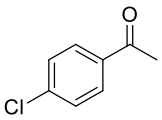
B-0 (99 %)



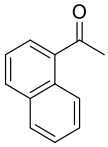
B-1 (86 %)



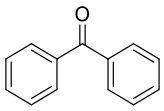
B-11 (99 %)



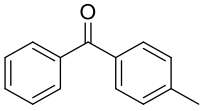
B-5 (67 %)



B-13 (89 %)



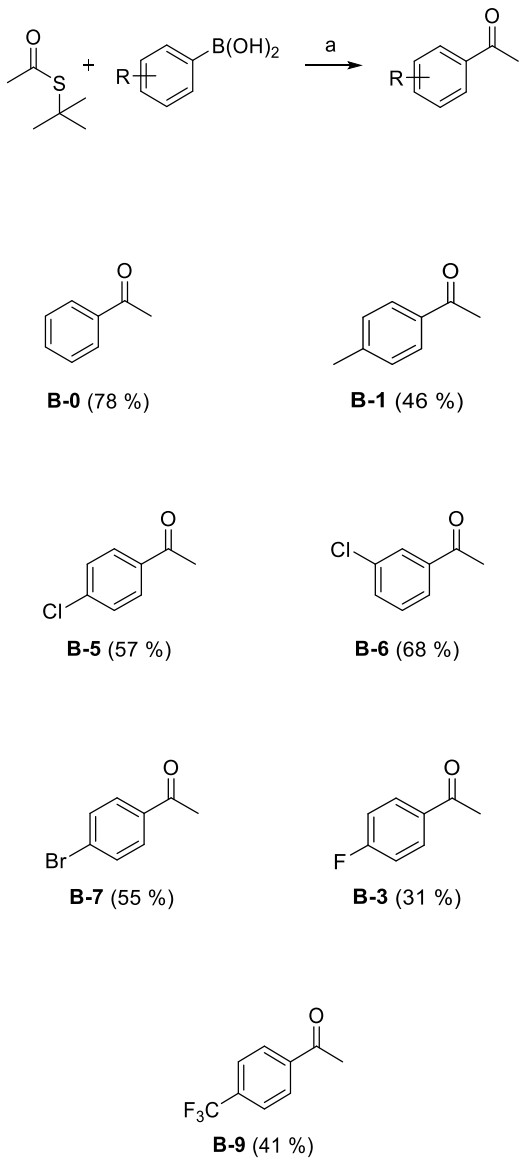
B-15 (99 %)



B-16 (99 %)

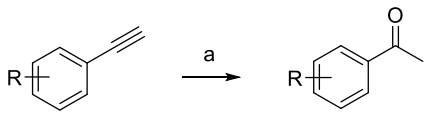
Scheme A-3 Reagents and conditions: a) CuTC, $Pd(PPh_3)_4$, heptane

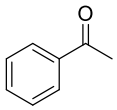
A.III.2 $Pd_2(dba)_3$ catalyzed ketone synthesis



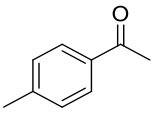
Scheme A-4 Reagents and conditions: a) CuTC, $Pd_2(dba)_3$, $P(OEt)_3$, H_2O/*i*-PrOH

A.IV Substrate synthesis by Au(III) catalyzed hydration of alkynes

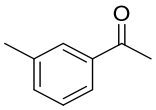




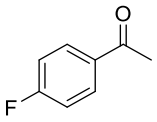
B-0 (98 %)



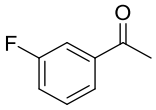
B-1 (98 %)



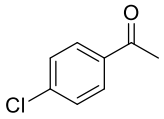
B-2 (99 %)



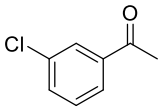
B-3 (71 %)



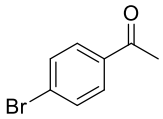
B-4 (86 %)



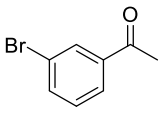
B-5 (72 %)



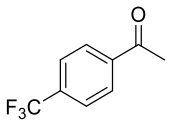
B-6 (76 %)



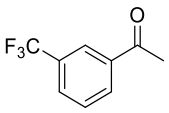
B-7 (70 %)



B-8 (79 %)



B-9 (49 %)

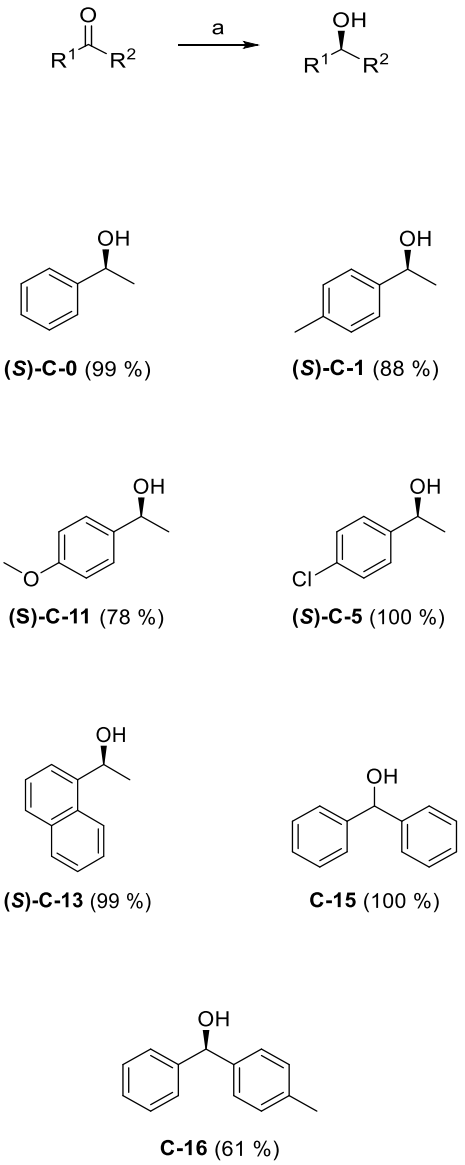


B-10 (57 %)

Scheme A-5 Reagents and conditions: a) H_2O, $AuCl_3$, *i*-PrOH

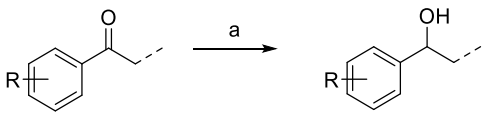
A.V Biotransformations: Alcohol dehydrogenases

A.V.1 Enzymatic reductions applying alcohol dehydrogenase from *Saccharomyces cerevisae*



Scheme A-6 Reagents and conditions: a) ADH, NADP+, glucose, glucose dehydrogenase

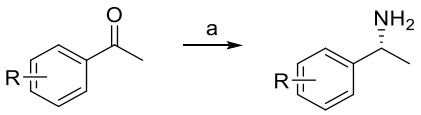
A.V.2 Enzymatic reductions applying ADH-A and LK-ADH as whole cell lyophilisate



(*S*)-and (*R*)-alcohol	ADH-A Conversion [%] (*S*)	LK-ADH Conversion [%] (*R*)	Cpd. No. (*S*)- or (*R*)-alcohol
1-Phenylethan-1-ol	96	95	**C-0**
1-(4-Methylphenyl)ethan-1-ol	90	83	**C-1**
1-(3-Methylphenyl)ethan-1-ol	85	17	**C-2**
1-(4-Fluorophenyl)ethan-1-ol	99	83	**C-3**
1-(3-Fluorophenyl)ethan-1-ol	95	91	**C-4**
1-(4-Chlorophenyl)ethan-1-ol	97	90	**C-5**
1-(3-Chlorophenyl)ethan-1-ol	92	88	**C-6**
1-(4-Bromophenyl)ethan-1-ol	98	90	**C-7**
1-(3-Bromophenyl)ethan-1-ol	99	45	**C-8**
1-(4-Trifluormethylphenyl)ethan-1-ol	99	99	**C-9**
1-(3-Trifluormethylphenyl)ethan-1-ol	99	0	**C-10**
1-(4-Methoxylphenyl)ethan-1-ol	99	40	**C-11**
1-Phenylpropan-1-ol	91	0	**C-12**
1-(2-Naphtyl)ethan-1-ol	77	0	**C-14**

Scheme A-7 Reagents and conditions: a) ADH, *i*-PrOH, Tris-HCl

A.VI Biotransformations: ω-Transaminases

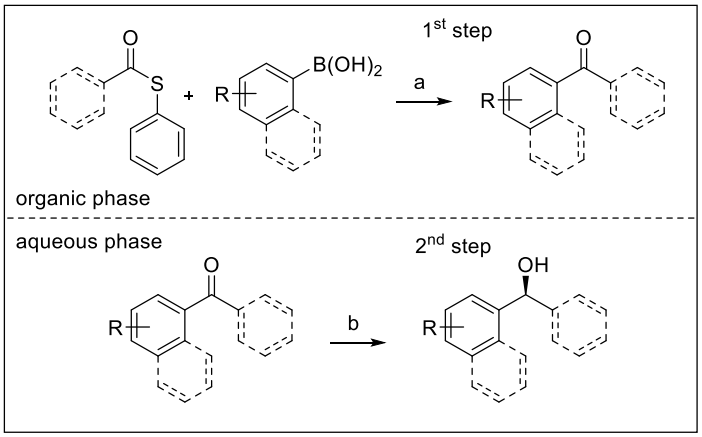


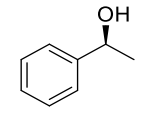
R	Conditions	Conversion [%]	Cpd. No.
H	a-1	92	**(*R*)-D-1**
H	a-2	86	**(*R*)-D-1**
CH_3	a-2	83	**(*R*)-D-2**
F	a-2	90	**(*R*)-D-3**

Scheme A-8 Reagents and conditions:
a-1) alanine, lactate dehydrogenase, NADH, glucose, glucose dehydrogenase
a-2) ω-ATA, PLP, isopropylamine, triethanolamine (codexis ω-transaminases)

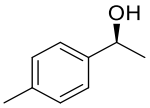
A.VII Chemoenzymatic one pot reactions

A.VII.1 Liquid-liquid biphasic system- Synthesis of chiral alcohols

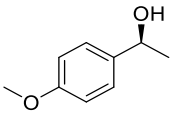




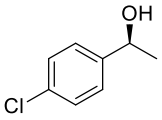
(*S*)-C-0 (57 %)



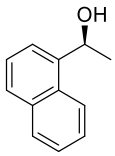
(*S*)-C-1 (44 %)



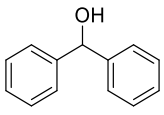
(*S*)-C-11 (63 %)



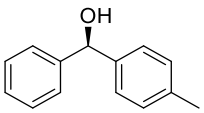
(*S*)-C-5 (91 %)



(*S*)-C-13 (49 %)



C-15 (80 %)

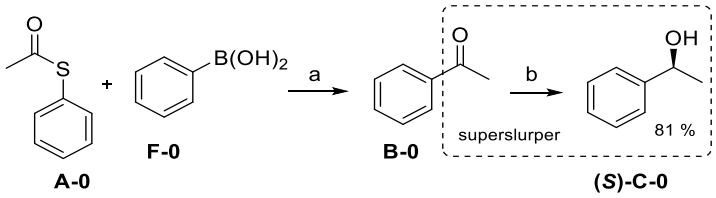


C-16 (5 %)

Scheme A-9 Reagents and conditions: a) CuTC, $Pd(PPh_3)_4$, heptane (10 % overall reaction volume)
b) ADH, NADP+, glucose, glucose dehydrogenase (90 % overall reaction volume)

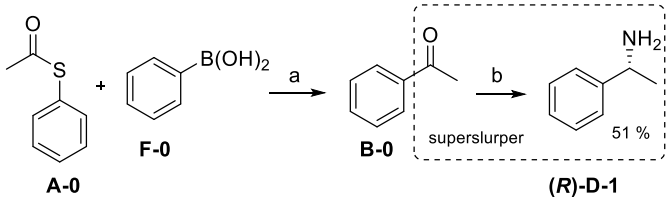
A.VII.2 Liquid-solid biphasic superslurper system- Synthesis of chiral alcohols and amines

A.VII.2.1 Alcohol dehydrogenase embedded in sodium polyacrylate



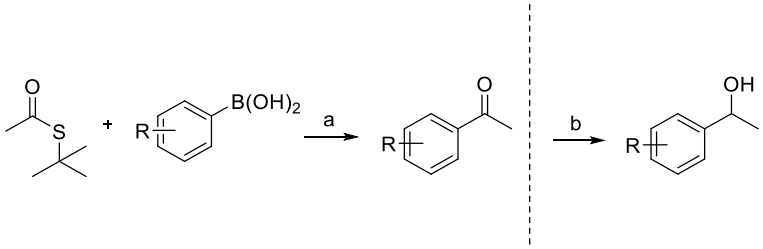
Scheme A-10 Reagents and conditions: a) CuTC, $Pd_2(dba)_3$, $P(OEt)_3$, heptane b) ADH, NADP+, glucose, glucose dehydrogenase, sodium polyacrylate

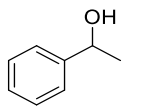
A.VII.2.2 ω-Transaminase embedded in sodium polyacrylate



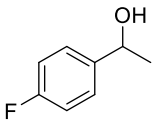
Scheme A-11 Reagents and conditions: a) Boronic acid, CuTC, $Pd(PPh_3)_4$, 50 °C, heptane b) ω-transaminase, codexis mastermix (0), sodium polyacrylate

A.VII.3 Membrane based system- Synthesis of chiral alcohols

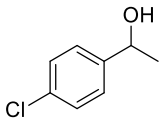




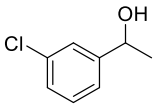
(***S***)-**C-0** (65 %)
(***R***)-**C-0** (81 %)



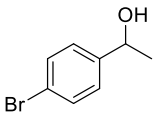
(***S***)-**C-3** (75 %)
(***R***)-**C-3** (64 %)



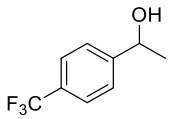
(***S***)-**C-5** (60 %)
(***R***)-**C-5** (57 %)



(***S***)-**C-6** (47 %)
(***R***)-**C-6** (53 %)



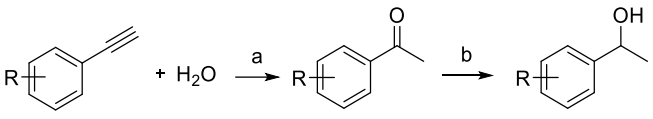
(***S***)-**C-7** (99 %)
(***R***)-**C-7** (50 %)

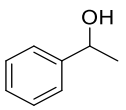


(***S***)-**C-9** (61 %)
(***R***)-**C-9** (53 %)

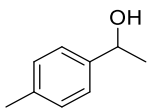
Scheme A-12 Reagents and conditions: a) CuTC, $Pd_2(dba)_3$, $P(OEt)_3$, H_2O/*i*-PrOH, b) ADH, *i*-PrOH, Tris-HCl

A.VII.4 Au(III) based single-phase system- Synthesis of chiral alcohols

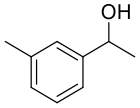




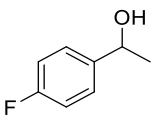
(***S***)-**C-0** (91 %)
(***R***)-**C-0** (93 %)



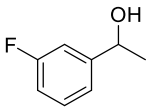
(***S***)-**C-1** (76 %)
(***R***)-**C-1** (73 %)



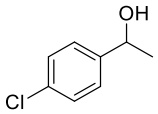
(***S***)-**C-2** (99 %)
(***R***)-**C-2** (15 %)



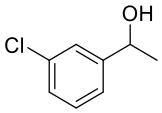
(***S***)-**C-3** (71 %)
(***R***)-**C-3** (65 %)



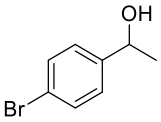
(***S***)-**C-4** (86 %)
(***R***)-**C-4** (74 %)



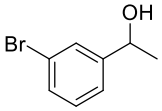
(***S***)-**C-5** (71 %)
(***R***)-**C-5** (69 %)



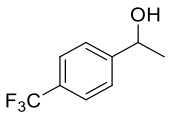
(***S***)-**C-6** (76 %)
(***R***)-**C-6** (29 %)



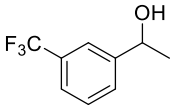
(***S***)-**C-7** (67 %)
(***R***)-**C-7** (67 %)



(***S***)-**C-8** (53 %)
(***R***)-**C-8** (17 %)



(***S***)-**C-9** (49 %)
(***R***)-**C-9** (49 %)



(***S***)-**C-10** (57 %)
(***R***)-**C-10** (0 %)

Scheme A-13 Reagents and conditions: a) H_2O, $AuCl_3$, *i*-PrOH, 65 °C, b) ADH, *i*-PrOH, Tris HCl buffe

B Introduction

The present thesis deals with the development of chemoenzymatic one pot processes in a cascade fashion. This comprises the precise investigation of both the chemical and the enzymatic reaction and, in the following, the approximation of the naturally not combinable reaction conditions of the two single cascade steps. Various strategies for a successful combination were examined and optimized. This included studies of several compartmentalization strategies and the related effect on scope and limitations of the single cascade reactions. In any case, the metal catalyzed step comprises the formation of a new functional group that subsequently undergoes the enzymatic transformation (Scheme B-1).

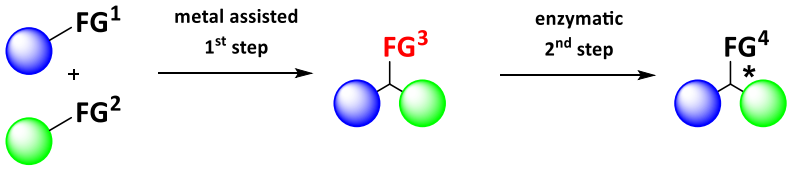
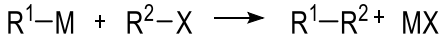


Figure B-1 Concept of a chemoenzymatic reaction cascade implying the formation of a new functionality (FG^3)

B.I Metal assisted C-C cross coupling reactions

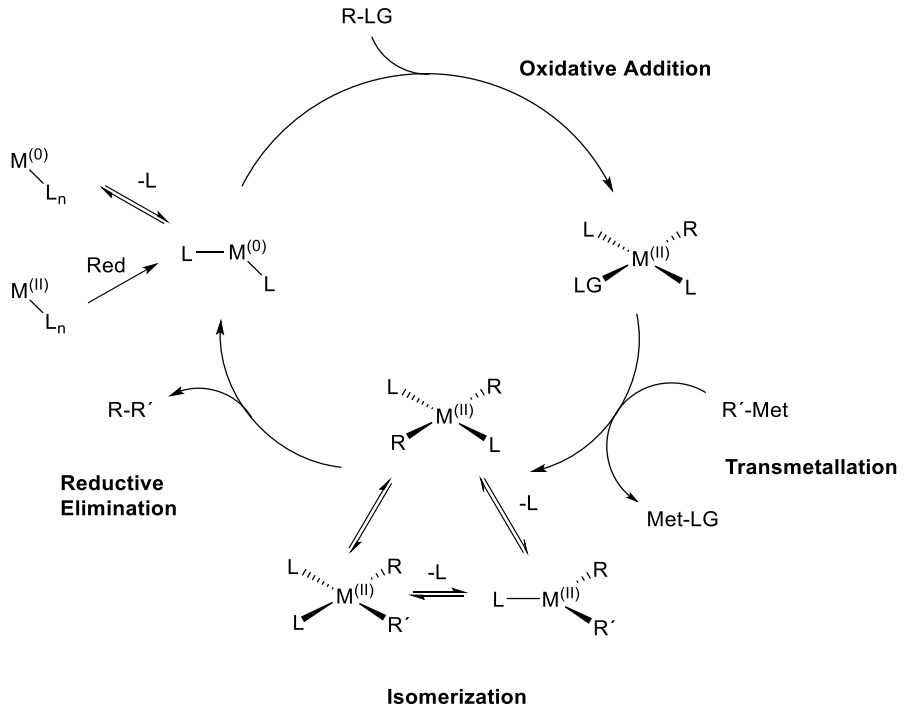
Transition metal catalyzed cross-coupling reactions had an enormous impact on C-C bond formation in organic synthesis which was rewarded with the noble prize in chemistry 2010.[1] Mild reaction conditions, high functional group tolerance and facile accessability of starting materials led to the tremendous impact of metal catalyzed cross coupling reactions. The coupling between organometals (R^1M) and organic electrophiles (R^2X) is undoubtedly one of the most straightforward methods for the formation of C-C bonds (Scheme B-1).



Scheme B-1 General concept of cross coupling reactions

However, the development of metal assisted cross-coupling procedures and their function in a stable manner was a long process. In the early development stage, the scope of cross-coupling regarding the organo-metal species was largely limited to Mg- and Li-organyls. Organometals containing these metals undergo cross-couplings only with certain relatively unhindered alkyl halides. In 1941, Kharasch and Fields cross-coupled an arylhalide with Grignard reagents using metal salts ($CoCl_2$, $CuCl_2$, $MnCl_2$, $NiCl_2$ and $FeCl_3$) as catalysts and thereby invented the first transition metal-catalyzed cross-coupling reaction[2]. Unfortunately, several side reactions including homo coupling occurred. Almost 30 years later, Tamao et al.[3] as well as Corriu and Masse[4] independently reported that Ni complexes, especially Ni-phosphine complexes are suitable catalysts for the reaction of Grignard reagents with alkenyl or aryl halides. Caused by the basicity and high nucleophilicity of Grignard reagents, they do not tolerate many functional groups. Thus, the scientific community was interested in developing milder alternatives. Hence, Murahashi and coworkers[5], Negishi and coworkers[6] and Fauvarque and Jutand[7] reported independently their investiagtions of Pd-catalyzed cross-coupling reactions. Due to moderately large atomic size of this transition metal a certain stability of organopalladiums is observed. Further, the preference for the 0 and +2 oxidation states separated by a narrow energy gap leads to a lower probability of one-electron or radical processes (compared to Ni) and thereby side reactions are limited to a certain extent. Moreover, a “ready to use” and reversible two-electron oxidation & reduction occurs, which is obviously fundamental for the catalytic activity. The relatively high electronegativity of Pd results in a high chemoselectivity. By the replacement of Mg by Al in the Kumada-Tamao-Corriu reaction, it was demonstrated that not only Grignard reagents are suitable organometallic species, but also metals other than Mg have the potential to participate in the transmetallation step[3-4]. Finally, several alternative organometallic coupling partners were investigated out of which the prominent coupling protocols involving zinc (Negishi)[8], tin (Stille)[9], boron (Suzuki)[10] and silicon (Hiyama)[11] emerged. The “classical” cross coupling methods were applied in a variety of synthetic sequences with outstanding success. However, these protocols suffer from certain limitations. In general, they follow the same reaction mechanism, which requires on the one hand an organic (pseudo)halide and on the other hand an organometallic species. Therefore, in reaction sequences where more than one halide is placed on a reaction partner, selectivity issues can occur.[12] This is even more problematically, since the trend in cross-coupling reactions goes into the direction of carrying out multiple cross-coupling steps in a one pot fashion.[13] The Kumada group was one of the first groups having a deeper look into the mechanism of cross coupling procedures and thereby proposed the widely adaptable three-stage catalytic cycle including oxidative addition, transmetallation and reductive elimination, which is fundamental for the general understanding of this reaction type[14].

The three-stage catalytic cycle published by the Kumada group in 1972 was in the following studied more intensively and the importance of the involved ligands was implemented (Scheme B-2), even if the initially proposed catalytic cycle is still valid.

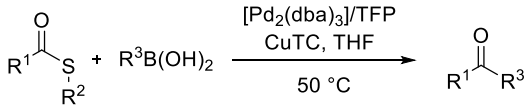


Scheme B-2 Revised version of the catalytic cycle for metal catalyzed cross coupling reactions

The main steps of the revised catalytic cycle remain unchanged. Thus, the initial step of the catalytic cycle is the oxidative addition, in which the coordinatively unsaturated zero-valent catalyst can be generated via either reduction of the divalent metal or via ligand loss and thus it is able to undergo the oxidative addition into the C-LG bond. The relative rate of the oxidative addition into the C-LG bond is largely dependent on bond strenghts which leads to the following order of reactivity: I > Br, OTf>>Cl>>F. Besides the leaving group, also the organic part of the C-LG coupling partner has a large influence on the rate of oxidative addition. The most reactive substituents are vinyl halides followed by aryl halides. In contrast, alkyl halides (sp^3 carbon) react very slowly and competing β-hydride elimination can occur. The rate of the transmetallation step is highly dependent on the applied organometallic species and the present reaction conditions. For Grignard reagents the transmetallation is extremely fast (for both Ni and Pd) but with less reactive metals (B, Si, Sn, Zn) it can be the rate determining step[15]. The reductive elimination as final step of the cycle regenerates the active catalyst and forms the coupling product. Since transmetallation gives the thermodynamically favoured *trans*-substituted intermediate and reductive elimination only takes place when the two coupling partners are in *cis*-configuration, the transmetallation step has to be followed by either isomerization or ligand dissociation.

B.I.1 Liebeskind-Srogl cross coupling reaction

Transition-metal-catalyzed C-C bond generating reactions have revolutionized the field of organic synthesis in the last two decades.[16] Classically, cross-coupling procedures usually involve the reaction of a nucleophilic organometallic reagent with an electrophilic organohalide (or related analogues). But also organosulfur compounds have been reported as potential electrophilic coupling partners. Although the oxidative addition of organosulfur compounds to a low-valent transition metal species has been extensively investigated, the key to catalytic turnover with organosulfur species is the activation of the very stable bond that is formed between the catalytically active metal and the soft sulfur atom. [17] Considering a variety of biochemical transformations, the selective activation of a stable carbon-sulfur bond is achieved in the presence of a great diversity of oxygen and nitrogen heteroatoms. To undestand Nature's use of biologically relevant thiophilic metals (e.g. Ni) in order to achieve selective disconnection of the carbon-sulfur bond in water, the concept of hard-soft acids and bases must be applied. As a consequence of these inspiring features, the scientific community is interested in laboratory-based synthetic procedures that comprise metal-catalyzed carbon-sulfur bond cleavage that lead to new C-C bond formation under mild conditions.[18] Moreover, cross coupling protocols involving boronic acids as coupling partners are prominent, due to their stability against air and moisture and their low toxicity. Moreover, boronic acids are commercially available in a broad variety.[16] In 2000, Liebeskind and Srogl took advantage of these key features, and reported the first example of a palladium-catalyzed, copper-mediated cross coupling between thioesters and boronic acids under base-free conditions.[18] This mechanistically unprecedented Pd catalyzed cross-coupling protocol leads to the formation of ketones from thioesters and boronic acids under neutral conditions (Scheme B-3).

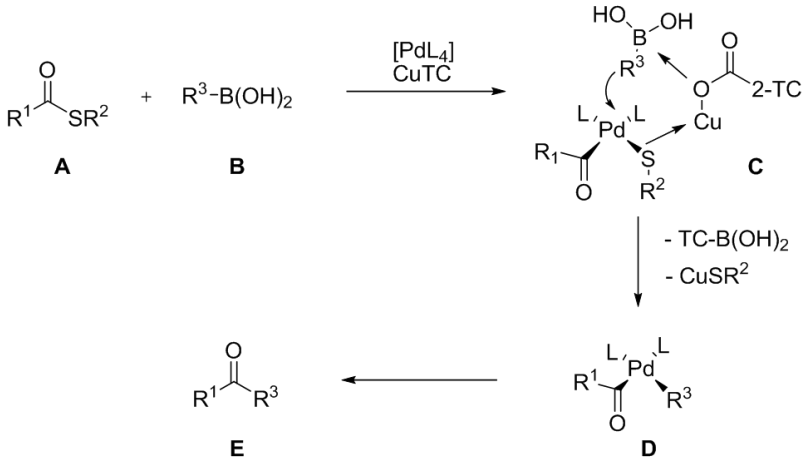


R^1, R^2 = alkyl, aryl
R^3=aryl, styryl

Scheme B-3 Initially published Liebeskind Srogl coupling[18]

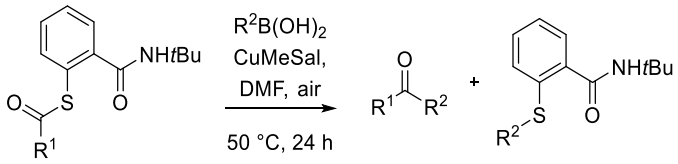
One of the main characteristics of this reaction is the requirement of a stoichiometric amount of a Cu^I-carboxylate species as a metal co-cataylst. The replacement by simple Cu^I sources such as Cu^I halides is not effective.

Best results were achieved, when Cu^{I} reagents like copper(I) thiophene-2-carboxylate (CuTC) and copper(I) 3-methylsalicylate (CuMeSal) were applied as cofactor. The proposed mechanism for the classical palladium catalyzed, Cu^{I}-mediated desulfitative cross-coupling is presented in Scheme B-4.[19]



Scheme B-4 Mechanism of the Liebeskind Srogl coupling [19]

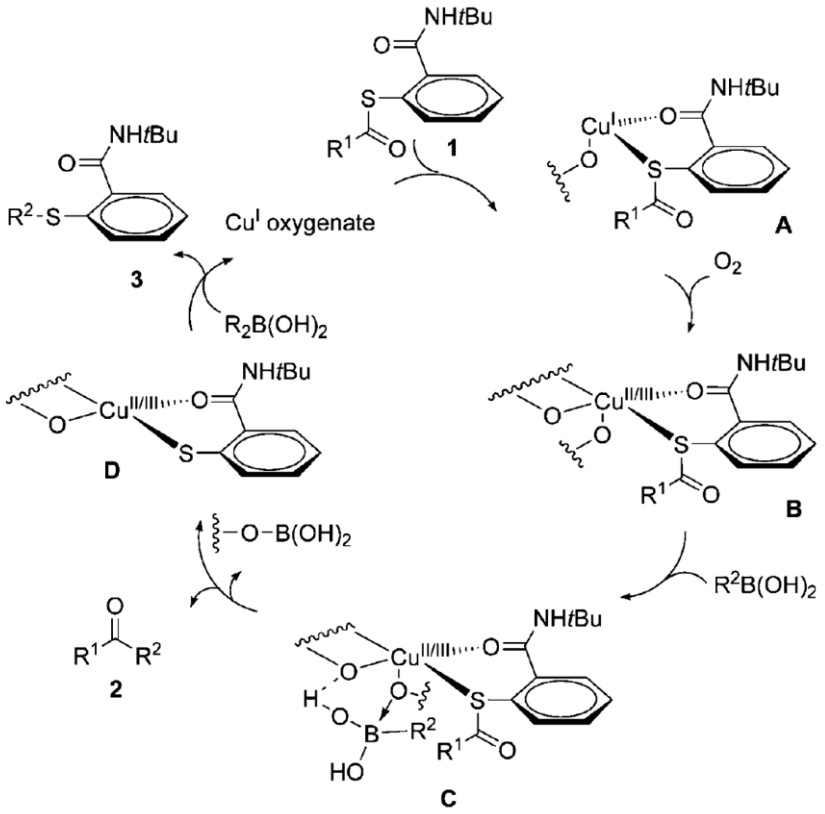
The first step is the oxidative addition of thioester **A** to the Pd^{0} catalyst. The copper(I) carboxylate polarizes the Pd-S bond through Cu^{I} coordination to the sulfur center. Simultaneously, the carboxylate coordinates to the boron, activating the trivalent boron compound. The nature of the hydrogen-bonded ternary complex **C** as reaction intermediate was confirmed.[20] A full equivalent of the Cu^{I} additive is required because it is needed as a scavenger for the thiolate released in the reaction. This unique, base free coupling protocol tolerates the involvement of base-sensitive starting materials and products and further it can even be utilized in the presence of Suzuki-active bromides. A successful application of the Liebeskind-Srogl coupling was demonstrated on a variety of thioorganic starting materials, like peptidylthiol esters,[21] heteroaromatic thioethers,[22] benzylthiocyanates,[23] thioalkynes,[24] bis(arylthiobutenediones),[25] methyl thiopseudourea derivatives[26] and cyclic thioamides [27] with either boronic acids or organostannanes. In 2002 Liebeskind and Srogl disclosed, that also π-deficient heteroaromatic thioethers (at a carbon center of oxidation stage +III) can undergo C-C bond formation under the standard conditions initially developed for thioesters.[28] This made the reaction a valuable tool for the decoration of heterocyclic compounds. Further, the synthesis of natural products (-)-d-*erythro*-sphingosine[29] and litseaverticillol B[30] was conducted via this ketone generating protocol. In 2007 Villalobos, Srogl, and Liebeskind described a mechanistically unique Cu-catalyzed coupling of thioesters and boronic acids under air in the absence of a Pd catalyst employing CuMeSal (Scheme B-5).[31]



R^1= Ph, *p*-tolyl, Me, *n*-propyl,ω–decycnyl, (*E*)-propenyl
R^2= alkyl, (hetero)aryl

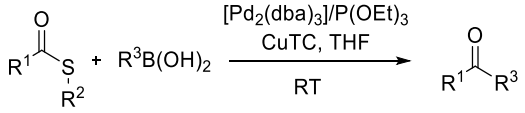
Scheme B-5 Cu catalyzed, aerobic coupling of boronic acids and thioesters

So far, in all coupling procedures applying boronic acids and thioesters as starting material catalytic quantities of Pd and a stoichiometric amount of Cu^{I} carboxylate was obligatory. Further, they were conducted under strictly anaerobic conditions. Contrary, this novel Cu-catalyzed protocol depends only on an active Cu species without any Pd source. The lack of Pd in the catalytic sequence and the absence of a precedent for oxidative addition of the thioester to Cu^{I} point to a formally related, but mechanistically unique C-C bond formation. A variety of investigations into the scope of this reaction have revealed that only thioesters, providing an appropriately positioned ligating *S*-pendant group generate the respective ketone efficiently. *S*-aryl-NH*t*Bu thiosalicylamides proved to be suitable reaction partners because of the steric bulk and favorable spatial orientation of the *S*-pendant group. The scope of this protocol has been studied by employing different *S*-aryl-NH*t*Bu thiosalicylamides and 2.5 equivalents of a boronic acid in the presence of 5 mol% CuMeSal in DMF at 50°C under air. Aromatic, aliphatic, and α,β-unsaturated thioesters were successfully coupled with aromatic, heteroaromatic, and alkenylboronic acids. In all cases, the *S*-arylated byproduct and the desired ketone were generated in approximately a 1:1 ratio. All Cu^{I} sources effectivly initiated the reaction regardless of the counterion in even catalytic amounts. In the case of Cu^{II} sources, only those bearing an oxygenate counterion (carboxylate or diphenylphosphinate) were able to initiate and support the aerobic reaction. It was suggested, that Cu^{I} must be disposable for efficient catalysis and thereby the in situ reduction of Cu^{II} to the active Cu^{I} by the boronic acid is required, which is facile in the presence of an oxygenate counterion, but not in the presence of a halide counterion. For regeneration of Cu^{I} a second equivalent of boronic acid must be added to cleave the strong Cu-S bond in the initially formed Cu–thiolate species, and to regenerate the key Cu–oxygenate species. In contrast to the intensively studied oxidative addition/transmetallation/reductive elimination pathway in transition-metal catalysis, this reaction obviously follows a novel mechanism templated by a higher oxidation state Cu species (Scheme B-6).[16]



Scheme B-6 Mechanism of the Cu catalyzed coupling of boronic acids and thioesters (adapted from Prokopcova et al.)[16]

Based on the knowledge of Cu^{I} dioxygen reactions, the authors proposed an initial aerobic activation of the Cu^{I}-coordinated thioester **A** to a higher oxidation state $Cu^{II/III}$ intermediate **B**. The nucleophilic R^2 moiety is directed towards the thioester by on the one hand Lewis acid activation of the thioester by coordination to Cu and on the other hand the activation of the trivalent boron by coordination of the oxygenate species to the boron center (**C**). Thus, the desired ketone and higher oxidation $Cu^{II/III}$-thiolate **D** was generated. Finally, the weakly coordinating thioether (byproduct) is formed via the reaction of $Cu^{II/III}$-thiolate **D** and the second equivalent of the boronic acid. Thereby, the key Cu^{I}–oxygenate species is generated and is accessible for the re-entry in the catalytic cycle. Further, Yang et al. developed a coupling procedure for thioesters and boronic acids at room temperature.[21, 29] Clearly, this protocol (Scheme B-7) was of special interest for us with regard to an ambient reaction temperature in a potential chemoenzymatic one pot process.

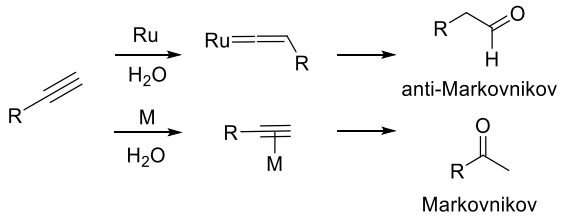


Scheme B-7 Liebeskind Srogl coupling at room temperature[29]

Via Liebeskind-Srogl coupling both a C-C bond is formed and a novel functional group is created without the addition of a base, like in many other coupling protocols. These characteristics and the creation of a highly interesting new functionality regarding a biocatalytic follow-up step gave rise to the idea of applying the Liebeskind Srogl coupling as a first step in a chemoenzymatic reaction cascade.

B.II Gold catalyzed hydration of alkynes

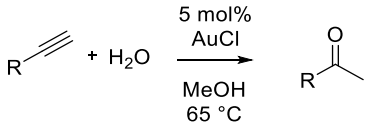
In various transformations such as enyne cyclization, polymerization and metathesis reactions, metal complexes of alkynes are essential intermediates. The classical Dewar–Chatt–Duncanson model[32] describes the bonding between the metal and the alkyne as a combination of a σ- and a π-interaction. [33] Moreover, σ donation and π acceptance are outlined as having a synergistic bonding effect.[34] In the case of late transition metals, such as Hg, Cu, Pd, Pt, Au, etc., a more important σ -contribution to the bond with almost no back-donation is observed. Thus, the electron density on the alkyne is minimized and thereby its susceptibility to a nucleophilic attack is increased. This reactivity mode has been intensively exploited over the pastdecades. Alkynes have been reported to undergo the addition of C, N, S and O nucleophiles via π-activation with transition metals. An excellent way to obtain access to aldehydes, ketones or the respective acetals, is the nucleophilic addition of water or alcohols to alkynes, according to a Markownikoff or anti-Markownikoff pathway.[33] When Ru(II) as catalytic species is used, the reaction favours the anti-Markownikoff manner, which is demonstrated in Scheme B-8.[35]



Scheme B-8 Metal catalyzed hydration of alkynes (adapted from Mokar et al.) [35]

One of the most valuable functionalization of unactivated alkynes is displayed by a hydration reaction to the respective ketone, which is often connected with a large access of acidic reagent.[36] However, a multitude of catalysts for this hydration reaction has been investigated. The “classical” catalytic system employs toxic mercury (II) salts in acidic media. [37] Naturally, these mercury salts had to be replaced, first because of its toxicity, but also the rapid formation of the reduced Hg (0) species, which is catalytically inactive, was a problem. Metals like Ag(I), Cu(I), Tl(III), Pd(II), Ru(III), Rh(III) and Os(II) were investigated, but the studied reactions suffered from a limited scope. In contrast, Pt(II), Au(I) and Au (III) showed a highly promising profile with regard to scope, selectivity and catalytic activity. Caused by the strong relativistic effects affecting the coordination behavior, the most efficient catalysts are based on gold.[33] Moreover, gold chemistry gained enhanced attention in the last years, due to the unpredicted properties of the supported metal in the size of nanoparticles and in general due to the reactivity characteristics.

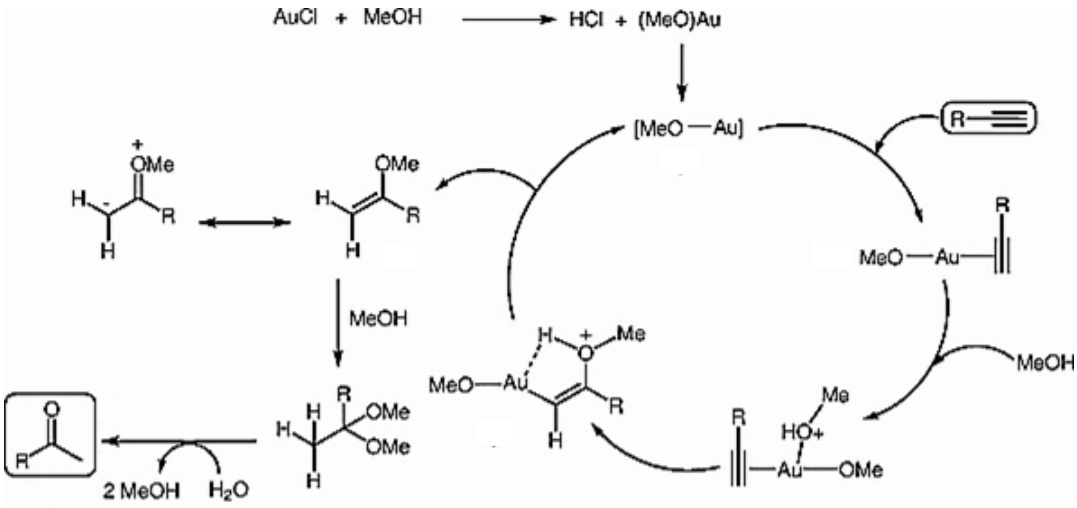
More specifically, gold shows a high affinity for alkynes as Lewis acid.[38] Further, the "alkynephilicity" of gold resembles that of mercury, which results from the common electronic properties of these two elements.[38] In general, good yields were reported when gold(I) catalysts were combined with strong acids under increased temperatures.[38-39] Further, procedures for the hydration of alkynes at room temperature [40] in eg. CH_3CN [41] or by application of gold isocyanide complexes [42] were developed. Moreover, the reaction was efficiently performed in aqueous media without using any organic solvents. Therefore, a novel bifunctional catalyst with Au nanoparticles covalently bonded to HS/SO_3H functionalized periodic mesoporous organosilica and a temperauture of 80 °C was needed.[43] In 2015 Das et al. demonstrated for the first time a ligand- and acid-free procedure for the synthesis of methylketones via hydration of terminal alkynes, which is catalyzed by gold(I) chloride. The presented method produces the respective Markovnikov ketones without any acid promoters or additives in moderate to high yields (Scheme B-9). This atom-economical and operationally easy procedure is extremely straightforward, inexpensive, environmentaly benign and provides an excellent functional group tolerance. Both aliphatic and aromatic alkynes, including functional groups such as alkoxy, chloro, bromo, fluoro, hydroxy, and tertiary amine were converted into the respective ketones.[36]



Scheme B-9 Goldchloride catalyzed hydration of alkynes[36]

The properties of the reaction medium significantly influence this reaction. MeOH was exchanged by EtOH and *i*-PrOH for the hydration of phenylacetylene. The conversion of 98 % by applying the standard conditions droped to 88% (EtOH) or 72% (*i*-PrOH). Further, several other solvent systems were tested.[36] In terms of a subsequent chemoenzymatic cascade reaction, this procedure shows excellent prerequisite. The first example of a gold(III) catalyzed transformation of unactivated alkynes into ketones was demonstrated in 1991 by Fukuda and Utimoto[44]. The treatment of terminal alkynes with 2 mol% of sodium tetrachloroaurate in aqeous methanol led to the formation of methyl ketones in excellent yields. Due to limited regioselectivity, the hydration of internal alkynes resulted in a mixture of ketones. Further, by using 2 eqivalents of MeOH without the addition of water to the reaction, the Utimoto group achieved direct formation of dimethyl acetals from alkynes under appropriate reaction conditions.[37] In 2003 Casado et al. proposed that the gold(III)-catalyzed hydration of alkynes occurred by the direct attack of water.[45]

Based on theoretical calculation, Lein et al. reported in 2010 for the first time the complete mechanism of the gold(III) catalyzed nucleophilic addition of water to an unactivated triple bond. Moreover, it was claimed that the reaction is not only catalyzed by gold(III) but also catalyzed by water at the same time.[46] However, Das et al. performed the reaction in large excess of water and in pure water and did not observe any product formation. This observation led to the disqualification of a direct attack by water to the alkyne moiety, irrespective of wether gold(I) or gold(III) serves as catalyst. Ketone formation was only observed applying alcoholic solvents and thus the mechanism presented in Scheme B-10 was proposed.[36]



Scheme B-10 Proposed mechanism for the hydration of alkynes (adapted from Das et al.) [36]

Catalyst bearing gold (III) centers were frequently applied in form of complexes like $BzPPh_3[Au(NHC_{mes})Cl_3]$ and $[Au(NHC_{IPr})Br_3]$ or salts (e.g. $NaAuCl_4$).[36] This can be explained by the fact that the efficiency of Au-catalysts seems to be enhanced by the addition of appropriate ligands, which thereby enabled a minimized loading of the precious catalyst.[47] However, especially Au(III) catalysts are interesting, since they provide catalytic properties unseen in other metals.[46] Further, this oxidation state is the most stable one, which is highly beneficial regarding the core idea of a chemoenzymatic one pot process. Moreover, as evaluated by in vitro cytotoxicity assays, gold ions are found to be less toxic or nontoxic toward cultured cells.[48]

B.III Biotransformations in organic chemistry

For centuries, chemists were inspired by the fact, that living organisms have the remarkable ability to convert simple starting materials to complex molecules. Due to the emerging field of biocatalysis, researchers now have the oportunity to mimic synthetic strategies that were so far only used by nature efficiently.[49] Whereas the development of new processes is the focus of attention, surprisingly less attention has been put on the combination of synthetic methodologies.[50] Undoubtly, biocatalysis is one of the greenest technologies for the synthesis of chiral molecules due to exquisite regioselectivity and stereoselectivity in water under extremely mild conditions. Moreover, protection and deprotection of functional groups is in general not required due to the outstanding chemoselectivity and the mild reaction conditions.[51] The reactions run under physiological temperatures and of course the use of organic solvents is drastically minimized.

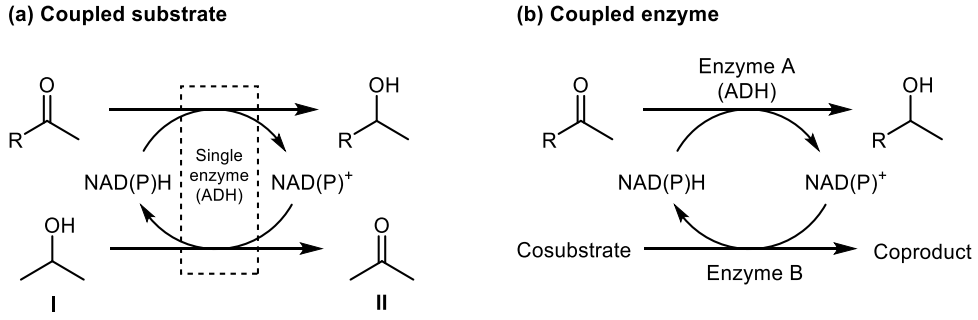
To truly empower the “green aspect” of biocatalysis, it is essential to integrate enzymatic transformations into chemical transformations on a retrosynthetic level.[51] The “natural synthetic strategy”, is based on three main aspects: the application of enzymes as catalysts, the compartmentalization of biocatalysts in cellular organells and the sequential application of enzymes in cascade reactions, represented by biosynthetic pathways. The field of biocatalysis uses the compartmentalization strategy as observed in living beings by applying different immobilization strategies.[49] Nowadays, novel chemoenzymatic routes are investigated to meet process requirements on cost-efficiency, waste reduction, energy consumption and throughput on the basis of the requirements for an optimal synthetic pathway. This involves a clear improvement towards the previous strategy of designing synthetic pathways around limited tool boxes of biocatalysts.[51] Further, the development of robust directed evolution and novel screening technologies to improve enzyme properties contributed to this development. One of the main advantages of enzymatic catalyzed reactions is a potential rate enhancement of as much as 10^{12}. Further, structurally diverse substrates can undergo biotransformations. Enzymes can be over-expressed and directed evolution can be performed to improve the efficiency and the substrate specificity. And obviously during biocatalytic processes only a small amount of chemical waste is produced, which leads togehther with the physiological conditions to “greener” reaction conditions as mentioned above. Moreover, the waste streams from fermentations are usually suitable for municipal sewers.[52]

In contrast to these beneficial characteristics, there are also some issues regarding the use of biocatalysts in organic chemistry. Especially, isolated enzymes suffer from instability and they require cofactors, as well as their recycling. Moreover, the purchase of specialized equipment for fermentations is cost-intensive and displacing established processes is often connected with difficulties.[52] However, often neglected by synthetic organic chemists beginning their investigations on a biocatalytic process, is to study the biocatalyst itself, whereas an understanding of the exact nature of the catalyst and the optimum way in which the catalyst should be presented to the reaction medium are crucial for the success of the methods.[53] By now, a variety of stable, "easy to use" enzymes can be purchased and used by synthetic organic chemists having no access to a culture collection, microbiology and do not have any fermentation skills. Biocatalysts are even increasingly designed for the application in organic synthesis.[53]

Especially in the context of a potential use of biocatalysts in organic chemistry, it is worth to describe the differences of applying isolated enzymes versus whole-cell transformations. For example commercially available hydrolases and lipases do not require cofactors and thereby reactions can be easily performed with isolated enzymes as long as they are stable in a pure state. Cofactors are non-protein components, such as metal ions or vitamins and are bound to the enzyme and required for biological activity. In contrast, when single or multiple cofactors (in stoichiometric quantities) are required whole-cell fermentations are preferred, since living cells already have all of the necessary cofactors in place as part of their metabolism. Further, a whole cell system provides enhanced enzyme stability due to the additional "barrier" of the cell wall. Of course, also problems can come up using biocatalysts. For example due to the presence of other enzymes, that metabolizes the substrate. Moreover, problems in transporting the substrate or product in and out of the cell are sometimes observed, or the substrate or product may be toxic to cells at increased concentrations in the range of organic reactions.[52] Especially in large-scale industrial preparation, the use of immobilized enzymes is advantageous. An immobilized enzyme is usually more active as well as more stable and the space-time yields of optimized reactions tend to be higher.[52] As an outlook into the future, the development of novel biocatalytic chemical and pharmaceutical processes, which might replace chemical routes with poorer process efficiency and higher manufacturing costs will gain tremendous attention.[51]

B.IV Alcohol dehydrogenases

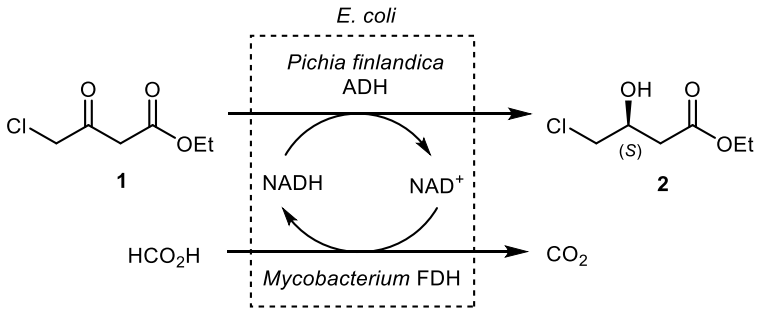
Chiral alcohols serve as valuable building blocks for the synthesis of many pharmaceuticals and agrochemicals.[54] The enzyme catalyzed synthesis can be realized starting from prochiral carbonyl compounds by asymmetric reduction using NAD^+- or $NADP^+$- dependent alcohol dehydrogenases.[55] Outstanding enantioselectivities in combination with an easily available (bio)catalyst and excellent productivity explains the growing appeal of alcohol dehydrogenases as biocatalysts for large scale applications.[56] Further, the transformation of ketones to the corresponding alcohols and vice versa is one of the most common redox-reactions in organic chemistry. Whereas traditional synthetic methods mainly use metals and cost-intensive complex hydrides, biotransformations offer diverse advantages.[57] As mentioned above, the majority of dehydrogenases and reductases used for the reduction of ketones and oxidation of alcohols require nicotinamide cofactors, such as NADH and NADPH. For the recycling of the nicotinamide cofactor two strategies are generally applied (Scheme B-11).



Scheme B-11 Strategies for nicotinamide-cofactor-recycling (adapted from Kroutil et al.) [57]

The most simple and sophisticated system employs a single enzyme, which simultaneously transforms substrate and co-substrate (Scheme B-11 a). To drive the equilibrium towards the direction of the product, the co-substrate must be utilized in large excess. Consequently, the "coupled-substrate" approach is frequently hindered by cosubstrate-inhibition. Thus, the main requirement for efficient, large scale coupled-substrate systems is the availability of chemo-stable dehydrogenases. Moreover, cofactor-recycling can be performed by using a second enzymatic reaction, which provides a more complex "coupled-enzyme" approach (Scheme B-11 b). This second enzymatic reaction is preferably irreversible. The coupled-substrate system is beneficial, because it can be used in the oxidation and reduction mode, whereas separate cofactor-recycling systems are required for the coupled-enzyme system. A very common regeneration system for NADH is

demonstrated by the application of formate dehydrogenase (FDH) and formate as hydrogen donor. By using these two enzymes not in an isolated form, but overexpressed in a single host, a much more elegant approach was displayed by Matsuyama et al., who demonstrated this for *Pichia finlandica* ADH and *Mycobacterium* FDH (Scheme A-12).[58] In general, the development of so called "designerbugs" (whole-cell organisms co-expressing all required enzymes) gains enourmous attention nowadays.



Scheme B-12 Coupled enzyme approach using ADH and FDH in E. coli (adapted from Kroutil et al.)[59]

The employment of *Escherichia coli* as host is very common and it is in general the most prominent example for microbial strains used for biocatalysis (Figure B-2). *E. coli* can be seen as one of the workhorses in biotechnology and the "standard" bacterium used for genetic engineering. The diameter of the *E. coli* cells varies between 0.5 and 1.5 µm.[59]

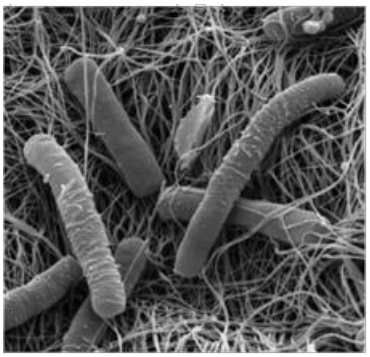


Figure B-2 *Escherichia coli*, the most important microbial strain in biocatalysis[60]

With regard to a potential previous metal assisted step, single enzyme systems, which are able to perform the substrate transformation and cofactor recycling simultaneously, are obviously enormously beneficial due to their plainness.

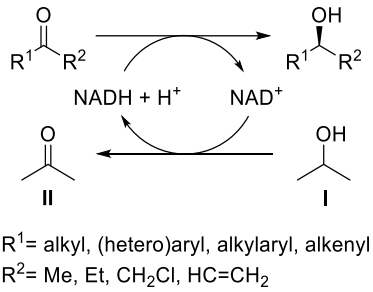
Moreover, the application of *i*-PrOH in access is not only important to shift the equilibrium to the product side, but it also enhances the solubility of lipophilic substrates in aqueous medium. As a consequence, the maximum tolerable concentration of *i*-PrOH applied for cofactor regeneration was constantly increased to maximize the productivity of such coupled-substrate approaches even more. This obviously requires extremely chemo-stable enzyme preparations.[57]

One of the most popular biocatalysts is baker´s yeast (*Saccharomyces cerevisiae*), which was investigated along this line for almost a century. The most important application of baker's yeast in organic synthesis is the asymmetric reduction of ketones. Due to the required nicotinamide cofactors (usually NADPH), the use of whole cells in comparison to isolated enzymes is preferred to avoid the need for enzyme purification and cofactor regeneration.[61] Notably, a NADPH-dependent reductase from baker´s yeast was one of the first biocatalysts used for the asymmetric reduction of carbonyl compounds, which led to alcohols of high enantiomeric purities (>98% *ee*).[62] Further, reductases from baker´s yeast have been used to reduce a β-keto-ester substituted by a secondary alkyl group at the alpha position. The corresponding β-hydroxy ester having three consecutive chiral centers was obtained in excellent stereoselectivity.[63] The Stewart group presented in 2004 a systematic investigation of carbonyl reduction catalyzing *Saccharomyces cerevisiae* enzymes. The reductases from baker´s yeast have been over produced in *Escherichia coli* as gluthathione S-transferase fusion proteins. A set of α- and β-keto esters were tested as substrates for each purified fusion protein. Both, the enzyme and the substrate structure had influence on the stereoselectivities of β-keto ester reductions. Also, sets of enantiocomplementary enzymes were discovered with largely overlapping substrate profiles, hence, enabling access to L- and D-alcohols with high stereoselectivities.[64] In general, individual yeast ketone reductases deliver the respective alcohols with very high stereoselectivities and most are specific for NADPH.[61] However, for our purpose alcohol dehydrogenases that can be used via a coupled substrate approach are clearly more attractive, since only the requirements of one single enzyme has to be met. Further, more potent enzymes were developed, which are most suitable for chemoenzymatic processes.

B.IV.1 (*S*)-Selective alcohol dehydrogenase "A" from *Rhodococcus ruber*

In 2003 a novel secondary alcohol dehydrogenase (ADH-A) with outstanding solvent and temperature stability from *Rhodococcus ruber* DSM 14855 was purified and characterized by the Faber group.[65] Due to the special characteristics, this biocatalyst appears to be highly interesting for industrial applications. Also today, many identified alcohol dehydrogenases require a second ADH or otherwise a set-up for co-factor recycling.

However, the simultaneous recycling of the co-factor and the reduction of the desired ketone by a single enzyme in the coupled substrate approach is only applicable with a few alcohol dehydrogenases. In the case of ADH-A, both reactions are catalyzed by the single enzyme in a hydrogen transfer like fashion. Another important feature of this biocatalyst is its impressive operational stability in the presence of high concentrations of organic compounds, for instance it is active in *i*-PrOH up to 80% v/v. This enables an effective shift of the equilibrium towards the product side, and further enhances the substrate solubility of more hydrophobic ketone substrates.[66] Further, the tolerance against co-solvents, primarily acetone and isopropanol, which act as co-substrates for ADH activity in the reductive and oxidative directions, respectively, serves for in situ recycling of the requisite nicotinamide cofactor.[67] In comparison to the wild type, lyophilized cells of *E. coli* containing the overexpressed ADH-A provided an outstanding enantioselectivity. Selected substrates, which were initially converted by the wild type-catalyst (WT) with low stereoselectivity, were converted by the partially purified ADH-A. Thereby, for instance enantiopure (S)-β-tetralol with > 99% *ee* was produced, which gave 83 % *ee* applying the wild type. This clearly indicates that further alcohol dehydrogenases competed for this substrate in the WT. In general, various building blocks that were not accessible by the wild type catalyst were now obtained in > 99% *ee*.[66] Further, the applicability of overexpressed ADH-A was investigated for reduction of a set of ketones (Scheme B-13) employing nonconventional aqueous-organic solvent systems like mono- and biphasic aqueous-organic solvent systems (50% v/v) as well as micro-aqueous organic systems (99% v/v). The extremely robust biocatalyst efficiently functions in micro-aqueous media composed of 99% of a very hydrophobic organic solvent and 1 % of buffer. The highest biocompatibility was found for hydrophobic solvents (log *P* > 2), such as toluene, cyclohexane and hexane. By using the micro-aqueous organic system high substrate loadings of up to 2.0 M were successfully converted.[68]



Scheme B-13 Biotransformations in high concentrations of organic solvent (adapted from Karabec et al.)[67]

The enzyme was purified from the wild-type strain of *Rhodococcus*, and found to be a protein with a monomer molecular weight of 38 kDa, containing one zinc atom per monomer. To gain more precise information about the connection of structure, substrate specifity and solvent tolerance of this ADH Karabec et al. conducted studies into its three-dimensional structure [67]. The solution of the structure has been solved from data collected on crystals grown at both pH 5.5 and pH 8.5, refined to resolutions of 2.0 and 2.8 Å respectively.The structure was in general similar in fold to other homotetrameric ADHs (Figure B-3), showing a strong similarity with enzymes from thermophilic organisms.

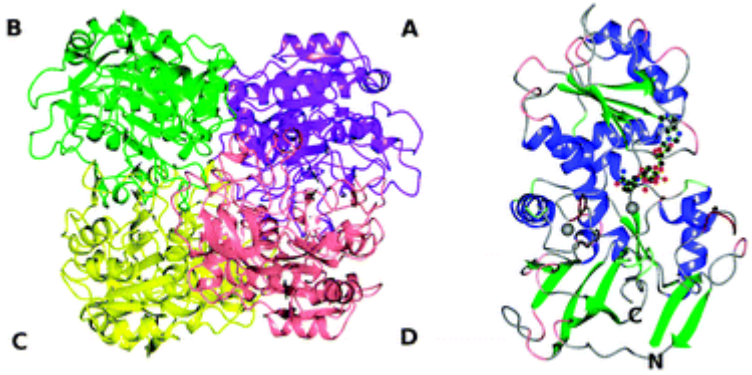


Figure B-3 Ribbon structure of ADH-A
Left: Structure of homotetramer presenting subunits A–D in purple (A), green (B), yellow (C) and coral (D).
Right: Monomer of ADH-A showing the cofactor NAD^+ in ball-and-stick representation. (adapted from Karabec et al.) [67]

Each subunit contained one structural zinc atom coordinated by a four-cysteine cluster, and additional zinc at the active site. This additional zinc was closely associated with the cofactor NAD^+ in the case of both structures. Furthermore, the substrate 2-octanone was docked into the active site using Autodock. In the two clusters with the lowest energy the carbonyl oxygen atom of the ketone was bound to the Zn ion (Figure B-4). Amino acids and the docked 2-octanone are shown in pink and green respectively, the cofactor in yellow.

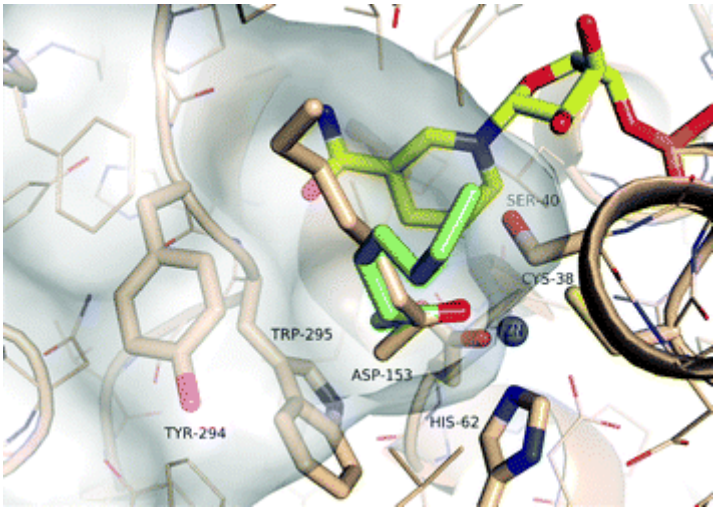


Figure B-4 2-Octanone docked into active site showing a comparison of representative binding modes from the two lowest energy docking clusters. (Active site pocket is shown in a semi-transparent surface representation) (adapted from Karabec et al.) [67]

The carbonyl carbon atom is in close proximity to the C4-atom of the cofactor. Thus, the hydride would be transferred onto the *re*-face of the carbonyl group, which is consistent with the (*S*)-selectivity of ADH-A. Moreover, the methyl group of the substrate interacts with the indole moiety of Trp-295, which might be the reason for the lower activity of the enzyme towards bulky-bulky ketones due to the apparent steric hindrance.[67] Previous protein engineering studies have suggested that the protein's stability in organic solvents might be defined by the proportion of hydrophobic residues at its surface and also the number of stabilizing interactions in the interior of the protein.[69] Karabec et al. thus compared the structure of ADH-A with the structures of structural related, known ADHs with a lower solvent tolerance. For this purpose the ADH from *Thermoanaerobium brockii* (Tb-ADH, 1YKF)[70] was selected. This ADH has been studied intensively, is commercially available and has already been subject of reports on its solvent stability. The resulting comparative analysis of the structures of ADH-A and Tb-ADH by the program PISA[71] displays that the Tb-ADH tetramer appears to be more closely packed than ADH-A. Further it has a larger surface area and more hydrogen bonds at the A–B or C–D dimer interface. Thereby, the surface of its tetramer is less hydrophobic. A superimposition of the structures also discloses some exposed loops in the region of Tb-ADH 321-326 where the ADH-A structure is more compact (Figure B-5).

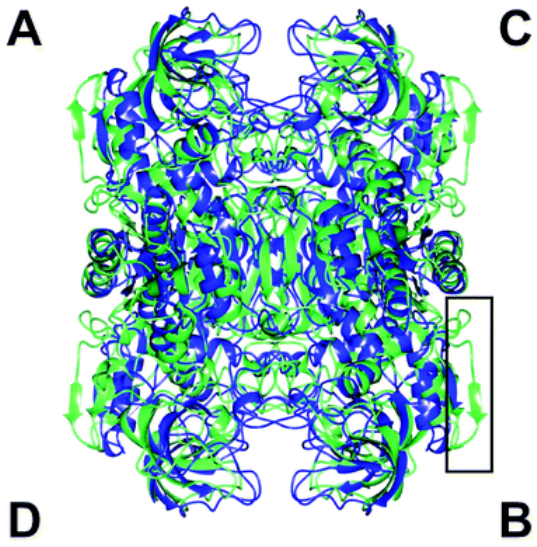


Figure B-5 Superimposition of ribbon structure of homotetramer of ADH-A (3JV7, blue) with alcohol dehydrogenase from T. brockii (Tb ADH, 1YKF, green). Exposed surface loops between residues 321–326 in each monomer of Tb-ADH are highlighted by the black box in monomer B. (adapted from Karabec et al.) [67]

Impressively, the major dimer interfaces between A–B and C–D in ADH-A, are each connected by ten salt bridges, whereas the equivalent dimer interfaces in Tb-ADH dimers have none. These investigations provide some of the first insights into the possible molecular basis for the remarkable chemotolerance of this ADH. Therefore, this ADH provides perfect characteristics for the combination with the classical chemical step of the envisaged cascade. Further, a variation of the enantioselectivity of the ADH would be of interest, to demonstrate the versatility of the chemoenzymatic process.

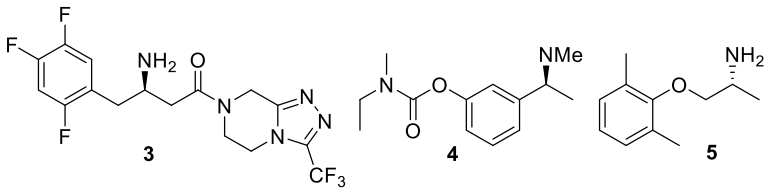
B.IV.2 (*R*)-Selective alcohol dehydrogenase from *Lactobacillus kefir*

A very early example of the practical application of an alcohol dehydrogenase as catalyst for organic synthesis was demonstrated by Hummel in 1990. Acetophenone was converted enantioselectively to (*R*)-phenylethanol by a novel (*R*)-selective alcohol dehydrogenase, found in a strain of *Lactobacillus kefir.* Magnesium ion (Mg^{2+}) addition was found essential for persistant enzyme activity and thus complete inactivation of the enzyme by addition of EDTA was observed. Further investigations delivered the key characteristics of this novel biocatalyst: it shows a pH optimum at 7.0 for the reduction of acetophenone and at 8.0 for the oxidation of phenylethanol and depends strongly on NADPH. Further, the K_m (acetophenone) 0.6 mM and K_m (NADPH) 0.14 mM was determined. A variety of other aromatic and long chained aliphatic secondary alcohols were found to be suitable substrates for this new alcohol dehydrogenase.

For the regeneration of NADPH glucose/glucose dehydrogenase, glucose-6-phosphat/glucose-6-phosphat dehydrogenase and *i*-PrOH were studied and all approaches proved to be functional (79-86 % conversion). Overall, this publication can be seen as a breakthrough in biocatalytic processes delivering chiral alcohols.[72] The batch-wise conversion of acetophenone was conducted with a substrate concentration of 10 mM, using cell free extract in the presence of 0.2 mM NADPH. In 1992 the overall stereochemistry of the enzymatic reduction catalyzed by *L. kefir* alcohol dehydrogenase (LK-ADH) and a wide extension of the substrate profile was demonstrated. A variety of cyclic, aromatic and aliphatic ketones were converted to the respective (*R*)-alcohols with high enantiomeric access (94-99 % *ee*) and good yields, by the transferation of the *pro-R* hydride from the cofactor to the si face of carbonyls. Like most alcohol dehydrogenases studied at that time, substrates bearing a methyl group as one side chain are preferred substrates over other side chains. But the successfull conversion of cyclic substrates implies an important novelty in the field. [73] Further, this biocatalyst was applied for the diastereoselective synthesis of optically active (2*R*, 5*R*)-hexanediol in quantitative yields with an enantiomeric excess and diastereomeric excess of >99 % by Haberland et al.[74] Weckbecker et al. were able to isolate the gene of the ADH produced by *lactobacillus kefir* DSM 20587 and determined the complete nucleotide sequence. The gene includes 759 bp and encodes a protein of 252 amino acids with a calculated molecular weight of 26 781 Dalton. The amino acid sequence suggests a high similarity to short-chain dehydrogenases. After cloning and expression in *Escherichia coli*, purification and characterization was carried out. The stereoselective reduction of aliphatic, aromatic and even β-keto esters was performed with the purified enzyme to prove its preparative applicability.[54]

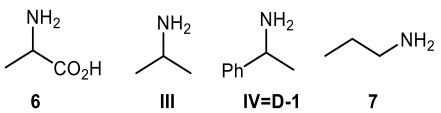
B.V ω-Transaminases

The first research publications dealing with ω-transaminases were published about 50 years ago.[75] However, it took until the end of the last century for this enzyme class to be taken into consideration as attractive biocatalytic species in synthetic applications.[76] As well as chiral alcohols, chiral amines, especially in enatiopure form also represent important chemical building blocks. They are particularly recognized in the pharmaceutical industry for imparting desirable biological activity to chemical entities. Some prominent examples are presented in Scheme B-14. (*R*)-Sitagliptin (**3**) is an antidiabetic drug[77], (*S*)-rivastimine (**4**) is applied in the treatment of dementia[78] and (*R*)-mexiletine (**5**) to treat ventricular arrhythmias.[79]



Scheme B-14 Chiral amine based pharmaceuticals: (*R*)-sitagliptin, (*S*)-rivastigmine, (*R*)-mexiletine (from left to right)

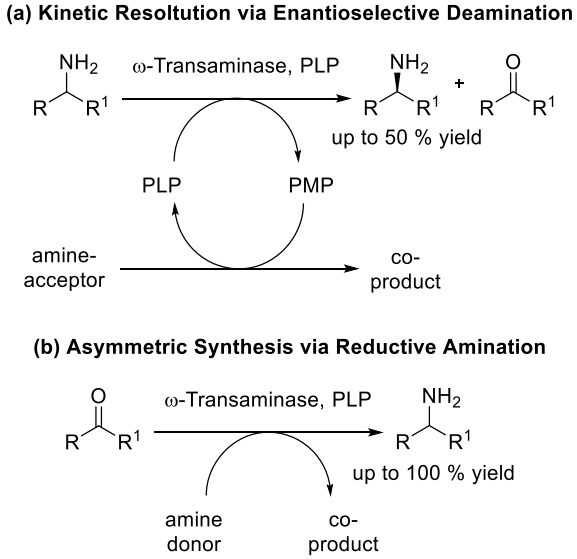
A variety of synthetic strategies to produce chiral amines via biocatalytic as well as chemical transformations have been developed. These days, ω-transaminases (ω -TAs) have attracted growing attention as promising catalyst, giving an environmentally benign access to chiral amines with exquisite stereoselectivity and excellent catalytic turnover.[80] These enzymes catalyze the deamination reaction of an amine donor (Scheme B-15) and the accompanying amination of a ketone or aldehyde (amine acceptor). Thus, during the reaction an amino moiety is transferred between two molecules.[76] Recently, the Turner group presented biocatalytic transaminations with near-stochiometric and inexpensive amine donors.[81]



Scheme B-15 Potential amine donors

In contrast to α-transaminases, ω-transaminases are not limited to α-amino and α-keto acids. In principle, ω-transaminases are able to aminate any ketone or aldehyde functionality. As a consequence, they are also referred to as amine transaminases.[76]

Due to the reversibility of the amination or deamination, ω-transaminases can be applied for the production of optically pure amines in two ways: (a) by kinetic resolution, in which one enantiomer of a racemic amine is transformed into the corresponding ketone, leaving the desired amine enantiomer unaffected (Scheme B-16, a), or (b) in asymmetric synthesis starting from the prochiral ketone (Scheme B-16, b).[76] Asymmetric synthesis is clearly the preferred process, since the product can be obtained in up to 100 % yield in contrast to only 50 % via kinetic resolution.[82]



Scheme B-16 Kinetic resolution (a) and asymmetric synthesis (b) applying ω-transaminases (adaped from Kroutil et al.)[83]

Each method has its advantages and suffers also from drawbacks. The kinetic resolution requires a single enzyme and is in general thermodynamically favored if the amine acceptor is pyruvate. But the enantiomeric excess of the amine depends on the conversion, and only 50 % yield can be reached at maximum in case of highest enantioselectivity. In contrast to that, the asymmetric reductive amination allows converting prochiral ketones and aldehydes in the best case in quantitative yield.[82] In any case these enzymes need pyridoxal 5-phosphate (PLP (**V**)) as cofactor, which acts as an intermediate amine acceptor and electron sink in the mechanism (Scheme B-17).[76] PLP (**V**) serves thereby as molecular shuttle for ammonia and electrons, transferring an amino group between the amine donor and the acceptor. But not only PLP is crucial for the mechanism, also its reductively aminated form PMP (**VI**) (pyridoxamine-5-phosphate).[76] PLP (**V**) forms a Schiff base with a lysine residue in the enzyme backbone (**F**). The C=N bond of the Schiff base is attacked by the substrate amine. Thereby, an imine is formed between substrate amine and PLP (**G**).

The attack is enabled by a water molecule in the active site and the phenolic oxygen of the PLP (**V**), while the phenolic hydroxy group in the *ortho*-position stabilizes the Schiff base in both cases through a hydrogen bond. A lysine residue then abstracts the proton at the amine carbon, which is now more acidic due to the imine formation, formally generating a carbanion. The abstraction of the proton leads to isomerization to the resonance methide form (**I**), which is rearomatized by the proton of the base from the first deprotonation step (**J**). The recently generated imine is then hydrolyzed, which liberates the ketone product and an enzyme-PMP complex (**K**). Since all steps of the mechanism are reversible, the PMP (**VI**) complex is able to transfer the amino group to another ketone substrate by the reverse pathway. The pyridine nitrogen of the cofactor plays an important role since this heterocycle, especially in its protonated form, can stabilize the postulated methide form (**I**) of the cofactor, which is fundamental for efficient catalysis. In the case of deaza-PLP as applied cofactor, the enzymatic activity drops by four to nine orders of magnitude. In terms of chiral information transfer, the substrate is positioned in a large (L) pocket and a small (S) pocket within the active site (Figure B-6), enabling a high degree of predictability for biotransformations.

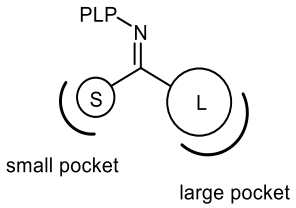
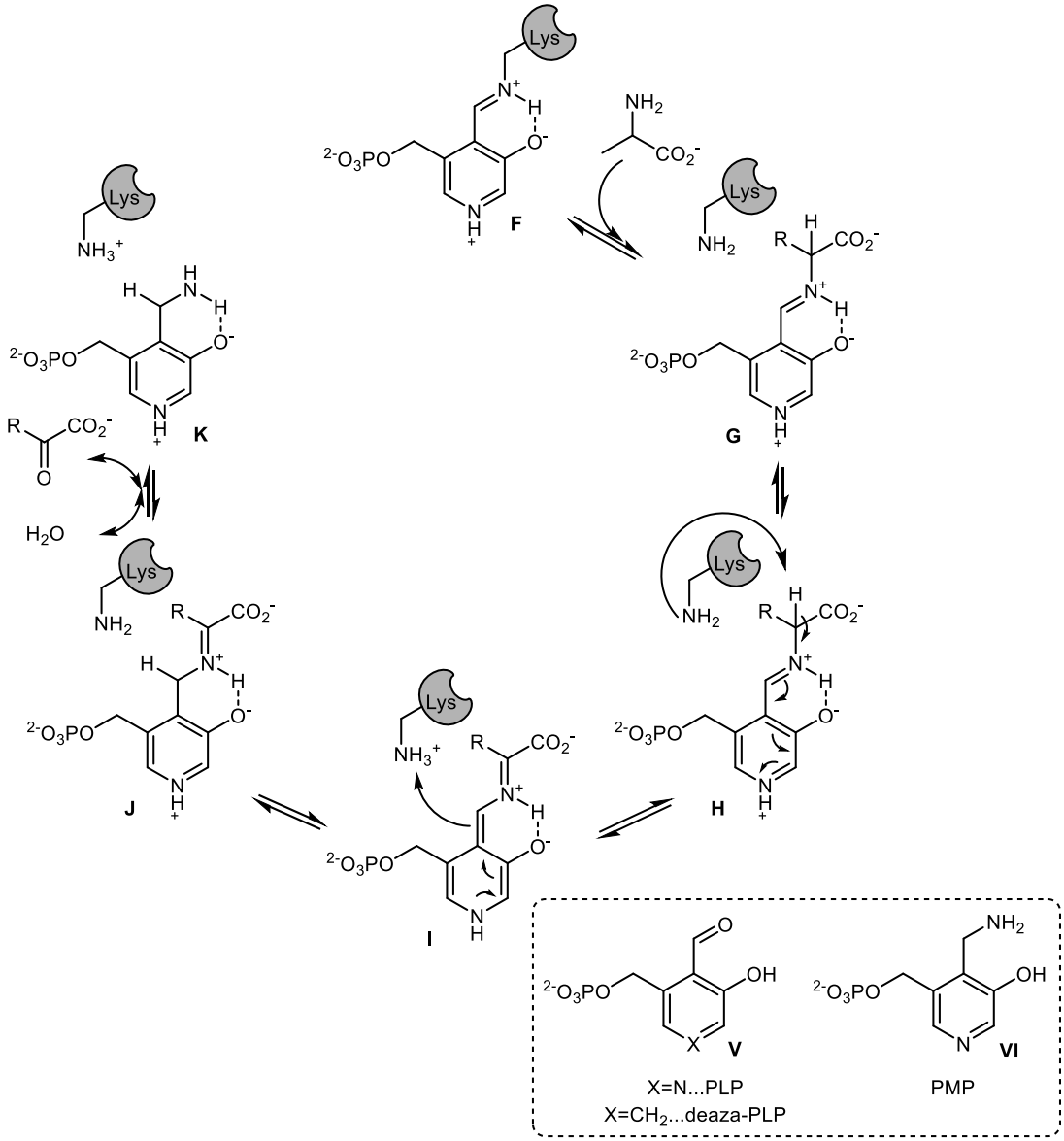


Figure B-6 Schematic view of the binding site of an ω-TA (adapted from Fuchs et al.)[76]

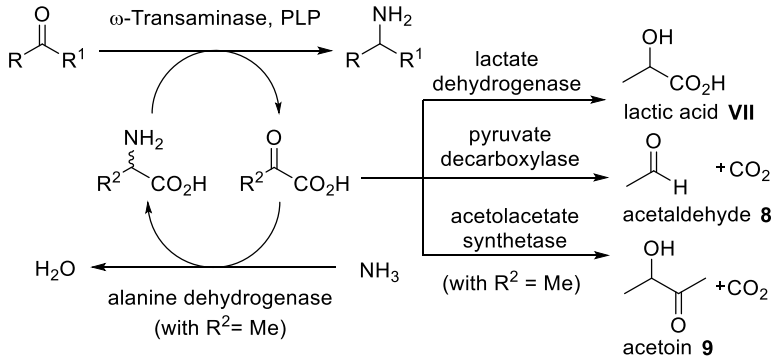
Thus, the wild-type enzymes do not accept substrates with two large substituents next to the carbonyl functionality. The Bornscheuer group overcame this major challenge by extensive protein engineering followed by optimization of the identified motif. The resulting biocatalysts demonstrated up to 8.900-fold higher activity in comparison with the starting scaffold and are highly stereoselective (up to >99.9% *ee*) in the asymmetric synthesis of a set of chiral amines holding bulky substituents.[84] Moreover, using bioinformatic tools, the Bornscheuer group identified variants of the ATA from *Vibrio fluvialis*, a frequently used (*S*)-ATA, with a widened binding pocket as exemplified for a range of ketones. The asymmetric synthesis of 2,2-dimethyl-1-phenylpropan-1-amine - not accessible by any wild-type ATA- was successfully performed. The best variant containing four mutations gave 100% conversion of the ketone and >99% *ee*, notably with a preference to the (*R*)-enantiomer. In silico modeling enabled the reconstruction of the substrate binding mode to the newly evolved pocket and hence the experimental results were expained.[85]



Scheme B-17 Structure of PLP (bottom) & mechanism for the transamination (adapted from Fuchs et al.)[76]

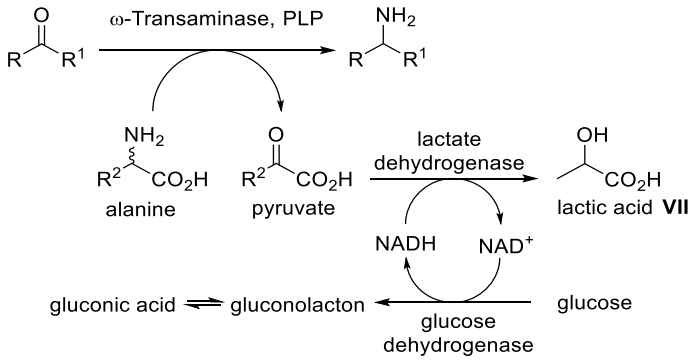
The substrates and products (amine donor and acceptor, product, co-product) exist in equilibrium with each other in the reation mixture. Hence, depending on the substrates' and products' nature reaction engineering or follow-up reactions might be obligatory to push the reaction towards the product side.[86] In fact, one of the major problems is, particularly in the case of asymmetric synthesis to shift the equilibrium to the product side, which lies naturaly strongly on the substrate side.[76] Therefore, the development of process-adapted enzymes or methods to shift the equilibrium was crucial for the success of amine synthesis using ω-transaminases.[82] Options to circumvent this issue are either the utilization of a large excess of amine donor and/or removal of the co-product. One way to solve that problem was recently demonstrated by the application of an artificial multienzyme network that was successfully expressed in a single host.[87]

However, to use ω-ATAs for a high-output asymmetric reductive amination the thermodynamic barrier has to be overcome to shift the equilibrium toward product formation. Currently, several convenient methods are available like (1) removing volatile co-products (e.g., acetone, propanone)[88] or (2) the application of designated amine donors,[89] or (3) the use of multienzyme networks (Scheme B-18) as well as (4) nonenzymatic spontaneous reactions. [82]



Scheme B-18 Enzyme coupled techniques to shift the equilibrium in ω-ATA catalyzed reactions

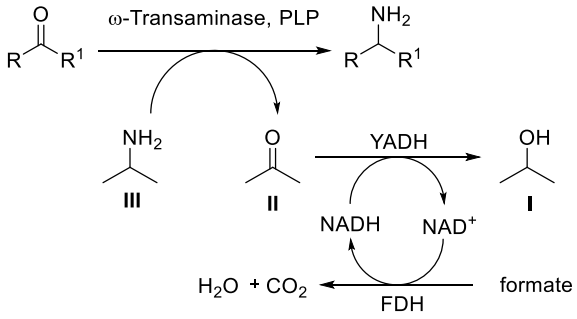
In the following, the most common "equilibrium shifting enzyme cascades" will be briefly explained. The applicability of lactate dehydrogenase was first proven by the incorporation of a glucose dehydrogenase (GDH) for recycling of the cofactor nicotinamide NADH in form of a ωTA/LDH/GDH coupled system (Scheme B-19).



Scheme B-19 LDH/GDH multi-enzyme network to shift the equilibrium towards product formation
(LDH = lactate dehydrogenase, GDH = glucose dehydrogenase)

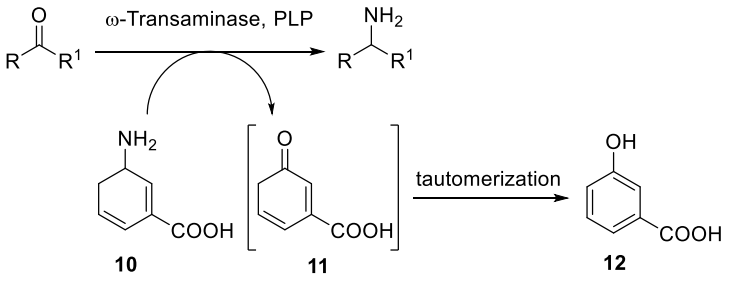
Another way to shift the equilibrium is the removal of pyruvate from the reaction medium by incorporation of a pyruvate decarboxylase (PDC), which converts pyruvate to acetaldehyde and CO_2. Thus, both species can easily be removed leading to a consequently irreversible system.[82] Hereby, only two enzymes and no additional NADH recycling is required.

While in the above described methods only alanine was used as amine source, an alternative cascade was established by using *iso*-propylamine as cheap and achiral amine donor. In this case, the coproduct acetone was removed employing a yeast alcohol dehydrogenase (YADH) from *Saccheromyces cerevisiae* in combination with a NADH recycling system including formate dehydrogenase (Scheme B-20).



Scheme B-20 Acetone removal by YADH

Further, nonenzymatic methods for the equilibrium shift were developed. One example is the application of amine donors such as 2-propan- and butanamine, which is highly attractive due to the volatility of the formed byproducts (acetone and butanone) and because this amine donors are very economical.[82] But not every ω-TA accepts every amine donor. Amine donors are limited to a restricted number of ω-TAs being able to accept the respective donor. This limitation can be bypassed by reaction engineering enabling very attractive single enzyme systems or by rational protein engineering.[88b, 89] Alternative amine donors are for example derivates of 1-phenylethylamine but also more complex amines as for example an amino-cyclohexadien-derivative (Scheme B-21).



Scheme B-21 Ketone removal via spontaneous tautomerization

During this shifting-approach the formal intermediate coproduct ketone resulting from the commercial available amino-cyclohexadien derivative undergoes an irreversible tautomerization to 3-hydroxybenzoic acid. In this case, only one enzyme is required and expensive cofactors such as NADH are completely avoided. However, the system suffers from the rather expensive amine donor which has to be added in equimolar amounts.[90]

B.VI Chemoenzymatic cascade reactions & combination strategies

Nature's elegance of efficiently assembling cascade type reactions inspired biologists and chemists all around the world. The understanding and mimicking of such networks are of highest interest for both academia and industry. By combining either the advantages of chemo-, regio-, and stereoselectivity of biocatalysts or promiscuity and productivity of chemocatalysts a few multistep one-pot reactions were recently developed. Thus, a variety of concepts, inspired by different disciplines (biocatalysis, metabolic engineering, synthetic chemistry, and material science), have been published.[91] Regarding process efficiency and sustainability, the combination of individual reaction steps has a tremendous potential for decreasing solvent consumption and waste production due to the decreased number of overall required work-up steps.[50] Further, space-time yields can be significantly improved, which leads to an increased general interest in this topic of one-pot processes from an industrial standpoint (see Figure B-7).

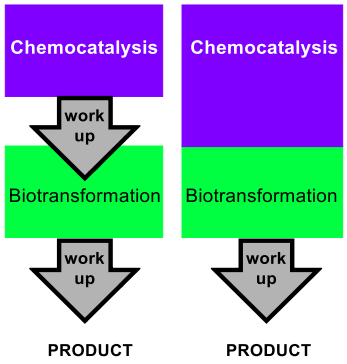


Figure B-7 Basic concept of chemoenzymatic one pot processes

Processes of the same type of technology, meaning chemocatalysis[92] (e.g. domino reactions)[93] on the one and biotransformations[94] (e.g. fermentations)[95] on the other hand were investigated, extensively. However, combinations of these two worlds are rather rare. With respect to a suitable solvent for a biocatalytic step, the majority of these processes are conducted in aqueous medium, which beneficially leads to "greener" processes in general and besides that, choosing water as a solvent enables in principle the use of the full range of enzymes as water represents the natural reaction media for all enzymes. Combinations of chemocatalysis and biocatalysis for consecutive reactions in aqueous media are of particular importance.[50]

Since the two reaction types traditionally require substantially different reaction conditions, particularly regarding solvent, reaction temperature and substrate concentration, it is obviously a challenging task to bring these apparently contradictive worlds together, that are naturally not combinable. The development of combination strategies can only be realized in consideration of enzyme activity maintenance on the one hand, and metal catalyst activity maintenanace on the other hand.

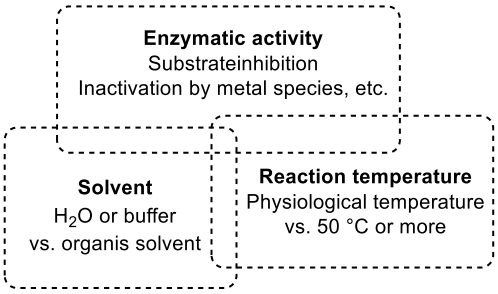
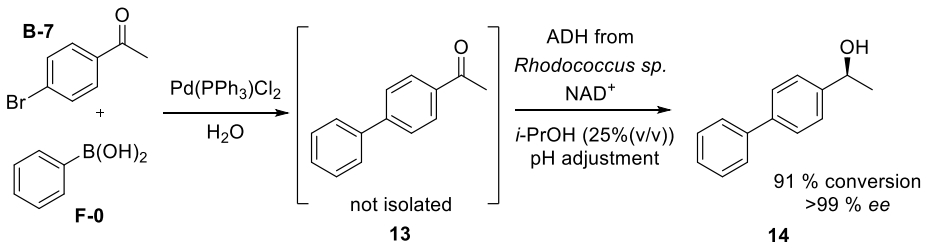


Figure B-8 General compatibility issues between metal- and biocatalysis affecting each other

Additionally the general process requirements such as high conversions, yields and selectivity must be taken into account.[50] In the early 1980s, the van Bekkum group elegantly combined a heterogenized metal-catalyzed hydrogenation with an enzymatic isomerization and thereby laid the foundation for this development[96]. The process comprises the synthesis of the sugar substitute D-mannitol starting from D-glucose, which is in situ transformed into D-fructose employing a D-glucose isomerase. Subsequent heterogeneous Pt-catalyzed hydrogenation delivered the desired D-mannitol as major product. Next major contributions made for dynamic kinetic resolutions by combining a chemocatalytic racemization and a hydrolase catalyzed resolution in water was done by the Williams group jointly with Harris[97] and the Wang as well as Beller group[98] utilizing metal catalysts and organocatalysts, respectively, for racemization. In parallel a range of dynamic kinetic resolutions of alcohol and amines based on metal catalyzed racemization and lipase catalyzed resolution through acylation was developed in organic solvent by the groups of Bäckvall, Kim, and Berkessel.[99]

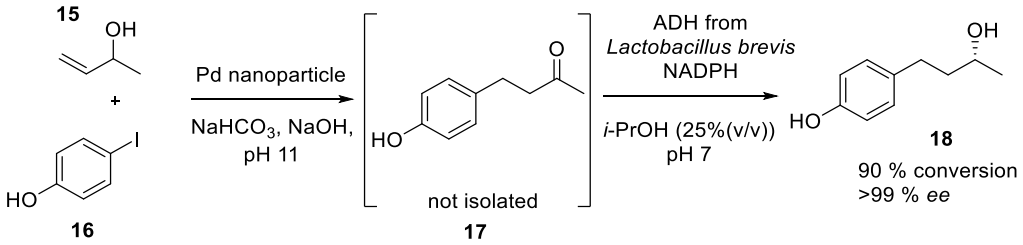
The efforts regarding the combination of metal catalysis and biocatalysis also result from the fact that both catalyze unique reactions that often cannot be conducted by the other catalytic discipline. Thereby, from a biocatalysis point of view those metal-catalyzed transformations for combinations are particularly interesting, for which enzymatic processes do not exist such as cross-coupling reactions (Pd catalysis for Heck reaction, Suzuki-reaction), metathesis (Ru-catalysis or Mo- catalysis) and Wacker-oxidation (Pd catalysis).

Further, organic reaction mixtures from heterogeneously catalyzed hydrogenations were combined with subsequent biotransformations. This was demonstrated by the Mihovilovic group for the hydrogenation of cyclic enones conducted in a flow reactor and a subsequent epimerization with an ion-exchange polymer, followed by a monooxygenase catalyzed Baeyer-Villiger oxidation to form a lactone with >99 % *ee*, >99 % de and a space-time-yield of 3.4 g/(L*d)[100]. Moreover, a Suzuki cross-coupling reaction was efficiently combined with an enantioselective biocatalytic reduction collectively by the Gröger and Hummel groups (Scheme B-22)[101]. The boronic acid which is required for the coupling reaction turned out to be a critical component due to its strong inhibition of the alcoholdehydrogenase.



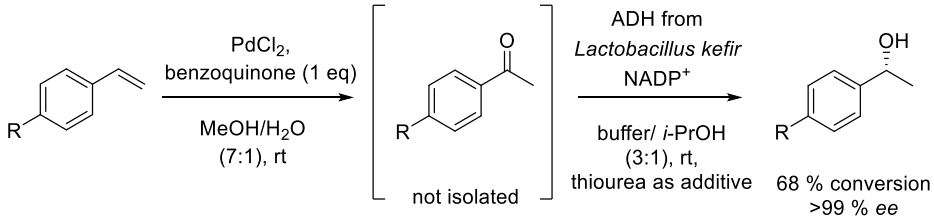
Scheme B-22 Combination of a Suzuki cross coupling reaction and a biocatalytic reduction (adapted from Gröger et al.)[50]

By application of a two-step one-pot strategy and thus addition of the enzyme to the reaction mixture after the completed biaryl formation and full consumption of the boronic acid (**F-0**), the biocatalyst was compatible with the reaction mixture from the Suzuki reaction and the biaryl-substituted alcohol (**14**) was formed with high conversion (91%) and >99% *ee*. An extension of this process was demonstrated by Prastaro et al. employing water-soluble palladium nanoparticles stabilized within the protein cavity of a thermostable DNA binding protein as the metal catalyst,[102] and Gauchot et al. demonstrated an efficient re-use of chemocatalyst and biocatalyst when using ionic liquid and buffer as a biphasic system[103]. The earlier contributions with Suzuki reactions at elevated temperatures were further investigated and a biocompatible Suzuki reaction at room temperature was developed based on a water-soluble palladium-TSPP catalyst [104]. However, to conduct both reactions under the same pH conditions was not realized at that time. Another Pd-catalyzed reaction of industrial interest is the Heck reaction, and its combination with an enzymatic reduction towards a one-pot process in aqueous medium was conducted by Boffi et al. [105] (Scheme B-23).



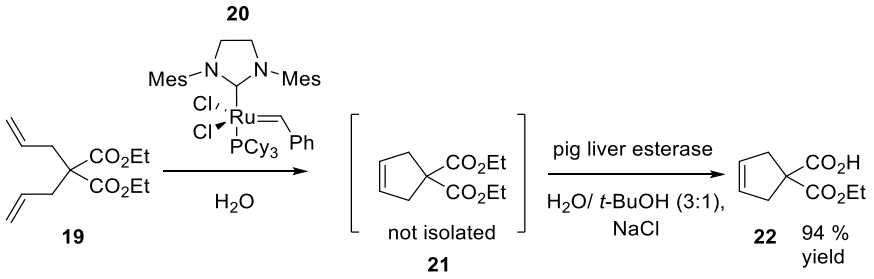
Scheme B-23 Combination of a Heck reaction and a biocatalytic reduction (adapted from Gröger et al.)[50]

Based on a former combination of a Heck reaction in organic media[106], this Pd catalysis was performed in aqueous medium using a phosphine-free perfluoro-tagged palladium nanoparticle[105] and combined with an ADH in a two-step one pot system. The allylic alcohol products were observed in yields of up to 92 % and with >99 % ee. Another interesting one pot process in aqueous media, which formally corresponds to an enantioselective hydration reaction, is a Pd-catalyzed Wacker oxidation starting from a prochiral alkene, giving an aldehyde or ketone, coupled with an enantioselective enzymatic reduction. Gröger and Hummel demonstrated this transformation of styrene into 1-phenylethanol via acetophenone (Scheme B-24).[107]



Scheme B-24 Combination of a Wacker oxidation with a biocatalytic reduction in a one-pot process (adapted from Gröger et al.)[50]

The compatibility of two reaction types was only achieved in the presence of a catalytic amount of an additive known to bind to the present palladium species (e.g., thiourea), even if the effect of this additive was not clear. A complexation of the palladium species which directly or indirectly (e.g. by interaction with the cofactor) inhibits the enzymatic process was suggested. In this chemoenzymatic process, the alcohol product was obtained in up to 68 % yield and with up to 99 % *ee*. [108] Further, olefin metathesis is another metal catalyzed method complementary to the reaction spectrum of enzyme catalysis and was combined with a biotransformation towards a one-pot process (Scheme B-25).[109]



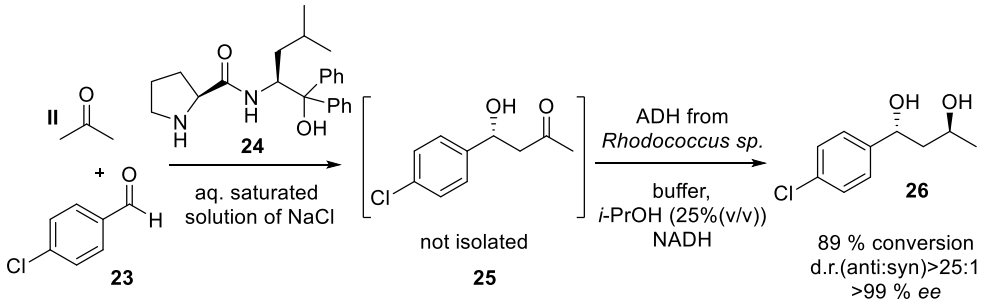
Scheme B-25 Combination of an olefin methathesis with an enzymatic hydrolysis (adapted from Gröger et al.)[50]

When starting from a diallylmalonate, ring-closure metathesis gave the cycloalkene derivative (**21**), which was then converted into the respective monoester (**22**) by pig liver esterase (PLE) in 94% overall yield. The PLE was added after the formation of the cycloalkene, and high compatibility of the metathesis catalyst with the enzymatic hydrolysis was demonstrated. Moreover, a metathesis reaction was combined with a P450-monooxygenase catalyzed oxidation in aqueous medium, generating various epoxides in a tandem fashion as recently reported by Denard et al.[110] The cooperative action of the two catalysis types enables the combination of a dynamic equilibration of the alkenes with a selective epoxidation of the desired cross-metathesis product to form the epoxidized carboxylic acid. With 27 % yield this one-pot tandem process gave a 1.5-fold higher yield compared to the hypothetical yield of 18 % resulting from a stepwise process comprising of the two reactions steps and the isolation of the intermediate.

In extension of the combination of classic types of chemocatalysts with biocatalysts, artificial metalloproteins can serve as chemocatalyst moieties in chemoenzymatic one-pot processes. This was demonstrated by Köhler et al. by applying an artificial transfer hydrogenase combined with various redox enzymes.[111] L-Pipecolic acid was synthesized in a deracemization-process starting from the bulk chemical L-lysine, which provides an example for such a process. An initial oxidation catalyzed by an L-amino acid oxidase and subsequent hydrogenation by the iridium-complex-containing metalloprotein generates the racemic amino acid, which then is resolved highly enantioselectively by a D-amino acid oxidase and catalase to decompose the formed hydrogen peroxide. The unchanged L-enantiomer of pipecolic acid is finally obtained in high enantiomeric excess. Up to three enzymes where thereby integrated in the cascade. An outstanding compatibility of such (chemo-)catalysts with enzymes is assumed, since artificial metalloproteins bear a chemocatalytic moiety surrounded by a protein structure.

Thus, a concurrent cross methathesis and enzymatic oxidation was demonstrated by Köhler et al. in 2014. A revesible ruthenium catalyzed reaction was thereby combined with an irreversible enzymatic transformation using cytochrome P450 BM3 monooxygenase.[112]

Notably, also an increasing number of efficient asymmetric organocatalytic methodologies combined with biocatalytic steps have been investigated in the last 20 years. A proof of concept for such a process was recently reported by Baer et al., consisting of a combination of an organocatalytic aldol reaction with a biocatalytic reduction of the aldol adduct leading to 1,3- diols [113]. This group also presented the first chemoenzymatic one-pot process, in which the organocatalytic aldol reaction as well as the enzymatic reduction proceeds in aqueous medium.[114] The desired 1,3-diols were observed with up to 89 % conversion and a diastereomeric ratio of up to >25:1 with enantioselectivities of up to 99 % *ee* (Scheme B-26). [114]



Scheme B-26 Asymetric organocatalytic reaction with a biocatalytic reduction (adapted from Gröger et al.)[50]

Two years later an extension of this process was published by the same group.[115] An elegant option for the compartmentalization of the two different catalyst types was applied using a commercially available superabsorbing material. The immobilization was conducted based on a literature known protocol, which was developed by Jeromin in 2009. Jeromin reported the finding of a "new catalyst" for asymmetric reduction, which ment a superabsorbed alcohol dehydrogenase. The immobilisate contains all components for the reaction, i.e. enzyme, the coenzyme $NADP^+$, buffer and cofactors. This "all inclusive catalyst" is easy to prepare by simply embedding the enzyme in the superabsorber matrix. Further, the superabsorbent polymer is economically attractive and commercially available. Prochiral ketones, acetophenone and 4-acetylpyridine were successfully converted into the respective (*R*)- and (*S*)-alcohols with a superabsorbed ADH from *Lactobacillus brevis* and a superabsorbed ADH from *Thermoanaerobicum sp.* in *i*-PrOH as organic solvent and at the same time the cofactor-regenerating substrate. Jeromin obtained yields from 97-100 % and achieved enantiomeric excesses of 99 %.[116]. This immobilization procedure was applied in the following by the Gröger group for the embedment of an ADH from *lactobacillus kefir* and its employment in the reduction of acetophenone as a model substrate, which led to high conversion (>95 %). A mixture of aqueous buffer/isopropanol (75/25 (v/v)) was used as solvent.

Further, the immobilized biocatalysts turned out to be suitable for the diastereoselective reduction of an organocatalytically prepared enantiomerically enriched aldol adduct. High conversion, diastereomeric ratio and enantioselectivity for the resulting 1,3-diols were observed via application of a "fixed-bed" reactor for the separation of the two catalyst types.[115]

In 2014 a further extension was published by Heidlindemann et al., which is based on a "one-pot like" process for 1,3-diols, again based on immobilized organo- and biocatalysts used in different compartments, but this time completely in organic media. The efficient biocatalyst provided good reusability and simple product separation from the immobilizate under avoidance of any tedious extraction steps during the process. During these studies it turned out, that the use of polar solvents (e.g. buffer/isopropanol) in the reaction mixture leads to a dramatic leaching of enzyme and cofactor, which can be explained by the fact, that *i*-PrOH extracts water from the superabsorber matrix and therefore substantially lowers the water activity (a_w) in the hydrogel compartment and thereby dries out the aqueous compartment. Further in the presence of *i*-PrOH the overall concentration of *i*-PrOH in the superslurper phase is enhanced and could thereby inactivate the ADH.[117] Thus, whenever this combination approach is applied, the use of *i*-PrOH must be avoided. Nevertheless, this protection strategy for the ADH has the beneficial effect, that the substrate concentration is lower in the superslurper matrix due to solubility reasons, which is highly desirable to avoid substrate inhibition. The discussed investigations were of high relevance for the in this thesis targeted chemoenzymatic one pot processes. A concept for a potential reaction setting is demonstrated in Figure B-9.

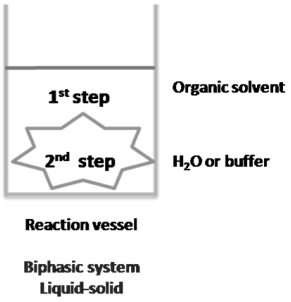


Figure B-9 Concept of a liquid-solid biphasic system for the targeted one pot process

Heidlindemann et al. adressed a very general issue by running this process completely in organic media, since the insufficient stability of biocatalysts under processing conditions displays one of the major barriers for the use of enzymes in industrial biotechnology. The use of organic solvents instead of aqueous media for enzymatic reactions would offer numerous advantages, such as an increased solubility of hydrophobic substrates or suppression of water-dependent side reactions (e.g. hydrolysis reactions). However, organic solvents in most cases inactivate enzymes.

Thus, industry and academia are highly interested in strategies to enhance the lifetime of enzymes in the presence of organic solvents.[118] Therefore the influence of organic solvents on enzymes, even though every biocatalyst has to be studied individually, was intensively investigated and discussed. However, the same effect of a beneficial distribution of a hydrophobic substrate between aqueous and organic phase can be expected for a strictly non miscible, liquid-liquid biphasic system (Figure B-10). Further, the organic solvent itself does not affect the biocatalyst to a critical amount due to it's highly apolar character.

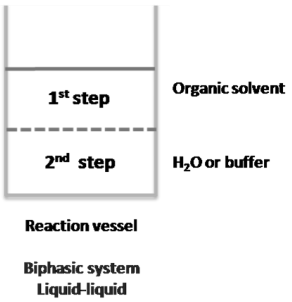
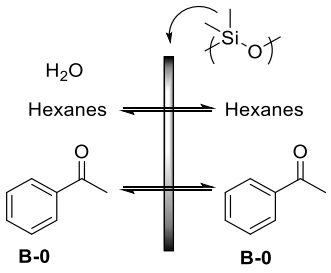


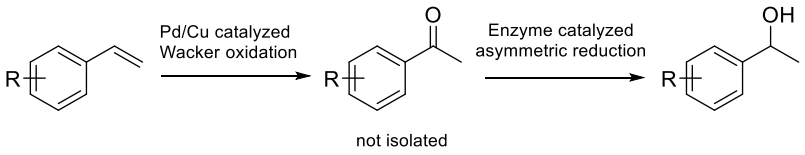
Figure B-10 Concept of a liquid-liquid biphasic system for the targeted one pot process

A different technical possibility using multiple catalysts or reagents in a cascade fashion is to site-isolate catalysts from each other by applying a semipermeable membrane system. One prominent example of reagents being completely incompatible is water and $LiAlH_4$ reacting rapidly with each other and therefore they can not be added to the same reaction vessel. In 2008 the Bowden group used polydimethylsiloxane (PDMS) to develop a general method to site-isolate water from $LiAlH_4$ and Grignard reagents to carry out a series of cascade reactions[119]. In this case PDMS timbles were employed to completely encapsulate water (Scheme B-27).



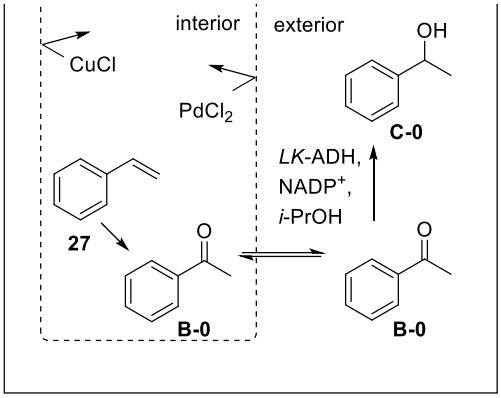
Scheme B-27 Diffusion of small non-ionic organic molecules through the PDMS membrane

PDMS is a hydrophobic, commercially available polymer which is used as a membrane in separation devices, due to the characteristic that small molecules can diffuse through it readily. The flux rates of non-ionic organic molecules through PDMS are typically high, whereas very polar molecules have low flux rates caused by their low solubilities in PDMS. Thus, water has a very low flux rate through PDMS owing to its highly polar and hydrogen-bonding structure which is incompatible with the hydrophobic matrix. PDMS can be purchased in form of a two-component kit and readily cast into the desired shape. The two components are mixed to form a viscous liquid that cures into a solid after a few hours at 65 °C. However, the difference in flux rates of polar and non-polar molecules allows incompatible reagents to remain on the interior or exterior of PDMS barrier for a long time. This method provides interesting opportunities to carry out cascade reactions using this material as tool in the synthesis of organic molecules because it allows multiple reagents and catalysts to be used without regard for whether they poison or affect in any other means each other.[119] Of course this provides an interesting concept for potential chemoenzymatic one pot reactions. In 2015, the Gröger group demonstrated a Wacker oxidation using CuCl/$PdCl_2$ as a catalyst system combined with a subsequent enzymatic ketone reduction. Styrene was converted enantioselectively into 1-phenylethanol in a one-pot process, although the two reactions conducted in aqueous media are not compatible due to enzyme deactivation by Cu ions (Scheme B-28).



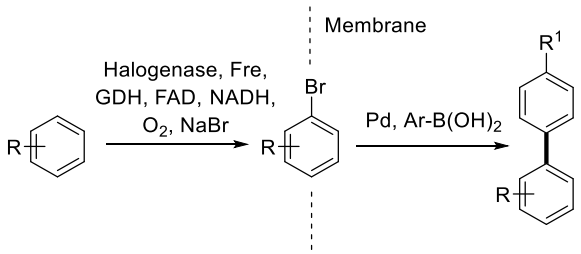
Scheme B-28 Combination of Wacker oxidation and enzymatic reduction (adapted from Sato et al.)[120]

Conducting the Wacker oxidation in the interior of a PDMS thimble enables diffusion of only the organic substrate and ketone product into the exterior where the biocatalytic step takes place. Thus, the Cu ions, which inactivate the enzyme, are withheld from the reaction media of the biotransformation. Via this sequential process, which formally corresponds to an asymmetric hydration of alkenes, a range of 1-arylethanols were generated with high conversions and 98–99 % *ee* (Scheme B-29). Additionally, the influence of the oxidation state of the copper was investigated, indicating that Cu^{II} ions are much more harmful regarding enzyme inactivation, than Cu^{I} species.[120]



Scheme B-29 Site-isolation of catalysts using a PDMS thimble to combine a wacker oxidation and an enzymatic reduction[120]

Another novel chemoenzymatic process applying the PDMS material was demonstrated recently by Latham et al.[121] In the field of C-H activation discrimination between two similar, unactivated C–H positions is beyond the scope of current chemocatalytic methods. Therefore, this group demonstrated that integration of a regioselective halogenase with Pd-catalysed cross-coupling chemistry in one-pot reactions successfully addresses this problem. The chemoenzymatic process delivers functionally diverse arylated products that are impossible to access using either enzymatic or chemocatalytic C–H activation separately under mild, aqueous conditions (Scheme B-30). The compatibility issues of enzyme and transition metal are overcome through membrane compartmentalization, with the optimized process requiring no intermediate isolation or purification steps.[121]



Scheme B-30 Combination of a halogenase catalyzed transformation and a Suzuki-Miyaura cross coupling using flavin reductase (Fre) and glucose dehydrogenase (GDH) enzymes for cofactor recycling[121]

This technology was clearly another interesting option for the development of a chemoenzymatic cascade reaction within our project (Figure B-11).

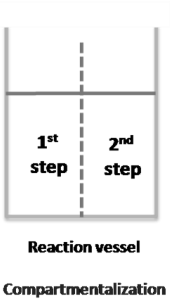
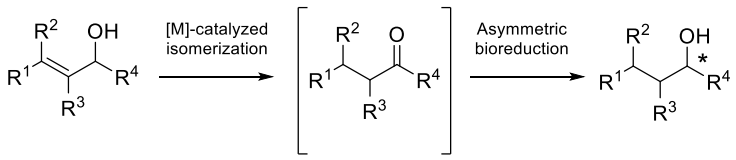


Figure B-11 Concept of compartmentalization by application of a semipermeable mebrane

In 2015 a combination of a ruthenium catalyzed isomerization of allylic alcohols with a ω-transaminase catalyzed reaction was demonstrated by Rios-Lombardia et al.[122] The resulting amines were observed with high yields and enantiomeric excesses, but the process had to be accomplished in a sequential fashion due to incompatibility issues of the catalysts. Moreover, the same group recently presented the first chemoenzymatic process without the requirement of a compartmentalization or separation of the two cascade steps or the two catalyst types. The process was conducted in aqueous medium, in both sequential and concurrent fashion, and it provides enantiomerically pure alcohols from racemic allylic alcohols. This was realized by combining a ruthenium(IV)-catalyzed isomerization with a bioreduction promoted by ketoreductases (Scheme B-31).



Scheme B-31 Concurrent ruthenium(IV)-catalyzed isomerization and bioreduction promoted by ketoreductases (adapted from Rios-Lombardia et al.)[123]

In case of the sequential process published before, ketoreductase and cofactor was added and a decrease of the temperature was required. Regarding the concurrent process, both the metal catalyst and biocatalyst were able to coexist and work simultaneously from the start of the cascade reaction, thus furnishing the final products with yields close to 85 %.[123]

C Project description

Despite the great attractiveness, the implementation of concurrent cascade reactions that combine an organometallic catalyst with a biocatalyst has proven challenging because of the mutual inactivation of both catalysts.[111] However, driven by the outstanding strengths of the contrarily appearing catalyst types, this work aims on a chemoenzymatic one-pot cascade reaction, which is based on a combination of a metal catalyzed reaction and a subsequent enzyme mediated transformation of the newly created carbonyl group. An option to solve the problem of the described poisoning effect is to site-isolate catalysts and reagents from each other in a way that they do not come into contact.[119] To realize this in a one pot fashion and thus without any intermediate step between the two partial reactions, the general compatibility issues of these different catalysis types, namely overall reaction concentration, inactivation of the enzyme by metal species, reaction temperature and solvent, have to be solved. To demonstrate the versatility of the developed method, we aimed on identifying two enantiodivergent alcohol dehydrogenases that provide access to both enantiomeric forms of the desired chiral alcohol (Figure C-1). Further, the employment of a transaminase was envisaged for the production of chiral amines.

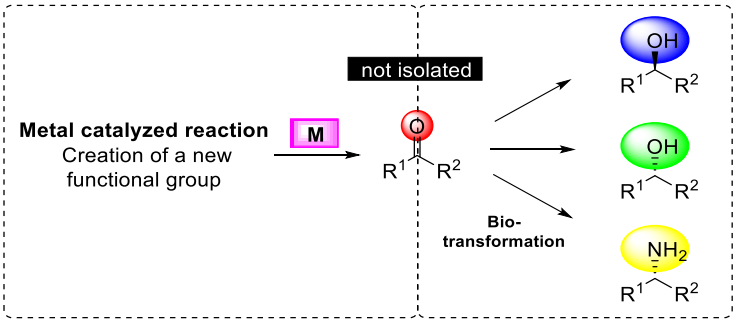


Figure C-1 Enantiodivergent biotransformations applying the by metal catalyzed reaction generated substrate

More specific, this thesis focuses on a concept to overcome the necessity of effortful intermediate steps during the two-step reaction sequence. In the following we envisaged a simultaneous combination of a coupling reaction, which generates a new functional group that subsequently undergoes the biotransformation leading to an enatiomerically pure compound. This should in general facilitate concomitant operation of both catalytic reactions in presence of each other, representing a Domino-type conversion. Moreover, the project was extended by combination of a gold catalyzed hydration reaction of alkynes, again generating a carbonyl group, and a subsequent reduction of the novel functionality in an extremely straightforward fashion.

D Results & Discussion

This chapter implies detailed investigations of the individual steps of a prospective chemoenzymatic cascade reaction (Figure D-1) meaning metal catalyzed step, enzymatic step and in the following the development of four different combination strategies. Therefore, the influence of the variation of several reaction parameters had to be studied in detail to bring these two contrary appealing worlds of metal- and biocatalysis together.

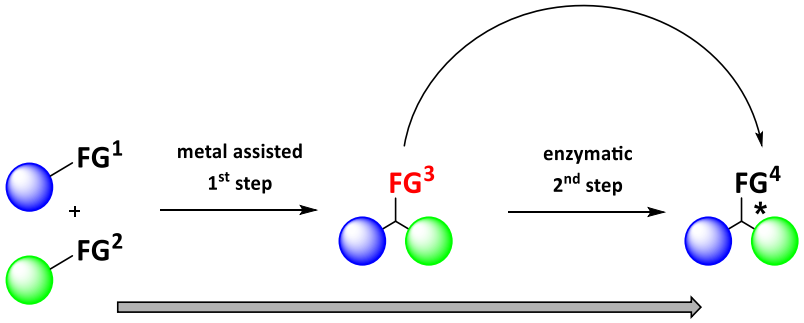


Figure D-1 Envisaged chemoenzymatic one pot cascade for the synthesis of chiral compounds

The general objective during the development of a successful chemoenzymatic one pot process is to indentify a commom denominator regarding the present reaction conditions for both catalysis types. Our approach was to initially study the metal catalyzed reaction to achieve “near-biocompatible” reaction conditions within the first cascade step that generates the new functionality (FG^3). Having the respective reaction conditions for the metal catalyzed transformation in hand, the biotransformation was studied with respect to a substrate overlap. As a result, four strategies based on different reaction settings were developed for the combination of both individual steps applying two different enzyme classes. The actually applied biocatalysts must be evaluated individually with respect to their unique characteristics and behavior in the present one pot system. Overcoming the general compatibility issues was very complex, especially since the adaption of single reaction parameters influences the impact of other ones (Figure D-2).

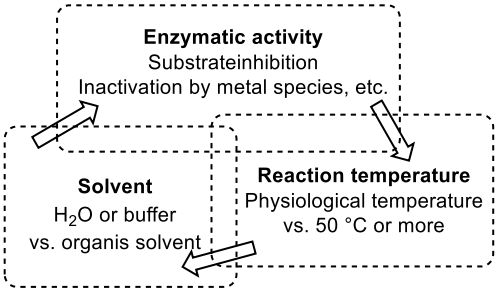


Figure D-2 General compatibility issues between metal- and biocatalysis affecting each other

The main criterion was of course to preserve the activity of both, the metal catalyst and the biocatalyst. First of all, the enzyme suffers from the high concentrations of starting materials within a metal catalyzed reaction and consequently the comparable high concentration of the potential substrate holding the novel functional group (FG^3). Further, the metal species itself displays a complexity regarding the combinability due to the general inhibitory action on various enzymes. Moreover, metal catalyzed reactions are classically performed in organic solvents. This is clearly the next task during the design of a chemoenzymatic one pot process, since biocatalysts in general only tolerate a limited content of organic solvent. Next, enzymes naturally perform under physiological conditions meaning reaction temperatures from about 20-37 °C, which is obviously not given in most metal assisted transformations.

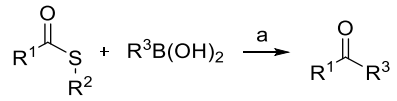
D.I Development of a liquid-liquid biphasic system

This chapter comprises the entire development of the first chemoenzymatic one pot process presented within this thesis and provides an overview about future design possibilities. The development of this liquid-liquid biphasic system is based on early observations within this project of both the coupling reaction and the enzymatic reduction.

D.I.1 Initial studies and adaption of the Liebeskind-Srogl coupling reaction catalyzed by tetrakis(triphenylphosphine)palladium

This section contains preliminary studies of the Liebeskind Srogl coupling reaction. The decision to first approach the reaction conditions of the applied enzymes within the metal assisted step is based on a narrow operational window for variations in the biocatalytic cascade step.

The mechanistically unprecedented coupling of a boronic acid and a thioester for the synthesis of ketones under neutral conditions was first demonstrated in 2000 by Lanny S. Liebeskind and Jiri Srogl. The C-C cross coupling procedure is in general performed under the presented reaction conditions (Scheme D-1).



R^1, R^2 = alkyl, aryl
R^3=aryl, styryl

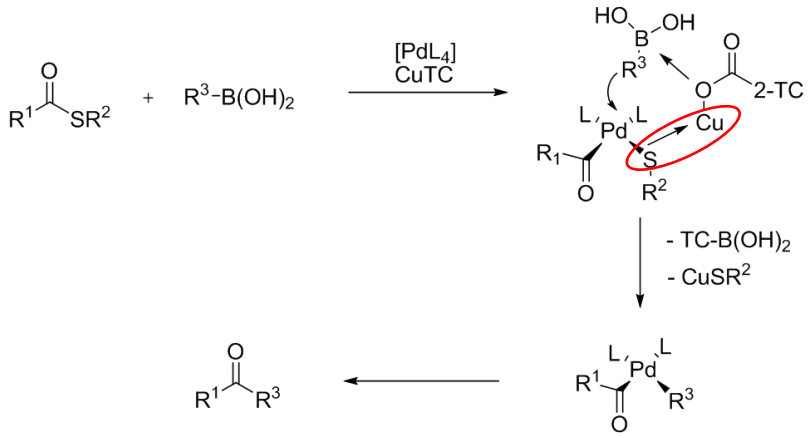
Scheme D-1 Reagents and conditions: a) Cu^I cofactor, Pd^0, THF, 50 °C

The fact that this metal catalyzed ketone synthesis does not require the addition of a base, which is obligatory for many other coupling protocols makes it potentially very attractive for our purpose. With regard to a combination with a biocatalyst it offers further advantageous characteristics. Within this reaction a carbonyl functionality is generated, which is a highly interesting functional group regarding a subsequent enzymatic step. The overall aim was to convert this newly created functional group by a subsequent biotransformation to a chiral product. Since most boronic acids show a good stability against air and moisture and are commercially available in a great diversity [16], the procedure was even more interesting.

Nevertheless, having a look on the initially published protocol the following critical properties regarding a potential combination with a biotransformation were determined:

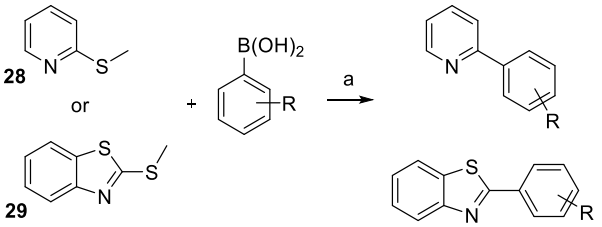
I. Cu (I) thiophene-2-carboxylate (CuTC) as cocatalyst
II. THF as solvent
III. 50 °C reaction temperature
IV. 250 mM initial thioester concentration

Preliminary experiments conducted by Moumita Koley indicated that CuTC inactivates the applied ADH (from *Saccharomyces cerevisae*) even at very low concentrations. This was also observed for the ADH from *lactobacillus kefir* by Sato et al.[120] and in general the influence of copper species on enzymes, especially alcohol dehydrogenases is rigorous.[124] However, a key feature of the Liebeskind Srogl coupling is the requirement of at least stoichiometric amounts of Cu^{I}-carboxylate species as a cofactor,[16] which is related to the mechanism of this protocol. The copper cofactor plays the dual role of both, polarizing the Pd-S bond through Cu^{I} coordination to the S center and at the same time, activates the trivalent boron center through coordination of the carboxylate group to the Boron atom (Scheme D-2).



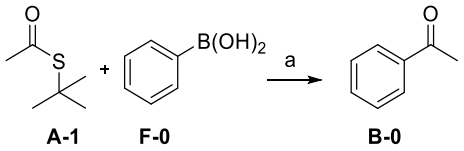
Scheme D-2 Role of CuTC in the mechanism of the Liebeskind Srogl coupling

As a consequence, the minimization or elimination of the copper species was no option during the cascade design. Even the procedure using only 5 mol% CuMeSal and no palladium catalyst (see Introduction, Scheme B-5) developed by Villalobos et al. [31] holds a copper content that is not tolerated by the respective alcohol dehydrogenases. Based on this knowledge, a separation or compartmentalization strategy of biocatalyst and copper ions was mandatory, using this coupling procedure as first cascade step. The next assignment was the identification of a solvent system that is tolerated by both catalyst species. In 2012 our research group (Koley et al.) presented the first Liebeskind Srogl coupling in water.[125] Within this protocol 2-(methylthio)pyridine and 2-(methylthio)benzothiazole were reacted in the presence of a Pd(0) catalyst and copper(I) thiophene-2-carboxylate with a series of arylboronic acids. The coupling procedure gave moderate yields, especially when electron-rich boronic acids were employed as coupling partners.



Scheme D-3 Reagents and conditions: a) CuTC (1.2 equiv.), $Pd(PPh_3)_4$ (5 mol%), AcOH (0.25 equiv.), 50 °C, H_2O

Based on this protocol, which was in the following transferred to our approach, *S-tert*-butyl thioacetate (**A-1**) was coupled with phenylboronic acid (**F-0**) using a mixture of water and *i*-PrOH (9:1) to synthesize the model substrate acetophenone (**B-0**) applying the respective reaction conditions (Scheme D-4). **B-0** served as model substrate for all initial studies, since it is a commonly used substrate for alcohol dehydrogenases and ω-transaminases, which we took into account for the biotransformation step. Thus, it could at the same time serve as model substrate in the envisaged biotransformation step.



Scheme D-4 Reagents and conditions: a) Boronic acid (1.1 equiv.), CuTC (1.2 equiv.), $Pd(PPh_3)_4$ (5 mol%), 50 °C
H_2O/i-PrOH

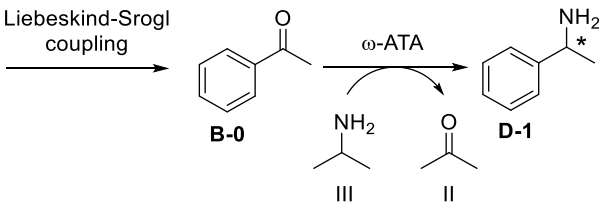
Therefore, the temperature was set to 50 °C and different solvent ratios of *i*-PrOH and H_2O were investigated (Scheme D-4, Table D-1) to achieve enhanced substrate solubility in the inhomogeneous reaction mixture. The addition of 10 % *i*-PrOH (v/v) delivered the best result (entry 1), whereas a higher content of *i*-PrOH did not lead to satisfying yields at 50 °C (entries 2, 3). Thus, exchanging THF against this aqueous solvent system led in the best case to an isolated yield of 46 % and indicated that one of the three main issues can in general be solved (Figure D-3), even if only a moderate yield was achieved.

Table D-1 Solvent studies of the Liebeskind Srogl coupling in mixtures of H_2O/i-PrOH

Entry	H_2O/ *i*-PrOH ratio	Yield [%]
1	9:1	46
2	3:1	39
3	1:1	16

Figure D-3 Combination issues between metal catlysis and biotransformation

Applying this aqueous solvent system, the influence of indispensable compounds of the biocatalytic step was investigated (Table D-2). Especially when ω-transaminases are applied this was of interest, since in this case an amine donor must be employed in excess to achieve a shift of the equilibrium towards the amine product. A classical and economically attractive amine donor is isopropylamine, which is converted to acetone during the transamination reaction that generates α-methylbenzylamine (**D-1**) from acetophenone (**B-0**). Isopropylamine is thereby used in high excess, in fact up to 100 equiv. amine donor (see general procedure F.XIV), to achieve an attractive result within the amine formation.



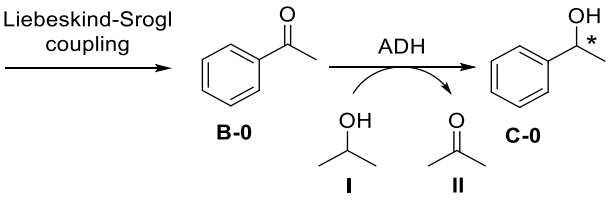
Scheme D-5 Potential chemoenzymatic cascade reaction applying ω-transaminases

Table D-2 Addition of components comprised in transamination reaction, 250 mM thioester concentration, H_2O/*i*-PrOH (9:1), 50 °C

Entry	Added component	Added amount [equiv.]	Yield [%]
1	Isopropylamine	1	37
2	Isopropylamine	10	8
3	Acetone	1	55
4	Methylbenzylamine	1	46

The generated results indicated, that the addition of isopropylamine, especially when it is applied in excess (here only 10 equiv. instead of 100 equiv.) has a negative influence on the product formation (entries 1, 2). This observation might result from hydrolysis of the thioester starting material and also the complexation of the required copper species under the respective conditions is concievable.

In contrast to that, the addition of 1 equiv. acetone leads to a slightly increased yield of 55 %, whereas 1 equiv. methylbenzylamine (**D-1**) has no influence, which might result from a lower water solubility compared to isopropylamine. Based on these results, a successful combination of Liebeskind-Srogl coupling and ω-transaminase using a classical one pot reaction setting was improbable. In contrast to this, the key compounds needed for cofactor recycling in an enzymatic reduction (*i*-PrOH and acetone) applying a coupled substrate approach do not inhibit the coupling reaction (Scheme D-6). Moreover, when a recycling system is applied in form of a coupled enzyme approach, inhibiting effects on the coupling reaction are not apparent.



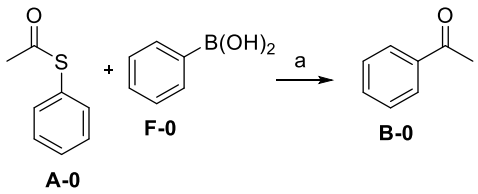
Scheme D-6 Potential chemoenzymatic cascade reaction applying alcohol dehydrogenases

The next issue to investigate was the reaction temperature which had to be decreased to reach physiological conditions. Thus, it was first set to 40 °C, which led to an unsatisfying result of 18 % yield. To compensate this decrease in yield the percentage of *i*-PrOH was increased to 50 %, which resulted in only 14 % isolated yield (**B-0**) (Table D-3). Thus, the minimization of the reaction temperature did not lead to satisfying results. Therefore, the adjustment of more than one parameter at the same time was in this case not expedient.

Table D-3 Temperature studies of the Liebeskind Srogl coupling in mixtures of H_2O/i-PrOH

Temperature [°C]	H_2O/ *i*-PrOH ratio	Yield [%]
50	9:1	46
40	9:1	18
40	1:1	14

Driven by these results, the development of a biphasic system was intended to approach the respective conditions of every individual step at once and further to achieve an advantageous partition between the two phases. Therefore it was important to identify a solvent, suitable for the Liebeskind Srogl coupling, which could serve as second phase in a one pot process. To successfully protect the biocatalyst in a biphasic system, the application of a solvent that is strictly water immiscible is obligatory. In the last decades some promising biphasic systems for biocatalytic reactions were reported [68, 126]. Amongst other strictly water immiscible solvents heptane showed promising characteristics as organic phase [127] Thus, we envisaged a biphasic system using heptane as second phase and thereby as solvent for the metal catalyzed cascade step. To ensure that also the thioester and the resulting leaving group is predominantly content of the organic phase, the thioester was changed from *S*-*tert*-butyl thioacetate (**A-1**) to the slightly more lipophilic *S*-phenyl thioacetate (**A-0**). First, we performed the model reaction in pure heptane and thereby observed a conversion of 99 % to **B-0** applying the specified conditions (Scheme D-7, Table D-4).



Scheme D-7 Reagents and conditions: a) Boronic acid (1.1 equiv.), CuTC (1.2 equiv.), $Pd(PPh_3)_4$ (5 mol%), heptane

Table D-4 Acetophenone synthesis by Liebeskind-Srogl coupling in heptane

Thioester Cpd.No.	Initial thioester concentration [mM]	Temperature [°C]	Conversion (GC), 24 h[%]
A-0	250	50	99

Consequently, a partition study of key components of the Liebeskind-Srogl coupling was conducted starting from a concentration of 125 mM of all components referred to the heptane phase (Table D-5).

Table D-5 Partition study applying a biphasic system, H_2O/ heptane (1:1)

Entry	Reactant of the coupling procedure	Partition between the two phases
1	*S*-Phenyl thioacetate	1:180
2	Acetophenone	1:15
3	1-Phenylethanol	1:1.3

To our delight, we found a partition equilibrium that is extremely beneficial, since substrate inhibition (entry 2), product inhibition (entry 3) as well as potential inactivating effects caused by the thioester (entry 1) could be circumvented using this biphasic system. The resulting concentrations in the aqueous phase containing the biocatalytic cascade step were in a range that is well tolerated by the applied ADH. Thus, the reaction in heptane was further investigated at 37 °C. Additionally, 2-methyltetrahydrofurane was considered as second phase. Consequently, the Liebeskind Srogl coupling reaction starting from *S*-phenyl thioacetate (**A-0**) was conducted in both potential organic phases (Table D-6).

Table D-6 Acetophenone synthesis via Liebeskind-Srogl coupling at 37 °C in solvents potentially serving as organic phase in a chemoenzymatic process

Initial thioester concentration [mM]	Solvent	Temperature [°C]	Conversion (GC), 24 h [%]
250	Heptane	37	30
125	Heptane	37	24
250	2-Methyl-THF	37	80

The use of heptane as solvent for the coupling reaction delivered 24-30 % conversion to **B-0** at 37 °C, depending on the initial thioester concentration. Conversely, 2-methyltetrahydrofuran led to a conversion of 80 %. However, the solvent was unfortunately not tolerated by the applied ADH (see section D.I.2). The same effect of this solvent on enzymatic activity was observed by Bornadel et al[126d]. Therefore, further investigations were conducted in a biphasic system consisting of heptane and water using two different ratios of the immiscible solvents (Table D-7).

Table D-7 Acetophenone synthesis by Liebeskind Srogl coupling in biphasic systems at 37 °C

Initial thioester concentration [mM]	Heptane/H_2O ratio	Conversion (GC) [%]
250	1:1	21
250	1:9	0

Driven by these results a sequential process was envisaged, since the temperature issue could not be solved using a biphasic system. Further, the copper ions predominantly present in the aqueous phase that potentially contains the biocatalyst ruled out a simultaneous biphasic process. As a consequence, the focus was set on a sequential chemoenzymatic reaction applying heptane as solvent for the Liebeskind Srogl reaction performed at 50 °C (Figure D-4), complemented by a subsequent centrifugation step after the metal assited reaction to remove copper species prior to the enzymatic reduction.The respective reaction setting is presented in Figure D-4.

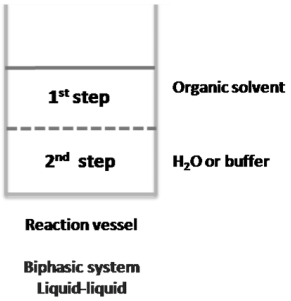
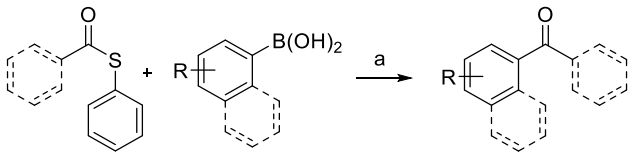


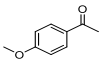
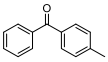
Figure D-4 Concept of a sequential liquid-liquid biphasic system

Consequently, potential ketone substrates were synthesized (Table D-8) using the specified conditions (Scheme D-8) with regard to a substrate overlap with the applied alcohol dehydrogenase from *Saccharomyces cerevisae*. Good to excellent conversions from 67-99 % were observed, depending on the electron withdrawing or donating effect of the respective substituent of the boronic acid, as well as the thioester.



Scheme D-8 Reagents and conditions: a) Boronic acid (1.1 equiv.), CuTC (1.2 equiv.), $Pd(PPh_3)_4$ (5 mol%), 50 °C, heptane

Table D-8 Synthesis of prochiral ketones applying heptane as organic phase in a sequential chemoenzymatic one pot process

Entry	Cpd. No.		Conversion (GC), 24 h [%]
1	**B-0**		99
2	**B-1**		86
3	**B-11**		99
4	**B-5**		67
5	**B-13**		89
6	**B-15**		99
7	**B-16**		99

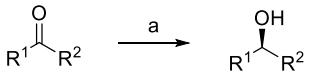
The hereby generated reaction mixtures bearing the ketone substrates (entries 1-7) for the subsequent biocatalytic reduction were applied within the liquid-liquid biphasic chemoenzymatic one pot system presented in section D.I.3.

D.I.2 Initial studies and adaption of the biotransformation applying alcohol dehydrogenase from *Saccharomyces cerevisiae*

Within this chapter, the characteristics of the applied alcohol dehydrogenase will be investigated in the context of a liquid-liquid biphasic system, which was presented above for the metal assisted step. For this approach, a key reductase from baker's yeast (*Saccharomyces cerevisiae*) was chosen. This methylglyoxal reductase (YOL151w, pIK3) has been overproduced in *Escherichia coli* as glutathione S-transferase fusion protein by the Stewart group in 2004.[64] Based on preliminary experiments conducted by Moumita Koley, which indicated that a combination within a chemoenzymatic process is feasible under certain conditions, this (*S*)-selective ADH from yeast was applied. The biocatalyst was either applied as cell free extract requiring a recycling system containing an additional enzyme (see B.IV) or as resting cells in M9 medium. Via the application of resting cells, the induction of an additional protection barrier in form of the cell wall was intended.

Initially, a temperature screening was conducted applying resting cells in M9 medium with an OD of 10, which also excludes a potential influence of glucose dehydrogenase on the overall process (

Scheme **D-9**). This screening, using the model substrate (B-0) with a concentration of 4 mM, clearly indicated decreased enzymatic activity under temperatures higher than 37 °C. Between 37 °C and 39 °C the conversion drops from 86 % to 48 % (Figure D-5).



Scheme D-9 Reagents and conditions: a) ADH, M9 medium (OD: 10)

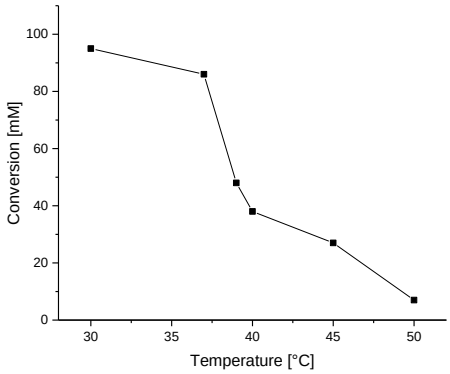


Figure D-5 Influence of the reaction temperature on enzyme performance

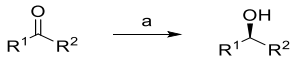
Further, intending to design a biphasic system the tolerated content of organic solvent was of interest. Initially, a system containing 50 % (v/v) organic solvent was envisaged. A variety of organic co-solvents were tested using cell free extract as well as resting cells, but the high solvent contents in general led to rapid enzyme inactivation in any case see (Table D-9). Since heptane was already investigated as second phase in a biphasic process with regard to the Liebeskind-Srogl reaction in the section above, the heptane content in the biotransformation was lowered to 20 % or 10 %, respectively. Finally, the reaction with 10 % heptane content delivered an acceptable conversion in the enzymatic reduction of 89 % when cell free extract was applied (entry 8). All approaches using resting cells gave no conversion to the desired alcohol. Thus, further experiments were conducted applying cell free extract as lyophilized powder. Consequently, a heptane content of 10 % in the chemoenzymatic process turned out to be feasible. The next step was to determine ketone substrates that can be synthesized by Liebeskind Srogl coupling and at the same time deliver good

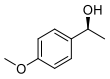
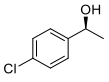
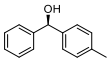
conversions within the biocatalytic step, thus a substrate screening was conducted first. Within this screening diverse highly suitable substrates were identified (Table D-10).

Table D-9 Influence of the diverse organic solvents on enzymatic reduction applying resting cells

Entry	Solvent content in H_2O	Conversion (GC), 24 h [%]
1	50 % MTBE	0
2	50 % 2-Methyl-THF	0
3	50 % Diethylether	0
4	50 % DCM	0
5	50 % Dodecane	0
6	50 % Heptane	0
7	20 % Heptane	0
8	10 % Heptane	89

Table D-10 Enzymatic reduction of potential substrates in a chemoenzyamtic one pot process (4 mM initial ketone concentration)

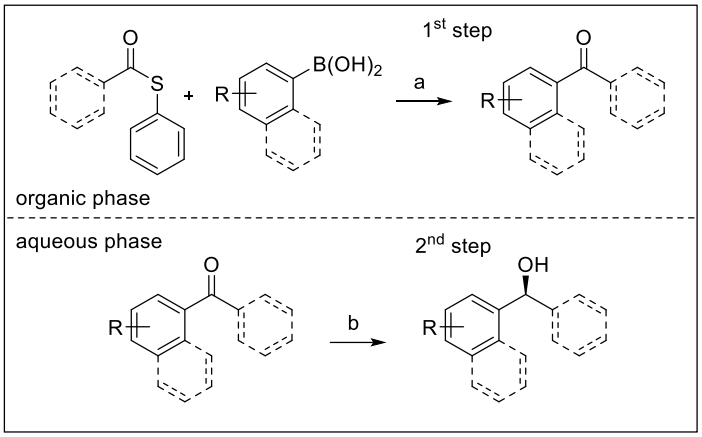


Cpd. No.		Conversion (GC), 24 h [%]	*ee* [%]
(*S*)-C-0		99	>99
(*S*)-C-1		88	>99
(*S*)-C-11		78	>99
(*S*)-C-5		100	>99
(*S*)-C-13		99	>99
(*S*)-C-15		100	n.a.
(*S*)-C-16		61	-*

*No separation observed for enantiomers of reference alcohol
Reagents and conditions: a) ADH (YOL151w, pIK3), $NADP^+$, glucose, glucose dehydrogenase

D.I.3 Design of a liquid-liquid biphasic system

Driven by these results the focus was put on a sequential chemoenzymatic one pot reaction applying heptane as solvent for the Liebeskind Srogl reaction performed at 50 °C. After centrifugation of the resulting reaction mixture, bearing the ketone that will be reduced during the subsequent biotransformation, the supernatant was added to the aqueous phase containing enzyme and buffer and the required recycling system (Scheme D-10). The attempt to combine the ADH with an initial not centrifuged reaction mixture containing acetophenone failed in any case due to the present copper salts in the coupling reaction mixture. However, as a result of a heptane content of 10 % in the chemoenzymatic process, a beneficial dilution of the metal catalyzed reaction mixture was achieved.



Scheme D-10 Reagents and conditions: a) Boronic acid (1.1 equiv.), CuTC (1.2 equiv.), $Pd(PPh_3)_4$ (5 mol%), 50 °C, heptane
b) Centrifuged coupling reaction 10 % (v/v), ADH, $NADP^+$, glucose, glucose dehydrogenase

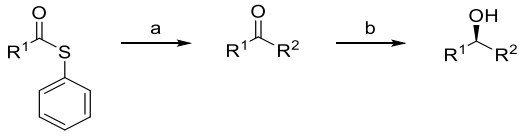
Next, the optimal concentration of the Liebeskind-Srogl coupling applied in the subsequent biotransformation was determined by adjusting different concentration in the centrifuged coupling reaction (Table D-11). Quantification was done after extraction of the overall biphasic reaction with EtOAc containing methylbenzoate as internal standard. The results show an excellent conversion of 97 % starting from 5 mM ketone substrate. Higher substrate concentrations deliver 57 % (entry 2), or 34 % conversion (entry 3). Nevertheless, using a higher lyophilisate loading, this effect can be overcome (entry 4).

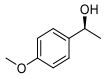
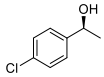
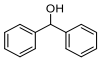
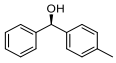
Table D-11 Sequential one pot reactions applying different concentrations of the centrifuged coupling reaction (10 % (v/v) coupling reaction in heptane, 30 °C)

Entry	Amount of cell free extract lyophilisate [mg]	Acetophenone concentration [mM]	Conversion (GC), 24 h [%]
1	10	5	97
2	10	10	57
3	10	15	34
4	20	15	74

Since a dilution of the coupling reaction was anyway mandatory within this process, we set the initial ketone concentration in the coupling reaction to 50 mM, which results in a concentration of 5 mM in the subsequent chemoenzymatic process. Now the reaction mixtures bearing different ketone substrates were utilized after the reaction mixtures were centrifuged and diluted to achieve a ketone concentration of 5 mM in the one pot process (Table D-12).

Table D-12 Sequential one pot reactions (5 mM ketone concentration, 10 % (v/v) coupling reaction, 30 °C)



Entry	Cpd. No.		Conversion (GC), 24 h [%]	*ee* [%]
1	**(*S*)-C-0**		57	>99
2	**(*S*)-C-1**		44	>99
3	**(*S*)-C-11**		63	>99
4	**(*S*)-C-5**		91	>99
5	**(*S*)-C-13**		49	>99
6	**(*S*)-C-15**		80	n.a.
7	**(*S*)-C-16**		5	-

Reagents and conditions: a) Boronic acid (1.1 equiv.), CuTC (1.2 equiv.), $Pd(PPh_3)_4$ (5 mol%), 50 °C, heptane
b) Centrifuged coupling reaction 10 % (v/v), ADH, $NADP^+$, glucose, glucose dehydrogenase

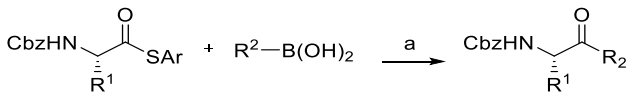
Within this liquid-liquid biphasic reaction setting, the desired alcohols were observed from 5 % to **(*S*)-C-16** (entry 7) up to 91 % conversion to **(*S*)-C-5** (entry 4) depending on the bulkiness and steric orientation of the substrates. All chiral alcohols were produced with outstanding optical purities. This development delivered the first chemoenzymatic one-pot process within this thesis. Even though a centrifugation step and a dilution of the coupling reaction were required, a successful sequential one-pot process for the production of enantiopure alcohols was achieved. The results of this section can be interpreted as proof of concept for the desired chemoenzymtic cascade reaction.

D.II Development of a liquid-solid biphasic superslurper system

The following section comprises the design of a chemoenzymatic one pot process applying a superabsorber material (sodium polyacrylate), containing the aqueous phase of the envisaged biphasic one pot system. Within this reaction setting the biocatalyst as well as the complete recycling system is embedded in the superslurper phase and benefits from the additional protecting barrier between the immiscible phases. The basic functionality of the applied material was introduced above in section B.VI (Figure B-9). Further, the design of this process implies the application of an additional Liebeskind-Srogl reaction variant running at room temperature.[21, 29]

D.II.1 Initial studies and adaption of the Liebeskind-Srogl reaction catalyzed by Tris(dibenzylideneacetone)dipalladium

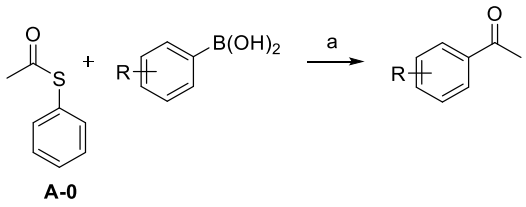
In 2007 the Liebeskind group presented a protocol for the coupling of thioesters and boronic acids which proceeds at room temperature. [21, 29] α-Amino acid thiol esters derived from *N*-protected mono-, di-, and tripeptides were coupled with aryl, π-electron-rich heteroaryl, or alkenyl boronic acids in the presence of stoichiometric amounts of CuTC and catalytic $Pd_2(dba)_3$/triethylphosphite to generate the corresponding *N*-protected peptidyl ketones in moderate to excellent yields (48-99 %) (Scheme D-11).



Scheme D-11 Reagents and conditions: a) Boronic acid (1.5 equiv.), CuTC (1.6 equiv.), $Pd_2(dba)_3$ (2.5 mol%), 20 mol% $P(OEt)_3$, RT, THF [21, 29]

This peptidyl ketone formation also proceeds under nonbasic conditions and demonstrates a high tolerance to several functionalities. Triethylphosphite plays thereby an important role as a supporting ligand by mitigating an undesired palladium-catalyzed decarbonylation-β-elimination of the α-amino thiol esters. [21] Naturally, this protocol represents an attractive option to finally solve the temperature issue we had to overcome to design a more productive sequential or even simultaneous chemoenzymatic process.

Besides the decreased reaction temperature, also a decreased thioester (**A-0**) concentration of 100 mM compared to 250 mM in the initially published variant[18] is applied within this procedure. Thus, the model substrate was synthesized utilizing the specified conditions (Scheme D-12). When THF was applied as solvent 69 % conversion was observed, while heptane gave 99 % conversion to acetophenone.



A-0

Scheme D-12 Reagents and conditions: a) Boronic acid (1.5 equiv.), CuTC (1.6 equiv.), $Pd_2(dba)_3$ (2.5 mol%), 20 mol% $P(OEt)_3$, RT

Based on these considerations, this procedure turned out to be highly interesting, since now the solvent as well as temperature issue was solved using a biphasic setting (Figure D-6).

Figure D-6 Combination issues between metal catlysis and biotransformation

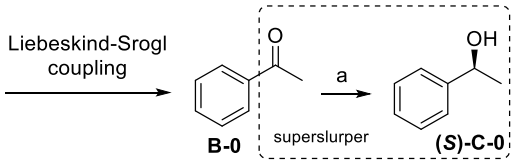
Nevertheless, the liquid-liquid biphasic process was so far limited regarding the overall content of the organic solvent in the final one pot reaction and the general incompatibility of both catalyst species. Thus, further protection of the biocatalyst was desirable. Having a closer look on the utilization of enzymes in industrial processes, their insufficient stability under processing conditions, for instance in organic solvents, poses a major problem. However, the application of enzymes in organic solvents would offer numerous advantages, such as increased solubility of hydrophobic substrates or suppression of water-dependent side reactions. Considerable effort into the development of effective strategies to increase the lifetime of enzymes in the presence of organic solvents has been made in the last decades. The approaches are based on isolation of novel enzymes functioning under extreme conditions, modification of enzyme structures to enhance their resistance or modification of the solvent environment to decrease its denaturing effect on enzymes. [118] Especially immobilization strategies proved to achieve enhanced protection of the biocatalytic species.[117-118, 128]

Inspired by the work of Jeromin[116] which comprises a successful and straightforward immobilization of an alcohol dehydrogenase, we aimed on a liquid-"solid" biphasic superslurper system.

D.II.2 Initial studies and adaption of the biotransformation applying ADH from *Saccharomyces cerevisiae*

To develop a proliferous chemoenzymatic system of this type we started with investigations of the required reaction conditions to ensure unrestricted enzymatic activity within this embedment strategy. As superabsorber sodium polyacrylate was applied. A general concept of the envisaged liquid-solid system is presented below (Scheme D-13). The crucial parameters during the design of a suitable reaction setting are presented below:

I. Ratio between organic phase and aqueous phase
II. Amount of sodium polyacrylate to ensure optimal interchangeability at the phase boundary
III. Substrate concentration within the organic phase to achieve a suitable diffusion rate into the aqueous superslurper phase
IV. Optimal distribution of enzyme and cofactor recycling system within the superslurper material



Scheme D-13 Reagents and conditions: a) Coupling reaction in heptane, ADH, NADP+, glucose, glucose dehydrogenase, sodium polyacrylate

We started our investigations by first mimicking a chemoenzymatic one pot reaction applying an embedded ADH surrounded by a heptane phase containing the respective concentration of the model substrate. Substrate concentrations are in the following calculated with regard to the overall reaction volume, even if the substrate is initially located in the organic phase. First, the substrate (**B-0**) concentration was set to 4 mM, which corresponds to the substrate concentration applied in the activity control of this enzyme. The amount of cell free extract lyophilisate was maintained (see F.XI), whereas the temperature was set to 37 °C. Based on the literature, polar solvents aside from H_2O were strictly avoided to prevent leaching effects of the superslurper phase.[117]

In the following, the term superslurper phase is used for an aqueous phase containing ADH, $NADP^+$, glucose and glucose dehydrogenase, embedded in the superslurper material sodium polyacrylate.
Using 10 % (v/v) superslurper phase surrounded by heptane under the below defined conditions only unsatisfying conversion was observed (Table D-13, entry 1). Via addition of the four fold amount of only the content of the superslurper phase (biocatalyst and the recycling system), not the polyacrylate amount, a conversion of 41 % was achieved (Table D-13, entry 2).

Table D-13 Systematic development of a superslurper system;
Determination of a suitable ratio between the two phases (4 mM ketone concentration, 37 °C)

Entry	Amount of cell free extract lyophilisate [mg]	Ratio (superslurper phase/ heptane phase)	Sodium polyacrylate amount [mg]	Conversion (GC)[a] 24 h [%]
1	10	1:9	5	12
2	40	4:6	5	41

[a] Quantification after extraction of the overall biphasic reaction with EtOAc

Next, the model substrate (**B-0**) concentration was increased to 16 mM following the enhanced amount of superslurper phase content. Further, by enhancing the substrate concentartion in the heptane phase we aimed on a suitable diffusion rate into the aqueous superslurper phase (Table D-14, entry 1). In addition, the influence of the sodium polyacrylate amount was studied (Table D-14, entries 2, 3) and applying the initial amount of superabsorber material, a conversion of 81 % to 1-phenylethanol was monitored (Figure D-7). Consequently, we set the sodium polyacrylate amount to 5 mg for further investigations.

Table D-14 Systematic development of a superslurper system (40 mg cell free extract lyophilisate, Ratio (superslurper phase/heptane phase) 4:6, 37 °C)

Entry	Amount of sodium polyacrylate [mg]	Acetophenone concentration [mM]	Conversion (GC), 24 h [%]
1	5	16	81
2	7	16	64
3	10	16	49

[a] Quantification after extraction of the overall biphasic reaction with EtOAc

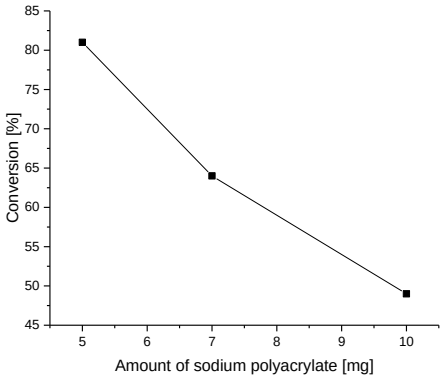


Figure D-7 Investigation of sodium polyacrylate amount containing the biocatalytic reaction of the cascade

To determine if the reaction is completed after 24 hours, a time course was performed using the biphasic reaction presented in Table D-14 (entry 1). Since an extraction of the overall reaction mixture was required, it was performed in triplicate and extracted after 18, 24 and 72 hours, which indicated that no further product formation occured after 24 h. To push the reaction further, a second equal superslurper phase, containing exactly the same amounts of biocatalyst as well as components of the recycling system, was added after 24 h, which led to a conversion of 91 % within 48 h. When the two phases were added from the beginning (T_0), the desired alcohol was observed with 88 % conversion after 48 h. This result indicates an effective protection of the biocatalyst against the inactivating surrounding of the filled superslurper over a period of 48 h.

Moreover, we were interested in converting higher substrate concentrations, which was studied starting from 32 mM and 48 mM **B-0**, respectively. In comparison with the reaction that gave 81 % conversion starting from 16 mM substrate concentration (Table D-14, entry 1), the results of this concentration study are demonstrated in Figure D-8. Thus, the system was suitable to convert concentrations up to 48 mM.

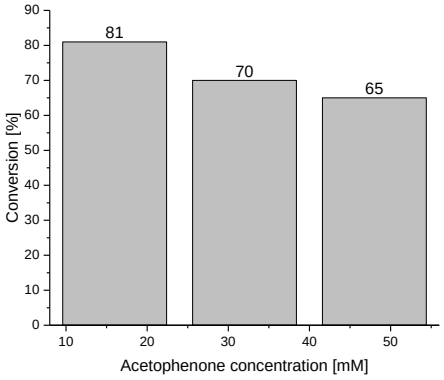
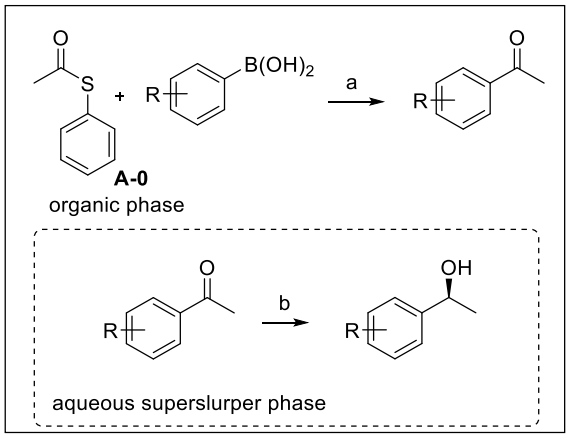


Figure D-8 Variation of the model substrate concentration

Moreover, the content of $NADP^+$ was doubled to verify a sufficent disposability within the superslurper phase. Since, this did not improve the conversion; the $NADP^+$ was used in the initial concentration of 1.2 mM.

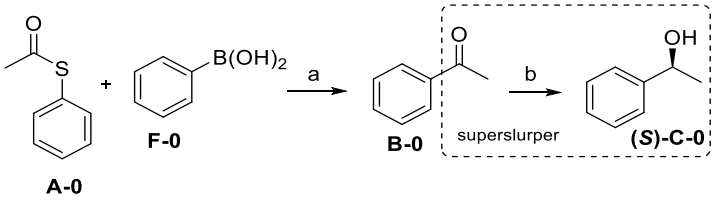
D.II.3 Design of a liquid-solid biphasic superslurper system applying an alcohol dehydrogenase

This chapter focusses on the development of a liquid-solid superslurper system, now applying the real coupling reaction as organic phase. The general concept of the novel chemoenzymatic one pot system is presented below.



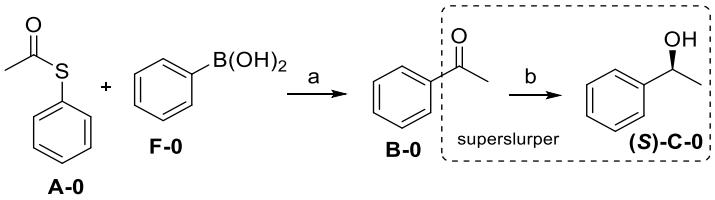
Scheme D-14 Reagents and conditions: a) CuTC, $Pd_2(dba)_3$, $P(OEt)_3$, 50 °C, heptane
b) ADH, NADP+, glucose, glucose dehydrogenase, sodium polyacrylate

Based on the preliminary results (D.II.2, Table D-14, entry 1), the developed reaction setting and conditions (5 mg sodium polyacrylate, 40 mg cell free extract lyophilisate, ratio superslurper phase/heptane phase (4:6)) were applied and a sequential combination of metal- and enzyme catalyzed reaction step was envisaged first. To create similar conditions to the developed liquid-liquid system, a centrifuged coupling reaction catalyzed by $Pd(PPh_3)_4$ (F.VI.1.1), adjusting **B-0** in a concentration of 16 mM, was used. This gave a conversion of 37 % in a first attempt. By addition of a second supersluperphase at T_0 (start of the reaction), the reaction delivered the alcohol **C-0** in a conversion of 81 %.



Scheme D-15 Reagents and conditions: a) Boronic acid, CuTC, $Pd(PPh_3)_4$, 50 °C, heptane
b) ADH, NADP+, glucose, glucose dehydrogenase, sodium polyacrylate

However, even more successful was the application of the $Pd_2(dba)_3$ catalyzed coupling reaction mixture (F.VI.2.1). This reaction yielded in a conversion of 74 % over two steps (Scheme D-16) with an *ee* of >99. Since in this case 99 % conversion was observed within the coupling procedure, a concentration of 59 mM (60 % heptane content) of **B-0** was successfully converted using the superslurper system. Thus, immobilizing the ADH using sodium polyacrylate enables higher substrate loadings as well as an extremely high content of organic solvent (60 %).



Scheme D-16 Reagents and conditions: a) CuTC, $Pd_2(dba)_3$, $P(OEt)_3$, 50 °C, heptane
b) ADH, NADP+, glucose, glucose dehydrogenase, sodium polyacrylate

All attempts using an initial, untreated coupling reaction failed, since copper was accumulating in the lower aqueous superslurper phase (see blue discoloration in Figure D-9) and led thereby to complete enzyme inactivation.

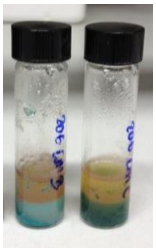
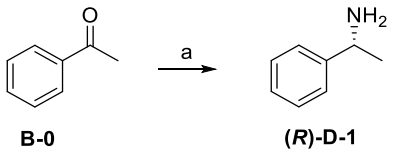


Figure D-9 Copper accumulation within the aqueous superslurper phase

D.II.4 Design of a liquid-solid biphasic superslurper system applying an ω-Transaminase

To demonstrate the versatility of the developed system, we were interested in a chemoenzymatic process utilizing ω-transaminases (ATAs) as biocatalytic species. Thus, for three ω-transaminases out of the Codexis screening kit (received from the Bornscheuer group) an activity test was conducted (Scheme D-17, Table D-15).



Scheme D-17 Reagents and conditions: a) ω-ATA (codexis screening kit), PLP, isopropylamine, triethanolamine

Table D-15 Comparison of different (*R*)-selective ω-transaminases

Applied ω-ATA	Conversion (GC)[a], 24 h [%]	*ee*[b], [%]
ATA 025	86	>99
TA P2- A07	75	>99
ATA 033	78	>99

[a] Quantification after pH-value adjustment to ≥11
[b] Determined after acetylation with acetyl chloride

Following the strategy above, a mimicked chemoenzymatic one pot reaction applying embedded ATA025 and ATA033, surrounded by a heptane phase containing the respective concentration of **B-0** was utilized. Since ATA025 delivered the most promising results (Table D-16, entry 2), the substrate concentration was increased to 50 mM, yielding in a decreased conversion of of 31 %.

Table D-16 Systematic development of a superslurper system,
20 mg ATA, ratio (superslurper phase/heptane phase) 4:6, 37 °C, 5mg sodium polyacrylate)

Entry	Applied ATA (codexis)	Acetophenone concentration[mM]	Conversion (GC), 24 h [%]
1	033	10	69
2	025	10	72
3	025	50	31

[a] Quantification after after pH-value adjustment to ≥11
and extraction of the overall biphasic reaction with EtOAc

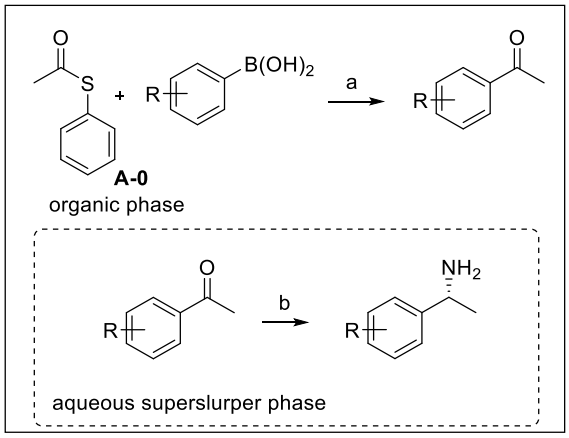
Moreover, to study the influence of the components of the Liebeskind-Srogl coupling reaction, the single components of the coupling catalyzed by $Pd_2(dba)_3$ were added to the transamination which in general delivers methylbenzylamine in a conversion of 86 % (F.XIV.1). Moreover, a biphasic system containing 60 % heptane content was applied without any further additives.

Table D-17. Preliminary studies of ATA 025 regarding a combination with a Liebeskind-Srogl coupling reaction (10 mM ketone concentration, 37 °C)

Entry	Addition of	Conversion (GC)[a] 24 h [%]
1	*S*-phenyl thioacetate [100 mM]	0
2	*S*-*tert*-butyl thioacetate [100 mM]	62
3	Phenylboronic acid [170 mM]	0
4	$Pd_2(dba)_3$ [2.5 mol %]	78
5	Triethylphosphite [20 mol %]	8
6	Triethylphosphite [10 mol %]	18
7	Thiophene-2-carboxylic acid [160 mM]	47
8	Heptane [60 %]	59

[a] Quantification after pH-value adjustment to ≥11 and extraction of the overall biphasic reaction with EtOAc

This screening clearly indicated that the presence of *S*-phenyl thioacetate (**A-0**), triethylphosphite and phenylboronic acid pose a major problem in this transamination reaction. As a consequence, the thioester was exchanged to *S*-*tert*-butyl thioacetate (**A-1**) and the $Pd(PPh_3)_4$ catalyzed coupling reaction was utilized in the following. Since the phenylboronic acid was crucial for the process, an exchange was not feasible but applying a biphasic system, the phenylboronic acid (**F-0**) is potentially predominantly located in the organic phase. A general concept of the reaction setting is presented in Scheme D-18. Thus, using a doubled amount of the biocatalyst, first the centrifuged coupling reaction (F.VI.1.1) was utilized as organic phase and the embedded ATA025 along with the respective mastermix as aqueous phase (Table D-18, entry 1, 2).



Scheme D-18 Reagents and conditions: a) Boronic acid, CuTC, $Pd(PPh_3)_4$, 50 °C, heptane
b) ω-transaminase, codexis mastermix (0), sodium polyacrylate

When the **B-0** concentration was set to 16 mM, a conversion of 51 % was achieved. A higher substrate loading was not converted efficiently. However, in any case outstanding optical purity was observed for the amine product. However, the Bornscheuer group conducted preliminary experiments indicating a certain tolerance of the transaminases against copper ions. Even when a simultaneous process could not be executed effectively using this superslurper system, we were interested in applying the untreated, not centrifuged coupling reaction in a one pot process generating enantiopure amines. Thus, the reaction was performed with untreated coupling reactions holding different acetophenone concentrations (Table D-18).

Table D-18 Sequential liquid-solid biphasic system for the production of amines, coupling reaction, 40 mg ATA025, ratio (superslurper phase/heptane phase) 4:6, 37 °C, 5mg sodium polyacrylate)

Entry	Form of applied coupling reaction	Acetophenone concentration [mM]	Conversion (GC)[a] 24 h, 48[b] h [%]
1	centrifuged	16	51
2	centrifuged	32	31
3	untreated	16	27
4	untreated	32	19
5	untreated	44	0
6	untreated	16	47[b]

[a] Quantification after after pH-value adjustment to ≥11 and extraction of the overall biphasic reaction with EtOAc
[b] Addition of second superslurper phase after 24 h, 48 h reaction time

Thereby, the best result was achieved starting from 16 mM concentration of **B-0** by addition of a second superslurper phase after 24 h and an overall reaction time of 48 h (Table D-18, entry 6).

D.III Development of a liquid-liquid membrane based system

Regarding a successful simultaneous process the problem of rapid enzyme inactivation by present copper ions was not solved so far. To overcome this issue, a strict spatial separation of copper species and biocatalyst was obligatory. Therefore a reaction setting employing a semipermeable membrane to ensure this separation was intended (Figure D-10).

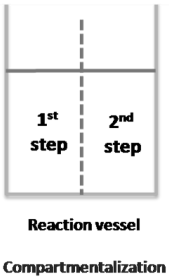


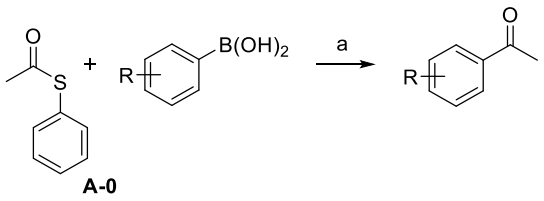
Figure D-10 Concept of compartmentalization by application of a semipermeable mebrane

As already introduced, a promising material for the design of a chemoenzymatic one pot process like this is polydimethylsiloxane (PDMS), which allows the diffusion of lipophilic compounds, in contrast to metal ions, that are not able to pass the membrane[119-121, 129]. Utilizing this strategy in combination with the coupling reaction at room temperature, it was possible to address all isues at once to design an effective chemoenzymatic one pot process in a simultaneous fashion (Figure D-11).

Figure D-11 Combination issues between metal catlysis and biotransformation

D.III.1 Continual studies and adaption of the Liebeskind-Srogl reaction catalyzed by Tris(dibenzylideneacetone)dipalladium with regard to a membrane based system

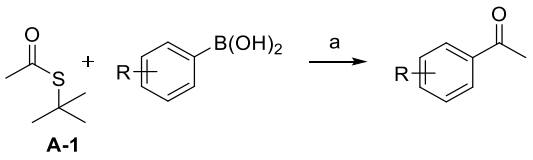
Using the coupling procedure as part of a membrane based system implies a complete change of the reaction setting. To perform the metal catalyzed ketone synthesis in organic solvent results in a good solubility of substrates and product and complies with the original reaction conditions[18]. However, polydimethylsiloxane is swelling against various organic solvents[130] and thereby looses its functionality, which means an effective separation of both reaction chambers can not be assured anymore. Consequenetly, we were interested in identifying a solvent or solvent mixture that does not lead to membrane swelling and thereby to the diffusion of copper ions through the membrane and further it should of course deliver satisfying results in the coupling reaction. The first attempt was to perform the ketone synthesis in H_2O according to the initial approach of the single phase reaction. To ensure a certain substrate solubility 10 % *i*-PrOH was added (Scheme D-19). Within this reaction a yield of only 28 % was isolated, which was not expected since die GC chromatogram indicated a complete consumption of **A-0**. To investigate the influence of the applied aqueous solvent system, the coupling reaction was conducted both in H_2O with 10 % i-PrOH and in THF. The reaction performed in the aqueous solvent system led to 41 % conversion to acetophenone and an overall recovery of thioester and ketone of 45 % determined by GC. In contrast, the Liebeskind-Srogl coupling performed in THF gave 69 % conversion to acetophenone and an overall recovery of starting material and product of 83 %. Thus, it was likely that the starting material (**A-0**) suffers from hydrolysis under these reaction conditions.



Scheme D-19 Reagents and conditions: a) Boronic acid (1.5 equiv.), CuTC (1.6 equiv.), $Pd_2(dba)_3$ (2.5 mol%), 20 mol% $P(OEt)_3$, H_2O/*i*-PrOH, RT

Consequently, hydrolysis studies for **A-0** and **A-1** were conducted by mimicking a coupling reaction applying the reaction conditions determined above omitting the Pd catalyst. Starting from **A-1** 80 % starting material was detected after 24 h, in case of **A-0** only 1 % could be recovered after this time period. Consequently, S-*tert*-butyl thioacetate (**A-1**) served henceforth as starting material.

Moreover, in the following experiments conducted in the aqueous solvent system not only conversion to product is given, but also the recovery to survey potential hydrolysis. By exchange of the thioester we observed a conversion of 78 % with full recovery (Scheme D-20, Table D-19, entry 1). Thus, the reaction conditions presented in Scheme D-20 were specified as prospective standard conditions.



A-1

Scheme D-20 Reagents and conditions: a) Boronic acid (1.5 equiv.), CuTC (1.6 equiv.), $Pd_2(dba)_3$ (2.5 mol%), 20 mol% $P(OEt)_3$, RT

Table D-19 Solvent screening for the coupling reaction using *S-tert*-butyl thioacetate as starting material in polar, protic and aprotic solvents for a potential application in PDMS membrane reactor

Entry	Solvent content in H_2O	Conversion (GC), 24 h [%]	Recovery [%]
1	10 % *i*-PrOH	78	100
2	50 % *i*-PrOH	30	100
3	10 % EtOH	69	76
4	100 % EtOH	74	94
5	100 % Polyethylene glycol	25	47
6	10 % THF	85	85
7	10 % DMSO	61	66
8	10 % Dioxane	57	71
9	10 % DMF	56	72
10	10 % DMPU	54	70

Driven by this promising result, the coupling reaction was conducted in both protic and aprotic polar solvents and in mixtures with H_2O, with respect to potential PDMS membrane compatibility. *S-tert*-Butyl thioacetate, phenyl boronic acid, CuTC, triethylphosphite and $Pd_2(dba)_3$ were stirred in the respective solvent mixture for 24 h at room temperature (Scheme D-20, Table D-19). The screening with protic solvents delivered the best result in a mixture of H_2O and 10 % (v/v) *i*-PrOH (Table D-19, entry 1) followed by pure EtOH (entry 4). Further, a high recovery was obtained in these cases, which indicates a low occurrence of thioester hydrolysis. Applying aprotic cosolvents (entries 6-10), the best conversion (85 %) was achieved using 10 % THF in H_2O (entry 6), but only 85 % of the material was recovered in this case, which consequently points towards starting material hydrolysis to a certain extent.

Moreover, the influence of the triethylphosphite was investigated, since this compound was assumed to be critical regarding the combination with a biocatalyst, which indicated that 20 mol% $P(OEt)_3$ are required in the coupling procedure at room temperature (Table D-20).

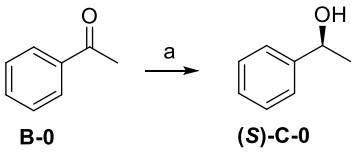
Table D-20 Influence of triethylphosphite amount on the Liebeskind Srogl coupling

$P(OEt)_3$ amount [mol%]	Conversion (GC), 24 h [%]
5	52
10	56
20	76

D.III.2 Initial studies and adaption of the biotransformation applying ADH from *Rhodococcus ruber*

In 2003 the Faber group purified and characterized the novel secondary alcohol dehydrogenase (ADH-A), that was introduced in section B.IV.1. This ADH from *Rhodococcus ruber* provides an outstanding solvent and temperature stability.[65] With respect to the idea of a chemoenzymatic one pot process, performed in two chambers, applying *i*-PrOH as cosubstrate in the coupling reaction, these characterisics offer great opportunities. In comparison with the ADH from *Saccharomyces cerevisiae*, this biocatalyst is much more appropriate for the purpose of a chemoenzymatic process since a second enzyme is not required within the enzymatic reduction.

First of all, to exclude general incompatibilities the single components of the coupling reaction were added to an enzymatic reduction (Scheme D-21) in the respective concentrations present in the Liebeskind-Srogl coupling (Table D-21). Moreover, a substrate concentration of 100 mM was investigated to work with the ketone concentration that will be present in the case of full conversion within the metal catalyzed step.



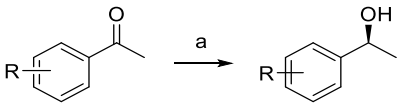
Scheme D-21 Reagents and conditions: a) ADH, *i*-PrOH, Tris-HCl

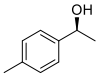
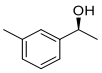
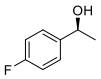
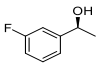
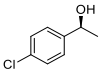
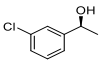
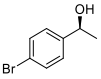
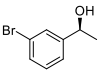
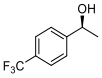
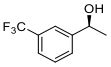
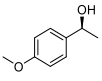
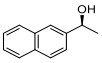
Table D-21 Preliminary studies of ADH-A regarding a combination with a Liebeskind-Srogl coupling reaction; Addition of components from the coupling reaction (5 mg whole cell lyophilisate, 100 mM substrate concentration, 16 % (v/v) *i*-PrOH, Tris-HCl buffer pH 7.5, 50 mM 30 °C)

Entry	Addition of	Conversion (GC), 4 h [%]
1	S-*tert*-butyl thioacetate [100 mM]	95
2	Phenylboronic acid [170 mM]	93
3	Triethylphosphite [20 mol%]	82
4	CuTC [160 mM]	0
5	CuTC [100 mM]	0
6	CuTC [160 mM], EDTA [160 mM]	0
7	$Pd_2(dba)_3$ [2.5 mol%]	70

These results clearly indicated that ADH-A is in general compatible with all components of the Liebeskind-Srogl coupling reaction except with CuTC. This is what was observed for all applied alcohol dehydrogenases in any combination strategy. However, the employment of a PDMS membrane would solve this crucial issue. Next, a substrate screening was conducted with respect to a potential substrate overlap with the coupling reaction (Scheme D-21).

Table D-22 Enzymatic reduction of prochiral ketones (100 mM substrate concentration)



Entry	Cpd.No. (*S*)-alcohol		Conversion (GC) 24 h [%]	*ee* [%]
1	**C-0**		96	>99
2	**C-1**		90	>99
3	**C-2**		85	>99
4	**C-3**		99	>99
5	**C-4**		95	>99
6	**C-5**		97	>99
7	**C-6**		92	>99
8	**C-7**		98	>99
9	**C-8**		99	>99
10	**C-9**		99	>99
11	**C-10**		99	>99
12	**C-11**		99	0
13	**C-0**		91	>99
14	**C-14**		77	>99

Reagents and conditions: a) ADH, *i*-PrOH, Tris-HCl

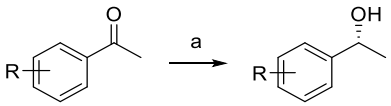
In general, excellent conversions to the chiral alcohols were observed even with this high substrate concentration. Depending on the bulkiness of the ketone substrates und the orientation of the substituents, the desired alcohol was prouced in lower amounts (Table D-22, entries 3 & 14) due to the respective fit into the active side of the enzyme. Moreover, ADH-A can be used as whole cell lyophilisate, which enables high enzyme concentrations and a storage period up to several months.

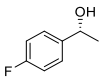
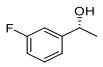
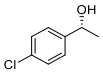
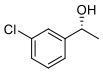
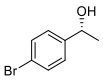
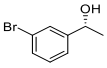
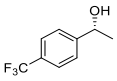
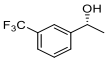
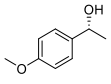
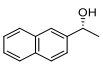
D.III.3 Initial studies and adaption of the biotransformation applying ADH from *Lactobacillus kefir*

Based on these promising results, it was now interesting to identify an enantiodivergent counterpart to the solvent stable (*S*)-selective ADH-A. Thus, the ideal candidate would be a strictly (*R*)-selective ADH that efficiently produces secondary alcohols via coupled substrate approach, provides a certain tolerance against *i*-PrOH and that can be applied in the form of whole cell lyophilisate. Therefore, the (*R*)-selective ADH from *lactobacillus kefir* was studied more intensively. First, it was tested if the enzyme, that was in-house only used as cell free extract (CFE), is able to convert a concentration of 100 mM acetophenone, when it is applied as whole cell lyophilisate. In a first shot experiment a conversion of 78 % was observed after 24 hours. This experiment was conducted using the same conditions as for ADH-A (see sectionF.IX). By simply doubling the amount of whole cell lyophilisate to 20 mg in 500 µl reaction volume, a conversion to the enantiopure (*R*)-alcohol of 95 % was achieved in the presence of 16 % (v/v) *i*-PrOH (Table D-23, entry 1). To sum up, this ADH was most promising as enantiocomplementary counterpart for ADH-A in a chemoenzymatic one pot process. Consequently, a substrate screening was performed to check if there is a substrate overlap with both, Liebeskind-Srogl coupling and the enzymatic reduction catalyzed by ADH-A (Table D-23).

The ADH from *lactobacillus kefir* is clearly less efficient for *m*-substituted aromatic ketone substrates, again depending on the bulkiness of the substituents and their orientation (see entries 3, 9, 11-14). This can be explained by sterical characteristics, impeding access to the active side of the enzyme. However, the enzymatic reduction delivered in general very good conversion up to 99 %. Further in all cases, where conversion was observed an *ee* of >99 % was determined.

Table D-23 Enzymatic reduction of prochiral ketones (100 mM substrate concentration)



Entry	Cpd.No. (*R*)-alcohol		Conversion (GC), 24 h [%]	*ee* [%]
1	**C-0**		95	>99
2	**C-1**		83	>99
3	**C-2**		17	>99
4	**C-3**		83	>99
5	**C-4**		91	>99
6	**C-5**		90	>99
7	**C-6**		88	>99
8	**C-7**		90	>99
9	**C-8**		45	>99
10	**C-9**		99	>99
11	**C-10**		0	-
12	**C-11**		40	>99
13	**C-0**		0	-
14	**C-14**		0	-

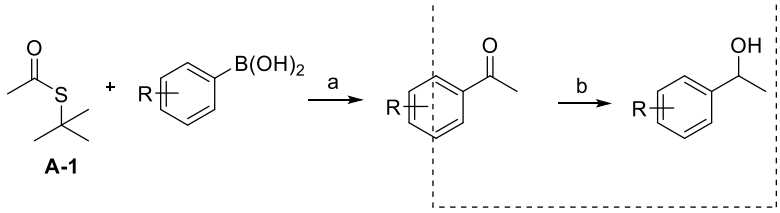
Reagents and conditions: a) ADH, *i*-PrOH, Tris-HCl

D.III.4 Chemoenzymatic one pot reactions in PDMS membrane reactor

In spite of concentration, temperature and solvent issues that were overcome at this point, a separation of CuTC from the biocatalytic reaction was still obligatory. Inspired by the work of Miller et al. [129b] and the Gröger group[120], we aimed on the application of a hydrophobic polydimethylsiloxane membrane, that prevents the contact of the biocatalyst with the strongly enzyme inhibiting CuTC, while lipophilic species, particularly the ketone substrate should retain the ablility to pass the membrane. Based on the method of Miller et al.[129a] PDMS thimbles were prepared (Figure D-12). The procedure is described in detail in section F.III.

Figure D-12 PDMS coated vials holding an aluminium foil attachment for later removal from the glas mould

In a first attempt, one of the prepared thimbles was filled with ADH-A whole cell lyophilisate in Tris-HCl buffer (50 mM, pH 7.5) and 16 % (v/v) *i*-PrOH. The thimble was attached utilizing safety pin and thread in a larger reaction vial that contained the coupling reaction in H_2O/*i*-PrOH (9:1) generating the model substrate (Scheme D-22, Figure D-13).



Scheme D-22 Reagents and conditions a) CuTC, $Pd_2(dba)_3$, $P(OEt)_3$, H_2O/*i*-PrOH, b) ADH-A, *i*-PrOH, Tris-HCl

Figure D-13 First chemoenzymatic one pot reaction setting

To our delight, this first simultaneous reaction delivered a conversion of 51 % to **(*S*)-1-C-0**. However, upon repeated usage of the thimble, it quickly became apparent that this design has some substantial limitations with respect to stability. Hence, a more convenient membrane-based one-pot reaction setting was developed for future investigations. With the support of Horst Lindenlaub the below presented PDMS membrane reactor (Figure D-14) was designed and manufactured. This reactor enables shaking with a high rpm number, it separates the chambers in an optimal way by providing some additional support to the membrane and, moreover, it provides the opportunity to sample from both chambers individually.

Figure D-14 Novel membrane reactor

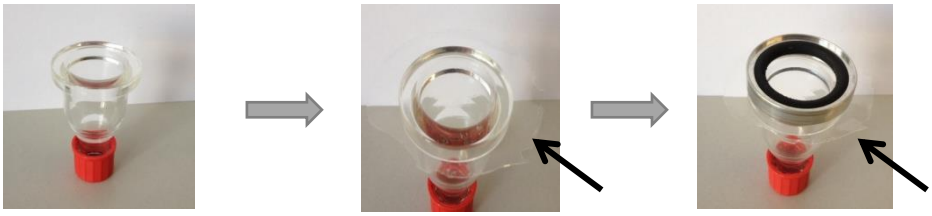


Figure D-15 Attachment of the PDMS Membrane (see black arrows) between the reaction chambers

Applying this novel PDMS reactor, a "mimicked" simultaneous membrane-reactor transformation was conducted. Therefore, one chamber was filled with ADH-A, Tris-HCl buffer and *i*-PrOH and the other chamber was charged with the potential solvent for the coupling reaction and the model substrate (**B-0**). This also gave insigths into the diffusion grade of the ketone substrate into the enzymatic chamber. The most promising solvent mixtures for the coupling reaction, identified in section D.III.1 (Table D-19) were consequently studied (Table D-24). Since100 % EtOH was not acceptable with regard to PDMS membrane swelling and therefore inactivation of the enzyme by high solvent concentration and accumulation of Cu species in the enzymatic chamber, we focused on a solvent content of 10 % in the chamber of the coupling reaction. Best results were achieved using 10 % *i*-PrOH or 10 % THF, whereas in the mixture of *i*-PrOH and H_2O, a full recovery of the starting material was observed. Thus the focus was set on this solvent system for the Liebeskind-Srogl reaction chamber.

Table D-24 Solvent studies for potential application in Liebeskind-Srogl coupling reaction chamber in membrane rector

Potential solvent system for coupling reaction chamber	Conversion (GC), 24 h [%]
100 % EtOH	0
10 % THF in H_2O	83
10 % *i*-PrOH in H_2O	80

Consequently, a chemoenzymatic one pot reaction generating 1-phenylethanol (**C-0**) (Scheme D-22) was performed using the same conditions applied in the reaction setting of the thimble. In this approach a slightly enhanced conversion (60 %) to the alcohol was observed, again using ADH-A as biocatalyst. A conversion in the same range was also achieved using the enantiocomplementary ADH from *lactobacillus kefir* (55 %), which indicated that also this enzyme can be used within the developed process.

After determination of the pH value in the individual reaction chambers, which was 5.2 in the enzymatic chamber and 4.8 in the chamber of the coupling reaction, which clearly led to complete enzyme inactivation, we were interested in a suitable Tris-HCl buffer concentration to adjust for the impact of present thiophenecarboxylic acid (160 mM) and boronic acid (170 mM). Thus, a screening utilizing different buffer concentrations was performed. However, the lowest effective concentration was consequently 330 mM. A reference reaction applying Tris-HCl concentrations of 50 to 350 mM and both ADHs indicated no influence on enzymatic activity. Conversions from 94-97 % were monitored after 4 hours reaction time.

Consequnetly, the initial chemoenzymatic one pot reaction utilizing a Tris-HCl buffer concentration of 350 mM was performed in the novel membrane reactor. Now a conversion of 71 % was achieved. Holding this promising process in hands, the substrate scope for this simultaneous chemoenzymatic one pot process was expanded and thereby several enantiodivergent alcohols were synthesized without any intermediate step in yields from 37-99 % with perfect optical purities. The results presented in Table D-25 are givenover two steps and reflect the influence of both, the coupling reaction and enzymatic reduction.

Table D-25 Simultaneous chemoenzymatic one pot reactions generating enantiopure alcohols

Cpd. No.	Product (*S*) or (*R*)-alcohol	ADH-A Conversion [%][a] (*S*)-alcohol	*ee* [%]	LK-ADH Conversion [%][a] (*R*)-alcohol	*ee* [%]
C-0	1-(Phenyl)ethan-1-ol	65	99	81	99
C-3	1-(4-Fluorophenyl)ethan-1-ol	75	99	64	99
C-5	1-(4-Chlorophenyl)ethan-1-ol	60	99	57	99
C-6	1-(3-Chlorophenyl)ethan-1-ol	47	99	53	99
C-7	1-(4-Bromophenyl)ethan-1-ol	99	99	50	99
C-9	1-(4-Trifluormethylphenyl)ethan-1-ol	61	99	53	99

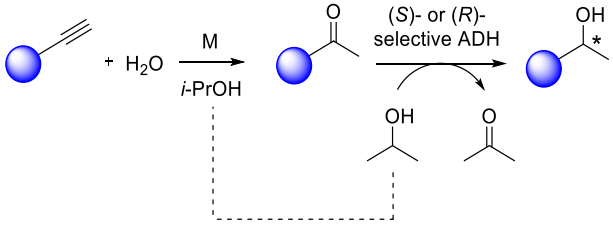
[a] Dilution with AcCN containing an internal standard and treated with metal scavenger, Conversions determined by UPC^2

D.IV Development of an Au(III) based single phase system

D.IV.1 Au(III) catalyzed hydration of alkynes

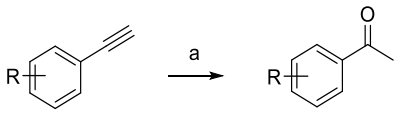
The synthesis of chiral molecules is nowadays not only reviewed in the sense of general feasibility, but also environmental and sustainable aspects are of high relevance [108, 131]. This equally applies for chemoenzymatic one pot cascades generating chiral compounds. As already discussed, the most challenging task is to arbitrate between the two parties of metal- and biocatalysis by finding a common ground with respect to general reaction conditions of the individual steps. Consequently, the choice of the metal catalyzed cascade step must take this into account to end up in a successful combination with a biocatalyst.

Since spatial separation of the copper species was obligatory for the previously described one pot procedures (F.XV.1, F.XV.2 F.XV.4), we were interested in a metal species that does not shut down the enzymatic activity being present at very low concentrations. Thus, the choice of the catalytic metal species for ketone synthesis was reconsidered with regard to an easier combination strategy. The core idea behind the new cascade reaction was to perform the metal catalyzed step in a solvent that serves as cosubstrate in the subsequent biotransformation. More precisely, the resulting reaction mixture from the metal catalyzed transformation comprises both the substrate, and at the same time, the required cosubstrate for the enzymatic step. As an alternative to the Liebeskind Srogl coupling, another excellent way to get access to the respective methylketone intermediates is the nucleophilic addition of water or alcohols to alkynes.[33] Further, these hydration reactions are classically conducted in alcoholic solvents.[37, 47a, 132] On the reverse side, a prominent example for an economical attractive cosubstrate is *i*-PrOH, which opens the opportunity to use alcohol dehydrogenases accepting *i*-PrOH as cosubstrate for the desired subsequent enzymatic reduction (see Scheme D-23).



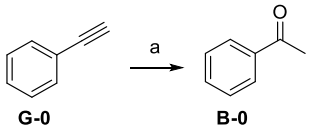
Scheme D-23 Envisaged chemoenzymatic one pot process using *i*-PrOH as solvent for the 1st cascade step and cosubstrat for the 2nd cascade step

The hydration reactions of unactivated alkynes to the respective ketones were in general connected with a large access of acidic reagent,[36] which is obviously critical regarding a potential combination with a biocatalyst. However, in 2015 Das et al. demonstrated for the first time a ligand- and acid-free procedure for the synthesis of methylketones via hydration of terminal alkynes, which is catalyzed by gold(I) chloride. In addition to phenylacetylene (**G-0**), other aliphatic and aromatic terminal alkynes, including those bearing functional groups such as alkoxy, chloro, bromo, fluoro, hydroxy, and tertiary amine, underwent hydration, affording moderate to excellent yields (Scheme D-24). Das et al. presented 14 examples with 20-98 % yield [36].



Scheme D-24 Reagents and conditions: a) H_2O, AuCl, MeOH, 65 °C

We took up this procedure and conducted some preliminary studies regarding solvent, temperature and oxidation state of the applied gold catalyst again producing **B-0** as model compound (Scheme D-25). First, we aimed at changing the catalyst to a gold(III) species, because this oxidation state is the most stable one, which is advantageous regarding the core idea of a chemoenzymatic one pot process. Further, we focused on *i*-PrOH as solvent, since this offers the opportunity to generate a reaction mixture, which can in total be used for a following enzymatic reduction, as substrate as well as cosubstrate are contained after the hydration reaction. Thus, by performing the hydration of **G-0** in *i*-PrOH applying $AuCl_3$ as catalyst, we were pleased to observe a conversion of 98 % to **B-0**.



Scheme D-25 Reagents and conditions: a) H_2O, $AuCl_3$, *i*-PrOH, 65 °C

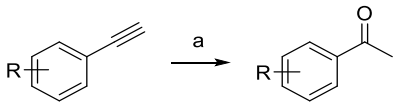
This result was used as starting point for further investigations regarding a future chemoenzymatic one pot process. Since, these studies (Table D-26) indicated a lower conversion in the hydration reaction, when either the reaction temperature is decreased to physiological conditions (37 °C), the reaction mixture is diluted to 100 mM substrate concentration, or the overall *i*-PrOH amount is decreased to a well-acceptable amount for the respective alcohol dehydrogenases (30 %), we decided to focus on a sequential chemoenzymatic process.

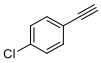
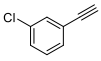
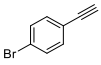
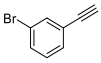
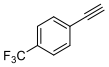
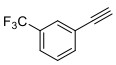
Table D-26 Variation of different reaction parameters in hydration of alkynes

Entry	Substrate concentration [mM]	*i*-PrOH content in H_2O [%]	Temperature [C°]	Conversion (GC), 24 h [%]
1	500	97	37	81
2	100	97	65	50
3	500	30	65	18

Additionally, the reaction was performed in 1-butanol, which turned out to be a suitable solvent for the hydration of **G-0**, resulting in 83 % conversion and at the same time offers the opportunity to serve as second phase in a potential biphasic chemoenzymatic system due to the very low water solubility. This approach was not successful due to enzyme inactivation caused by the presence of 1-butanol (see D.IV.2). Driven by these results, we investigated the substrate scope applying the modified conditions, and thereby synthesized a library of prochiral ketones starting from acetylenes bearing different substitution patterns. The modified hydration procedure gave moderate to excellent conversion (49-99 %) depending on the alkyne structure (Table D-27). **B-0, B-1** and **B-2** were observed in excellent conversions. Aromatic acetylenes bearing a halogenide gave in general lower conversions (70-86 %), especially the *para*-substituted substrates. Acetylenes carrying a trifluoromethyl group (**G-9, G-10**), clearly exhibiting the strongest –I effect, were observed only in moderate conversions (49-57 %).

Table D-27 Synthesis of prochiral ketones applying modified reaction conditions



Substrate Cpd.No.		Product Cpd.No.	Conversion (GC), 24 h [%][a]
G-0		**B-0**	98
G-1		**B-1**	98
G-2		**B-2**	99
G-3		**B-3**	71
G-4		**B-4**	86
G-5		**B-5**	72
G-6		**B-6**	76
G-7		**B-7**	70
G-8		**B-8**	79
G-9		**B-9**	49
G-10		**B-10**	57

[a] Reagents and conditions: a) H_2O, $AuCl_3$, *i*-PrOH, 65 °C

D.IV.2 Optimizing conditions for the transformation with ADH from *Rhodococcus ruber*

Furthermore, the ADH was investigated with respect to the new requirements within this process. In the following, the biocatalytic reduction was conducted with a substrate concentration of 500 mM (Table D-28,entry 1), an *i*-PrOH content of 80 % (v/v) (entry 2) and in the presence of **G-0** with a concentration of 100 mM (entry 3). These results pointed out that neither this high substrate loading, nor the presence of the starting material of the hydration reaction causes a major problem, in contrast to an i-PrOH content of 80 %, which led to only 5 % conversion.

Moreover, the feasibility of a biphasic system was studied by addition of alcohols with low solubility in water as potential phase for the hydration reaction (entries 5-8) and also as potential cosubstrate for the ADHs (entry 7) instead of *i*-PrOH. However, this did not deliver promising results, thus a single phase system applying *i*-PrOH and buffer was envisaged.

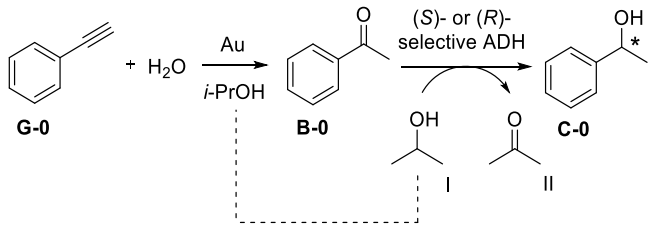
Table D-28 Preliminary studies of ADH-A regarding the combination with a gold catalyzed hydration reaction (10 mg whole cell lyophilisate, Tris-HCl buffer 350 mM, pH 8, 30 °C reaction temperature)

Entry	*i*-PrOH amount [%]	Ketone concentration [mM]	Addition of	Conversion (GC), 24 h [%]
1	16	500	-	50
2	80	100	-	5
3	16	100	Phenylacetylene 100 mM	90
4	16	100	-	96
5	10	100	1-Butanol 20 % (v/v)	3
6	10	100	2-Butanol 20 % (v/v)	0
7	-	100	2-Butanol 30 % (v/v)	3
8	10	100	1-Pentanol 20 % (v/v)	5

D.IV.3 Design of an Au(III) based single phase system

As discussed above, the application of potent biocatalysts within chemoenzymatic one pot processes gains enormous attention currently. Apart from the very rare examples e.g. the work of Rios-Lombardia et al. who presented a concurrent ruthenium catalyzed allylic alcohol isomerization and an asymmetric bioreduction,[123] most of these processes need a form of compartmentalization to separate metal- and biocatalyst from each other and thereby avoid the poisoning effect, respectively. For all examples presented in this thesis so far, a form of compartmentalization or seperation of enzyme and metal catalyst is required.

Building upon the results for the potential metal catalyzed cascade step presented in section D.IV, we started to investigate the combinability of gold catalyzed hydration of alkynes and a subsequent enzymatic reduction (Scheme D-26).



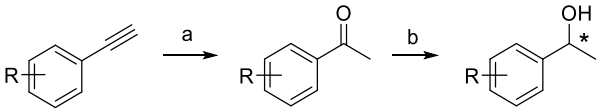
Scheme D-26 Envisaged chemoenzymatic one pot process using *i*-PrOH as solvent for the 1st cascade step and cosubstrat for the 2nd cascade step

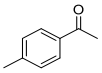
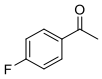
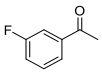
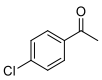
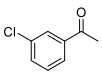
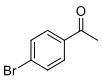
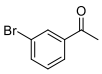
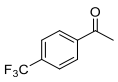
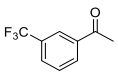
Initially, we applied the reaction mixture that gave 98 % conversion in the Au(III) catalyzed hydration, containing **B-0** (F.VII.1.1) and directly combined it with whole cell lyophilisate of ADH-A resuspended in Tris-HCl buffer (350 mM, pH 8). The high buffer concentration was necessary, because during the hydration reaction HCl is generated, which would lower the pH value in the subsequent chemoenzymatic one pot reaction. The final volume ratio was 30 % v/v hydration reaction mixture and 70 % v/v Tris-HCl buffer containing the ADH-A whole cell lyophilisate. This ratio was chosen since preliminary results indicated that 30 % *i*-PrOH was well tolerated by both alcohol dehydrogenases (ADH-A and LK-ADH).

Pleasantly, the enzymatic reduction gave 93 % conversion, which resulted in a conversion of 91 % to **(*S*)-C-0** with 99 % ee over two steps. This finding indicated that the present gold catalyst does not inactivate the (*S*)-selective ADH-A. Next, we conducted the one pot reaction using the ADH from *Lactobacillus kefir* and observed a conversion of 93 % to **(*R*)-C-0** over two steps, also with an excellent *ee* for the enantiocomplementary alcohol. Thus, nor the relatively high *i*-PrOH content of 30 %, neither the metal catalyst prevents an effective enzymatic reduction.

Having these interesting results in hands, we perfomed the enzymatic reduction for a variety of ketone substrates (**B-0 to B-10**) by direct use of the respective reaction mixtures. Thereby, we observed conversions over two steps from 49-99 % for the (*S*)-selective ADH-A. Employing the (*R*)-selective ADH, a conversion up to 93 % was achieved (Table D-29). All conversions are reported over two steps.

Table D-29 Synthesis of chiral alcohols via sequential chemoenzymatic one pot reaction



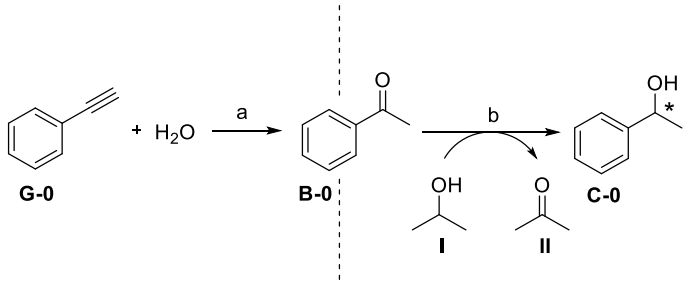
Substrate in reaction mixture Cpd.No.		Product Cpd.No.	Conversion (GC) 4 h [%][a] (*S*)-alcohol	(*R*)-alcohol	*ee* [%] (*S*)-and (*R*)-alcohol
B-0		**C-0**	91	93	>99
B-1		**C-1**	76	73	>99
B-2		**C-2**	99	15	>99
B-3		**C-3**	71	65	>99
B-4		**C-4**	86	74	>99[b]
B-5		**C-5**	71	69	>99
B-6		**C-6**	76	29	>99
B-7		**C-7**	67	67	>99
B-8		**C-8**	53	17	>99
B-9		**C-9**	49	49	>99
B-10		**C-10**	57	0	>99[b]

[a] Reagents and conditions: a) H_2O, $AuCl_3$, *i*-PrOH, 65 °C; b) ADH, Tris Hcl buffer
[b] Determined by chiral HPLC

Moreover, we give an insight into scope and limitation regarding suitable ketone substrates. The ADH from *lactobacillus kefir* is in general less appropriate for *m*-substituted aromatic ketone substrates, depending on the bulkiness of the substituent. This can be explained by sterical characteristics, impeding an efficient access to the active side of the enzyme. Both alcohol dehydrogenases delivered the respective secondary alcohols in excellent *ee* values.

Further, two preparative scale experiments were conducted and the alcohols **(*S*)-C-3** and **(*S*)-C-6** were isolated with a yield of 71 % **(*S*)-C-3** and 64 % **(S)-C-6** over two steps.

Further, the feasability of the developed cascade was tested applying a membrane reactor (Scheme D-27), also used for the simultaneous cascade presented in section D.III. This attempt was not successful, since the for the hydration reaction applied solvents (1-butanol and *i*-PrOH), which were placed in the respective reaction chamber in pure form, diffused through the PDMS membrane and thereby inactivated the alcohol dehydrogenase in the present amounts. In both cases no conversion to the alcohol was observed. A further problem is the lipophilicity of **G-0** that enables the substrate to pass the membrane and thus leads to an inconvienient partition between the two chambers, leading to lower phenylacetylene concentration in the hydration reaction chamber. Consequently, even the metal catalyzed step is impeded using a membrane reactor and gave in the best case 18 % to **C-0**.



Scheme D-27 Reagents and conditions: a) H_2O, $AuCl_3$, i-PrOH or 1-butanol, b) 200 mg ADH-A, Tris HCl buffer pH 8, 350 mM, 16 % *i*-PrOH in enzymatic chamber; (30 °C, 200rpm, 5000 µl)

In summary, we were able to present a very straightforward sequential chemoenzymatic one pot process for the production of enantiopure alcohols, in which the full reaction mixture from the metal catalyzed step is used in the following for the enzymatic reduction without any spatial separation of metal species and biocatalyst. This is realized by use of *i*-PrOH as solvent for the Au(III) catalyzed hydration, which functions in the subsequent enzymatic reduction as cosubstrate for the two enantiodivergent ADHs. Thereby we produced enantiopure alcohols with conversions up to 99 % over two steps with excellent optical purity within an extremely atom-economical, simple chemoenzymatic one pot process.

E Conclusion and perspective

Driven by the outstanding strengths of the complementary catalyst types, this work focused on a chemoenzymatic one-pot cascade reaction, which is based on a combination of a metal catalyzed reaction and a subsequent enzyme mediated transformation of the newly created carbonyl group. In summary, within this thesis four different chemoenzymatic one-pot processes are disclosed for the combination of a metal catalyzed transformation and a biocatalytic reaction to produce chiral alcohols or amines of outstanding optical purity. For the development of the desired one pot reaction, the general compatibility issues of these different catalysis types, namely overall reaction concentration, inactivation of the enzyme by the present metal species, reaction temperature and solvent, had to be solved.

First, a chemoenzymatic one-pot reaction for the combination of a metal (palladium and copper) catalyzed Liebeskind Srogl coupling reaction and a subsequent biotransformation of the newly created functional group, generating enantiopure alcohols was developed. Moreover, the employment of the two enantiocomplementary alcohol dehydrogenases provided access to both enantiomeric forms with excellent *ee* (Figure E-1). A further key feature is the utilization of lyophilized whole cells, which facilitates the conversion of this high substrate concentrations (100 mM) using only *i*-PrOH as economically attractive co-substrate.

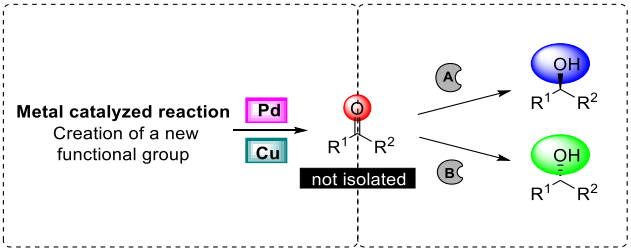


Figure E-1 Combination of Pd and Cu catalysis (Liebeskind Srogl coupling) with subsequent enzymatic reduction

For this chemoenzymatic process three different reaction settings (see Figure E-2) were applied: either a liquid-liquid biphasic system (**1**), or by embedding the applied enzyme in a superabsorber material (liquid-solid system (**2**)), or via compartmentalization by using a semipermeable membrane that enables a simultaneous process without any intermediate step (**3**).

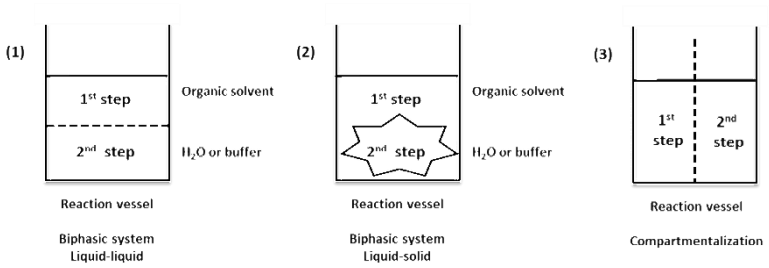


Figure E-2 Applied strategies for the combination of metal- and biocatalysis

Furthermore, the versatility of the system was demonstrated by variation of the enzyme class of the bio-catalyst. Depending on the applied enzyme (alcohol dehydrogenase or ω-transaminase), chiral alcohols as well as amines were produced without the need for any intermediate isolation. When ω-transaminases were used as biocatalytic species, the enzyme was placed in superslurper material (**2**) (see Figure E-3).

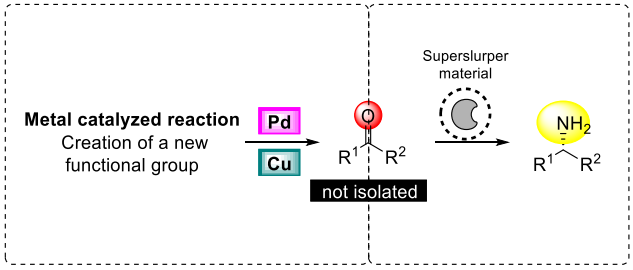


Figure E-3 Combination of Pd and Cu catalysis (Liebeskind Srogl coupling) with subsequent enzymatic transamination

In addition to these combination strategies, which generally require a sort of compartmentalization for both single cascade steps, a sequential single phase process consisting of a gold catalyzed hydration of alkynes and an enzymatic reduction was designed. Thus, a very straightforward chemoenzymatic one pot process for the production of enantiopure alcohols, in which the full reaction mixture from the metal catalyzed step can be used in the following for the enzymatic reduction without any spatial separation of metal species and biocatalyst, is presented here. Therefore, a process that is easily feasible also for researchers from other fields than biocatalysis is demonstrated. This is realized by applying *i*-PrOH as solvent for the Au(III) catalyzed hydration, which also plays the role of co-substrate for cofactor regeneration in the subsequent enzymatic reduction for two enantiodivergent ADHs. After resuspension of the respective whole cell lyophilisate, the enzyme can directly be added to the hydration reaction mixture, in which the substrate is already formed in a concentration up to 150 mM. This operationally easy procedure is environmentally benign, economically attractive, and provides a good functional group tolerance. (Figure E-4).

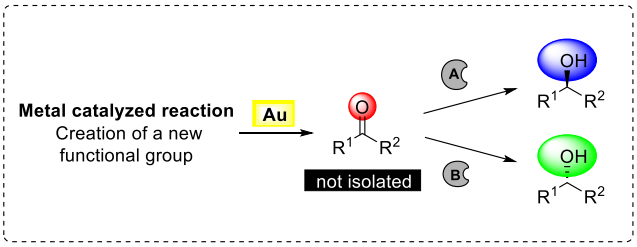


Figure E-4 Combination of Au catalysis (Alkyne Hydration) with subsequent enzymatic reduction

Regarding future chemoenzymatic one pot processes, the key challenge will remain the achievement of compatibility of the unequal catalytic species with each other. Further, more compatible (bio- and chemo-)catalysts with improved stability, reaction engineering like the application of immobilized biocatalysts and chemocatalysts in different compartments, will be of great significance in future developments for chemoenzymatic one pot processes.[108] However, the first example of a concurrent metal-catalyzed and biocatalyzed reaction presented by Rios-Lombardia et al. [123] opens up a new perspective in the field of chemoenzymatic one pot processes.

F Experimental part

F.I Materials and methods – chemical synthesis

Unless noted otherwise, all reagents were purchased from commercial suppliers and used without further purification. Chromatography solvents were distilled prior to use. For all other solvents quality grade is given in the reaction procedures. Column chromatography was performed on a Büchi Sepacore Flash System (2 x Büchi Pump Module C-605, Büchi Pump Manager C-615, Büchi UV Photometer C-635, Büchi Fraction Collector C-660) or standard manual glass columns using silica gel from Merck (40-63 µm) applying LP/Et_2O, EtOAc mixtures or DCM. Preparative TLC was performed applying Uniplate thin layer chromatography plates (20 × 20 cm, 1000 microns) and DCM. Signals were visualized with UV light (254 nm).

Table F-1. Recipes for TLC staining solutions

TLC staining solution1 (general purpose)		**TLC staining solution 2 (general purpose)**		**TLC staining solution for acidic compounds**		**TLC staining solution for carbonyl compounds**	
6 g	$KMnO_4$	10 g	phosphomolybdic acid hydrate	40 mg	bromocresol green	0.8 g	2,4-dinitro-phenylhydrazine
0.5 g	KOH	1 g	cerium ammonium nitrate	100 mL	dry EtOH	200 mL	2N HCl
40 g	K_2CO_3	20 g	H_2SO_4 conc.	0.1 M	NaOH until blue color appears	2 mL	EtOH
600 mL	deion. H_2O	300 mL	EtOH				

Desiccation of organic solvents after extraction in reaction workup was performed using anhydrous sodium sulfate and subsequent filtration. NMR spectra were recorded from $CDCl_3$ solutions on a Bruker AC 200 (200 MHz) or Bruker Advance UltraShield 400 (400 MHz) spectrometer and chemical shifts are reported in ppm. Whenever possible, calibration *via* residual solvent peaks was performed. Peak assignment is based on correlation experiments or software prediction.

General conversion control and examination of purified products were conducted by GC/MS using a Thermo Finnigan Focus GC / DSQ II equipped with a standard capillary column BGB5 (30m x 0.32 mm ID). Enantiomeric excess was determined *via* GC using a BGB175 (30 m x 0.25 mm ID, 0.25 µm film) or BGB173 (30 m x 0.25 mm ID, 0.25 µm film) column on a ThermoQuest Trace GC 2000 and a ThermoFocus GC, both with FID detector.

One of the following temperature profiles was used:

Table F-2. Applied temperature profiles

method	profile
A	80°C-2min→ 80-160°C, 5°C/min, 160°C-1min→ 160-220°C, 10°C/min, 220°C-8min
B	80°C-1min→80-220°C, 2°C/min, 220°C-5min
C	80°C-1min→ 80-220°C, 1°C/min, 220°C-5min
D	80°C-1min→ 80-90°C, 5°C/min, 90°C-12min→ 90-220°C, 10°C/min, 220°C-5min
E	65°C-0.5min→ 65-220°C, 25°C/min, 220°C-0min→ 220-290°C, 80°C/min, 290°C-3min

Further, enantiomeric excess was determined via HPLC using a ChiralPak AS-H (250 mm x 4.6 mm ID) on a Thermo Scientific/Dionex Ultimate 3000 HPLC using mixtures of n-heptane/i-PrOH or EtOH as mobile phase:

Table F-3. Applied profiles

method	profile
I	Hex_EtOH_99.5_0.5_1ml/min_25 °C_40min
II	Hex_i-PrOH_99.2_0.8_1ml/min_25°C_40min
II	Hex_i-PrOH_99.8_0.2_1ml/min_25°C_60min

Specific rotation was measured on an Anton Paar MCP500 polarimeter at the specified conditions. As general internal standard for GC and GC-MS analysis methylbenzoate was applied. Calibrations were conducted in a concentration range from 0.5 mM to 5 mM. Melting points were determined using a Kofler hot bench. For the preparation of polydimethylsiloxane sheets and thimbles, elastomer and curing agent (Sylgard 184, 10 gram clip-pak) purchased from Sigma-Aldrich was used.

The conversions of the membrane reactor based simultaneous one-pot reactions were determined using an UltraPerformance Convergence Chromatography (UPC^2) from Waters and methylbenzoate as internal standard. For the membrane reactor based production of 1-(4-fluorophenyl)ethan-1-ol and 1-(4-trifluormethylphenyl)ethan-1-ol 2-methylanisole was used instead as internal standard. As metal scavenger SiliaMetS Dimercaptotriazine (DMT) was applied.

F.II Materials and methods – biotransformations

Bacterial cultures were incubated in baffled Erlenmeyer flasks in orbital shakers (InforsHT Multitron 2 Standard) at 200 rpm. Bacteria on Agar plates were incubated in a Heraeus Instruments FunctionLine incubator under air. All materials and biotransformation media were sterilized by autoclaving at 121 °C for 20 minutes. Various aqueous stock solutions were sterilized by filtration through 0.20 µm syringe filters. Agar plates were prepared with LB_{amp} medium supplemented by 1.5% w/v Agar Agar. Cells lysis for the preparation of CCEs was affected using a Bandelin KE76 sonotrode connected to a Bandelin Sonopuls HD 3200 wave generator.

Table F-4. Constituents of all bacterial growth media used in this thesis.

LB Medium		TB Medium		M9 Medium		Trace Elemens Solution	
10 g	bacto-peptone	12 g	bacto-tryptone	0.8 g	$(NH_4)_2SO_4$	0.18 g	$ZnSO_4 \cdot 7H_2O$
5 g	yeast extract	24 g	yeast extract	0.5 g	NaCl	0.12 g	$CuCl_2 \cdot 2H_2O$
10 g	NaCl	16.4 g	$K_2HPO_4 \cdot 3\ H_2O$	7.5 g	$Na_2HPO_4 \cdot 2H_2O$	0.12 g	$MnSO_4 \cdot H_2O$
		2.3 g	KH_2PO_4	3.0 g	KH_2PO_4	0.18 g	$CoCl_2 \cdot 6H_2O$
				1.0 mL	1.0 M $MgSO_4$		
				1.0 mL	0.1 M $CaCl_2$		
				0.6 mL	0.1 M $FeCl_3 \cdot 6H_2O$		
				2.0 mL	1 mM thimine·HCl		
				10.0 mL	trace elements sol.		

Concentrations on 1000 mL deionized water; M9 salt solutions are separately sterilized by filtration and then added to the main salts and dissolved in deionized water.

F.II.1 Stock solutions

Reagent	Concentration in deionized H_2O
amp	50 mg mL^{-1}
kan	50 mg mL^{-1}
IPTG	839 mM
$NADP^+$ tetrasodium salt hydrate	0.1 M
G6P	0.5 M

F.II.2 Expression conditions

Table F-5 Growth and expression conditions for the biocatalysts used in this thesis.

Biocatalyst	Resistance	Inducer	Inducer Concentration	Expression Temperature [°C]
ADH-A	amp	IPTG	2 mM	20
LK-ADH	amp	IPTG	1 mM	30
ADH (YOL151w, pIK 3)	kan	IPTG	0.5 mM	24
Asp Fum	amp	IPTG	0.1 mM	20

F.II.3 ω-Transaminase in-house library

Table F-6 Overview about His-tagged "inhouse" ω-transaminases

Biocatalyst	Resistance	Inducer	Inducer Concentration	Expression Temperature [°C]
AspTer (*Aspergillus terreus*)	amp	rhamnose	0.2% (w/v)	20
AspFum (*Aspergillus fumigatus*)	amp	IPTG	0.1 mM	20
NeoFis (*Neosartorya fischeri*)	amp	IPTG	0.1 mM	20
MycVan (*Mycobacterium vanbaalenii*)	amp	rhamnose	0.2% (w/v)	20
3HMU	amp	IPTG	0.1 mM	20
3i5T	amp	IPTG	0.1 mM	20
Vfl H6	kan	IPTG	0.1 mM	20

F.II.4 Preparation of permanent cultures

E. coli strains were incubated at 37 °C on LB_{amp} plates for 12-24 h. A single colony was selected and a 10 mL preculture was inoculated and incubated (10 mL LB_{amp}, 50 mL shaking flask, 37 °C, 18 h). After addition of 2 mL glycerol, the mixture was vortexed, transferred in 1 mL aliquots into Eppendorf vials and stored at -80 °C.

F.II.5 Preparation of Codexis mastermix for transamination reactions

To 10 mL of deionized water, 1667 µl of isopropylamine, 295 µl of triethanolamine and 5.35 mg of pyridoxal 5-phosphate was added. The pH value was adjusted to 8 using HCl or NaOH. Finally, water was added to a final volume of 20 mL.

F.III Preparation of polydimethylsiloxane sheets

PDMS sheets were prepared according to Bowden[129a] with some modifications.

Precuring:

The elastomer and curing agent (Sylgard 184, 10 gram clip-pak, purchased from Sigma-Aldrich) were mixed and stored at room temperature for 2 hours for degassing.

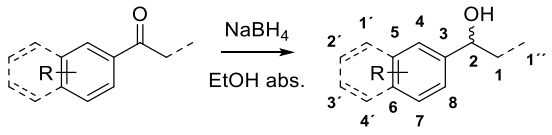
Coating and baking:

The PDMS mixture was evenly distributed (~8 cm diameter) on upside down crystallizing dishes and baked in oven at 65 degrees for 16 hours. The temperature was than increased to 110 °C for 1 hour.

Delaminating and washing:

The coated crystallizing dishes were placed in hexane for 5 min for delamination and the PDMS sheets were soaked twice for 2h in dichloromethane and finally dried in an oven at 110 °C for 2 h.

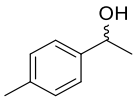
F.IV Synthesis of racemic alcohols - Reference compounds



General procedure:

To a stirred suspension of $NaBH_4$ (1.5 equiv.) in 6 mL of abs. EtOH the corresponding substituted ketone (1 equiv.) was added and the resulting mixture was stirred at room temperature until the reaction was completed as monitored by GC-MS. After concentration in vacuo to remove EtOH, H_2O (10 mL) was added and the reaction mixture was extracted with DCM (3 × 10 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and the solvent was removed to afford the desired racemic alcohol. If required, the purification was performed using preparative TLC or standard manual glass columns using silica gel from Merck (40-63 µm) and DCM.

F.IV.1 *rac*-1-(4-Methylphenyl)ethan-1-ol (C-1)

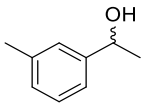


General procedure	**F.IV**
Reaction scale	100 mg (0.75 mmol) (**B-1**)
Reaction time	4 h
Yield	98 mg (98 %) colorless oil
Sum formula, m.w.	$C_9H_{12}O$, 136.19
^{1}H-NMR (200 MHz, $CDCl_3$)	δ = 1.46 (d, *J* = 6.5 Hz, 3H, H1), 2.11 (s, 1H, OH), 2.34 (s, 3H, Ph-CH_3), 4.83 (q, *J* = 6.4 Hz, 1H, H2), 7.07 – 7.31 (m, 4H, Ph).
^{13}C-NMR (50 MHz, $CDCl_3$)	δ = 21.0 (q, Ph-CH_3), 25.0 (q, C1), 70.1 (d, C2), 125.3 (d, 2C, C4, C8), 129.1 (d, 2C, C5, C7), 137.0 (s, C6), 142.8 (s, C3).

GCMS	136 (35, M^+), 121 (94), 93 (100), 92 (21), 91 (96), 77 (56), 65 (36), 63 (14), 60 (11), 51 (18)

Analytical data was in accordance with the literature. [133]

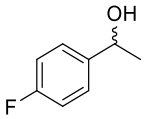
F.IV.2 *rac*-1-(3-Methylphenyl)ethan-1-ol (C-2)



General procedure	**F.IV**
Reaction scale	100 mg (0.75 mmol) (**B-2**)
Reaction time	4 h
Yield	101 mg (100 %) colorless oil
Sum formula, m.w.	$C_9H_{12}O$, 136.19
^{1}H-NMR (200 MHz, $CDCl_3$)	δ = 1.49 (d, *J* = 6.5 Hz, 3H, H1), 2.40 (d, *J* = 9.1 Hz, 4H, OH, Ph-CH_3), 4.83 (q, *J* = 6.5 Hz, 1H, H2), 7.01 – 7.40 (m, 4H, Ph).
^{13}C-NMR (50 MHz, $CDCl_3$)	δ = 21.3 (q, Ph-CH_3), 25.0 (q, C1), 70.1 (d, C2), 122.3 (d, C8), 126.0 (d, C6), 127.9 (d, C7), 128.2 (d, C4), 137.9 (s, C5), 145.8 (s, C3).
GCMS	136 (16, M^+), 121 (78), 119 (16), 118 (70), 117 (30), 115 (13), 93 (100), 92 (13), 91 (92), 77 (49), 65 (31), 63 (13), 60 (11), 51 (13)

Analytical data was in accordance with the literature.[134]

F.IV.3 *rac*-1-(4-Fluorophenyl)ethan-1-ol (C-3)

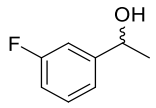


General procedure	**F.IV**
Reaction scale	100 mg (0.72 mmol) (**B-3**)
Reaction time	4 h

Yield	94 mg (91 %) colorless oil
Sum formula, m.w.	C_8H_9FO, 140.16
^{1}H-NMR (200 MHz, $CDCl_3$)	δ = 1.48 (dd, *J* = 6.5, 0.8 Hz, 3H, H1), 1.79 – 1.85 (m, 1H, OH), 4.89 (q, *J* = 6.4 Hz, 1H, H2), 6.97 – 7.08 (m, 2H, C5, C7), 7.29 – 7.39 (m, 2H, C4, C8).
^{13}C-NMR (100 MHz, $CDCl_3$)	δ = 25.3 (q, C1), 69.7 (d, C2), 115.2 (dd, 2C, J_{CF} = 21.3 Hz, C5, C7), 127.0 (d, 2C, J_{CF} = 8.1 Hz, C4, C8), 141.5 (d, J_{CF} = 3.2 Hz, C3), 162.1 (d, J_{CF} = 245.1 Hz, C6).
GCMS	140 (22, M^+), 125 (100), 123 (11), 97 (80), 96 (20), 95 (24), 77 (38), 75 (20), 51 (15)

Analytical data was in accordance with the literature. [133]

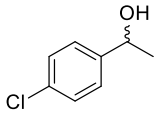
F.IV.4 *rac*-1-(3-Fluorophenyl)ethan-1-ol (C-4)



General procedure	**F.IV**
Reaction scale	296 mg (2.14 mmol) (**B-4**)
Reaction time	4 h
Yield	275 mg (92 %) colorless oil
Sum formula, m.w.	C_8H_9FO, 140.16
^{1}H-NMR (200 MHz, $CDCl_3$)	δ = 1.45 (d, *J* = 6.4 Hz, 3H, H1), 2.00 (s, 1H, OH), 4.84 (t, *J* = 6.5 Hz, 1H, H2), 6.82 – 7.36 (m, 4H, Ph).
^{13}C-NMR (50 MHz, $CDCl_3$)	δ = 25.2 (q, C1), 69.8 (d, J_{CF} = 1.8 Hz, C2), 112.3 (dd, J_{CF} = 21.8 Hz, C4), 114.2 (dd, J_{CF} = 21.2 Hz, C6), 120.9 (d, J_{CF} = 2.8 Hz, C8), 129.9 (d, J_{CF} = 8.2 Hz, C7), 148.5 (d, J_{CF} = 6.6 Hz, C3), 163.0 (d, J_{CF} = 245.8 Hz, C5).
GCMS	140 (15, M^+), 125 (58), 97 (100), 96 (28), 95 (23), 77 (36), 75 (17), 51 (10)

Analytical data was in accordance with the literature. [133]

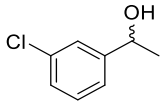
F.IV.5 *rac*-1-(4-Chlorophenyl)ethan-1-ol (C-5)



General procedure	**F.IV**
Reaction scale	100 mg (0.65 mmol) (**B-5**)
Reaction time	4 h
Yield	85 mg (84 %) colorless oil
Sum formula, m.w.	C_8H_9ClO, 156.61
^{1}H-NMR (200 MHz, $CDCl_3$)	δ = 1.37 (d, *J* = 6.4 Hz, 3H, H1), 1.83 (s, 1H, OH), 4.78 (q, *J* = 6.5 Hz, 1H, H2), 7.21 (s, 4H, Ph).
^{13}C-NMR (50 MHz, $CDCl_3$)	δ = 25.2 (q, C1), 69.7 (d, C2), 126.8 (d, 2C, C4, C8), 128.6 (d, 2C, C5, C7), 133.0 (s, C6), 144.2 (s, C3).
GCMS	158 (5, M^+), 156 (14), 143 (10), 141 (33), 113 (25), 78 (10), 77 (100), 75 (24), 74 (10), 51 (36), 50 (16)

Analytical data was in accordance with the literature. [133]

F.IV.6 *rac*-1-(3-Chlorophenyl)ethan-1-ol (C-6)

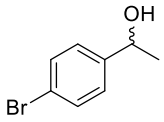


General procedure	**F.IV**
Reaction scale	100 mg (0.65 mmol) (**B-6**)
Reaction time	4 h
Yield	89 mg (88 %) colorless oil
Sum formula, m.w.	C_8H_9ClO, 156.61
^{1}H-NMR (200 MHz, $CDCl_3$)	δ = 1.49 (d, *J* = 6.5 Hz, 3H, H1), 1.85 (s, 1H, OH), 4.88 (q, *J* = 6.5 Hz, 1H, C2), 7.18 – 7.44 (m, 4H, Ph).
^{13}C-NMR (50 MHz, $CDCl_3$)	δ = 25.2 (q, C1), 69.8 (d, C2), 123.5 (d, C4), 125.6(d, C8), 127.5 (d, C6), 129.8 (d, C7), 134.4 (s, C5), 147.8 (s, C3).

GCMS	158 (3, M^+), 156 (10), 143 (11), 141 (35), 121 (12), 115 (11), 113 (37), 78 (10), 77 (100), 75 (14), 51 (17)

Analytical data was in accordance with the literature. [135]

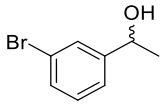
F.IV.7 *rac*-1-(4-Bromophenyl)ethan-1-ol (C-7)



General procedure	**F.IV**
Reaction scale	100 mg (0.50 mmol) (**B-7**)
Reaction time	4 h
Yield	92 mg (91 %) colorless oil
Sum formula, m.w.	C_8H_9BrO, 201.06
^{1}H-NMR (200 MHz, $CDCl_3$)	δ = 1.47 (dd, *J* = 13.3, 6.6 Hz, 3H, H1), 2.10 (s, 1H, OH), 4.85 (dq, *J* = 16.4, 6.5 Hz, 1H, H2), 7.15 – 7.53 (m, 4H, Ph).
^{13}C-NMR (50 MHz, $CDCl_3$)	δ = 25.2 (q, C1), 69.8 (d, C2), 121.2 (s, C6), 127.1 (d, 2C, C4, C8), 131.5 (d, 2C, C5, C7), 144.8 (s, C3).
GCMS	202 (7, M^+), 200 (8), 187 (24), 185 (29), 159 (14), 157 (21), 121 (13), 78 (50), 77 (100), 76 (21), 75 (22), 74 (20), 63 (13), 52 (12), 51 (52), 50 (32)

Analytical data was in accordance with the literature. [133]

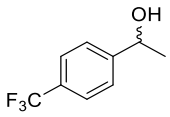
F.IV.8 *rac*-1-(3-Bromophenyl)ethan-1-ol (C-8)



General procedure	**F.IV**
Reaction scale	100 mg (0.50 mmol) (**B-8**)
Reaction time	4 h
Yield	100 mg (99 %) colorless oil
Sum formula, m.w.	C_8H_9BrO, 201.06
^{1}H-NMR (200 MHz, $CDCl_3$)	δ = 1.48 (d, *J* = 6.4 Hz, 3H, H1), 2.08 (s, 1H, OH), 4.86 (q, *J* = 6.5 Hz, 1H, H2), 7.09 – 7.59 (m, 4H, Ph).
^{13}C-NMR (50 MHz, $CDCl_3$)	δ = 25.2 (q, C1), 69.7 (d, C2), 122.5 (s, C5), 124.0 (d, C8), 128.5 (d, C7), 130.0 (d, C6), 130.4 (d, C4), 148.1 (s, C3).
GCMS	202 (10, M^+), 200 (11), 187 (29), 185 (35), 159 (24), 157 (28), 121 (24), 78 (42), 77 (100), 76 (12), 75 (11), 51 (18)

Analytical data was in accordance with the literature. [136]

F.IV.9 *rac*-1-(4-Trifluormethylphenyl)ethan-1-ol (C-9)

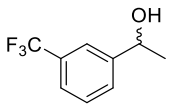


General procedure	**F.IV**
Reaction scale	100 mg (0.53 mmol) (**B-9**)
Reaction time	4 h
Yield	70 mg (69 %) colorless oil
Sum formula, m.w.	$C_9H_9F_3O$, 190.17
^{1}H-NMR (200 MHz, $CDCl_3$)	δ = 1.50 (d, J = 6.5 Hz, 3H, H1), 1.98 (s, 1H, OH), 4.96 (q, J = 6.5 Hz, 1H, H2), 7.40 – 7.65 (m, 4H, Ph).

^{13}C-NMR (100 MHz, $CDCl_3$)	δ = 25.4 (q, C1), 69.8 (d, C2), 122.8 (q, J_{CF} = 272.0 Hz, CF_3), 125.4 (q, 2C, J_{CF} = 3.8 Hz, C5, C7), 125.6 (d, 2C, C4, C8), 129.6 (dq, J_{CF} = 32.4 Hz, C6), 149.7 (s, C3).
GCMS	190 (9, M^+), 176 (9), 175 (100), 147 (23), 145 (16), 127 (92), 77 (17), 75 (12), 51 (13)

Analytical data was in accordance with the literature. [137]

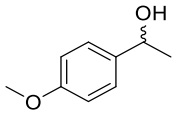
F.IV.10 *rac*-1-(3-Trifluormethylphenyl)ethan-1-ol (C-10)



General procedure	**F.IV**
Reaction scale	297 mg (1.6 mmol) (**B-10**)
Reaction time	4 h
Yield	285 mg (95 %) colorless oil
Sum formula, m.w.	$C_9H_9F_3O$, 190.17
^{1}H-NMR (200 MHz, $CDCl_3$)	δ = 1.24 (d, *J* = 6.5 Hz, 3H, H1), 1.90 (s, 1H, OH), 4.69 (q, *J* = 6.5 Hz, 1H, H2), 6.95 – 7.45 (m, 4H, Ph).
^{13}C-NMR (50 MHz, $CDCl_3$)	δ = 25.3 (q, C1), 69.8 (d, C2), 122.2 (q, J_{CF} = 3.8 Hz, C6), 124.1 (q, J_{CF} = 272.3 Hz, CF_3), 124.2 (q, J_{CF} = 3.8 Hz, C4), 128.8 (q, J_{CF} = 1.5 Hz, C7), 128.9 (d, C8), 130.8 (dq, J_{CF} = 32.2 Hz, C5), 146.7 (s, C3).
GCMS	190 (9, M^+), 175 (100), 147 (16), 45 (15), 127 (78)

Analytical data was in accordance with the literature (Sigma Aldrich).

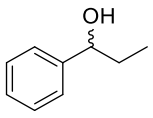
F.IV.11 *rac*-1-(4-Methoxyphenyl)ethan-1-ol (C-11)



General procedure	**F.IV**
Reaction scale	100 mg (0.67 mmol) (**B-11**)
Reaction time	4 h
Yield	101 mg (100 %) colorless oil
Sum formula, m.w.	$C_9H_{12}O_2$, 152.19
^{1}H-NMR (200 MHz, $CDCl_3$)	δ = 1.46 (d, *J* = 6.4 Hz, 3H, H1), 1.97 (s, 1H, OH), 3.79 (s, 3H, OCH_3), 4.83 (q, *J* = 6.5 Hz, 1H, H2), 6.79 – 6.92 (m, 2H, H5, H7), 7.30 (d, *J* = 2.2 Hz, 2H, H4, H8).
^{13}C-NMR (50 MHz, $CDCl_3$)	δ = 24.96 (q, C1), 55.22 (q, OCH_3), 69.86 (d, C2), 113.76 (d, 2C, C5, C7), 126.60 (d, 2C, C4, C8), 137.98 (s, C3), 158.88 (s, C6).
GCMS	151 (4, M^+), 150 (34), 135 (100), 107 (18), 92 (16), 77 (27)

Analytical data was in accordance with the literature. [138]

F.IV.12 *rac*-1-Phenylpropan-1-ol (C-12)

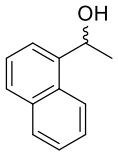


General procedure	**F.IV**
Reaction scale	100 mg (0.75 mmol) (**B-12**)
Reaction time	4 h
Yield	85 mg (84 %) colorless oil
Sum formula, m.w.	$C_9H_{12}O$, 136.19
^{1}H-NMR (200 MHz, $CDCl_3$)	δ = 0.92 (t, *J* = 7.4 Hz, 3H, H1´´), 1.69 – 1.84 (m, 3H, H1, OH), 4.60 (t, *J* = 6.6 Hz, 1H, H2), 7.35 (d, *J* = 3.8 Hz, 5H, Ph).
^{13}C-NMR (50 MHz, $CDCl_3$)	δ = 10.1 (q, C1´´), 31.9 (t, C1), 76.0 (d, C2), 125.9 (d, 2C, C4, C8), 127.5 (d, C6), 128.4 (d, 2C, C5, C8), 144.6 (s, C3).

GCMS	136 (21, M^+), 107 (100), 79 (60), 77 (25)

Analytical data was in accordance with the literature. [135]

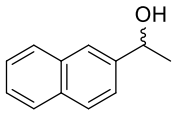
F.IV.13 *rac*-1-(1-Naphtyl)ethan-1-ol (C-13)



General procedure	**F.IV**
Reaction scale	100 mg (0.59 mmol) (**B-13**)
Reaction time	4 h
Yield	94 mg (92 %) colorless crystals
MP[139]	31-33 °C (Lit. 34 °C)
Sum formula, m.w.	$C_{12}H_{12}O$, 172.23
^{1}H-NMR (200 MHz, $CDCl_3$)	δ = 1.67 (d, *J* = 6.5 Hz, 3H, H1), 2.08 (s, 1H, OH), 5.67 (q, *J* = 6.5 Hz, 1H, H2), 7.36 – 8.21 (m, 7H, naphtyl).
^{13}C-NMR (50 MHz, $CDCl_3$)	δ = 24.3 (q, C1), 67.1 (d, C2), 121.9 (s, C3), 123.1 (s, C7), 125.5 (d, 2C, C8, C2´), 126.0 (d, C3´), 127.9 (d, C4´), 128.8 (d, C1´), 130.2 (d, C4), 133.8 (d, C5), 141.3 (s, C6).
GCMS	172 (55, M^+), 157 (56), 153 (10), 130 (12), 129 (100), 128 (53), 127 (28)

Analytical data was in accordance with the literature. [140]

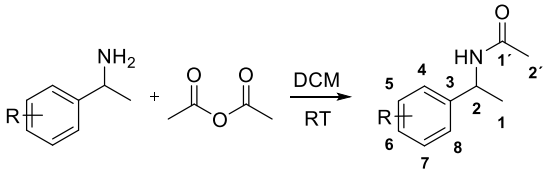
F.IV.14 *rac*-1-(2-Naphtyl)ethan-1-ol (C-14)



General procedure	**F.IV**
Reaction scale	100 mg (0.59 mmol) (**B-14**)
Reaction time	4 h
Yield	96 mg (94 %) colorless crystals
MP[141]	54 °C (Lit. 53-54 °C)
Sum formula, m.w.	$C_{12}H_{12}O$, 172.23
^{1}H-NMR (200 MHz, $CDCl_3$)	δ = 1.58 (d, *J* = 6.5 Hz, 3H, H1), 2.03 (s, 1H, OH), 5.06 (q, *J* = 6.5 Hz, 1H, H2), 7.49 (ddt, *J* = 9.8, 7.5, 3.3 Hz, 3H, C8, C2´, C3´), 7.71 – 7.93 (m, 4H, C4, C7, C1´, C4´).
^{13}C-NMR (50 MHz, $CDCl_3$)	δ = 25.1 (q, C1), 70.4 (d, C2), 123.7 (s, C3), 123.8 (s, C3´), 125.9 (d, C2´), 127.1 – 128.6 (m, C1´, C4, C4´, C5, C8), 133.0 (d, C7), 143.1 (s, C6).
GCMS	172 (55, M^+), 157 (49), 130 (12), 129 (100), 128 (50), 127 (28)

Analytical data was in accordance with the literature. [142]

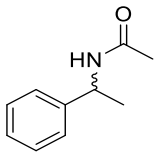
F.V Synthesis of racemic amides – Reference coumpounds



General procedure:

Acetic anhydride (2.1 equiv.) was added to a solution of the respective amine (1 equiv.) in DCM (10 mL). The reaction was stirred at room temperature for 6 hours. Excess acetic anhydride was hydrolyzed by washing the reaction mixture with $NaHCO_3$ (2 × 20 mL), until a basic pH value was observed. The organic phase was separated and the combined aqueous phases were extracted with DCM (3 × 10 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was removed to afford the desired amide.

F.V.1 *N*-(1-Phenylethyl)acetamide (E-1)

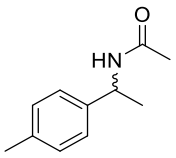


General procedure	**F.V**
Reaction scale	500 mg (4.13 mmol) (**D-1**)
Reaction time	6 h
Yield	547 mg (81 %) colorless crystals
MP[143]	102 °C (Lit. 101-102 °C)
Sum formula, m.w.	$C_{10}H_{13}NO$, 163.22
^{1}H-NMR (400 MHz, $CDCl_3$)	δ = 1.45 (d, J = 6.9 Hz, 3H, H1), 1.94 (s, 3H, H3´), 4.98 – 5.19 (m, 1H, H2), 6.27 (d, J = 7.7 Hz, 1H, NH), 7.16 – 7.44 (m, 5H, Ph).

^{13}C-NMR (100 MHz, $CDCl_3$)	δ = 21.7 (q, C1), 23.2 (q, C2´), 48.7 (d, C2), 126.1 (d, 2C, C4, C8), 127.2 (d, C6), 128.5 (d, 2C, C5, C7), 143.2 (s, C3), 169.2 (s, C1´).
GCMS	163 (36, M^+), 148 (23), 120 (26), 106 (100), 105 (17), 104 (36), 79 (30), 78 (16), 77 (39), 51 (24)

Analytical data was in accordance with the literature. [144]

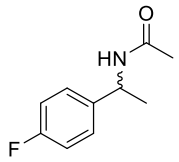
F.V.2 *N*-[1-(4-Methylphenyl)ethyl]acetamide (E-2)



General procedure	**F.V**
Reaction scale	500 mg (3.70 mmol) (**D-2**)
Reaction time	6 h
Yield	535 mg (82 %) colorless crystals
MP[145]	82 °C (Lit. 73-75 °C)
Sum formula, m.w.	$C_{11}H_{15}NO$, 177.25
^{1}H-NMR (200 MHz, $CDCl_3$)	δ = 1.46 (d, J = 6.9 Hz, 3H, H1), 1.96 (s, 3H, H3´), 2.33 (s, 3H, H Ph-CH_3), 5.07 (p, J = 7.1 Hz, 1H, H2), 5.99 (s, 1H, NH), 7.03 – 7.33 (m, 4H, Ph).
^{13}C-NMR (50 MHz, $CDCl_3$)	δ =21.0 (q, C-Ph-CH_3), 21.6 (q, C1), 23.3 (q, C2´), 48.5 (d, C2), 126.1 (d, 2C, C4, C8), 129.2 (d, 2C, C5, C7), 137.0 (s, C6), 140.1 (s, C3), 169.1 (s, C1´).
GCMS	177 (27, M^+), 162 (25), 134 (12), 120 (100), 119 (13), 118 (26), 117 (13), 93 (20), 91 (27), 77 (14), 65 (16)

Analytical data was in accordance with the literature. [146]

F.V.3 *N*-[1-(4-Fluorophenyl)ethyl]acetamide (E-3)

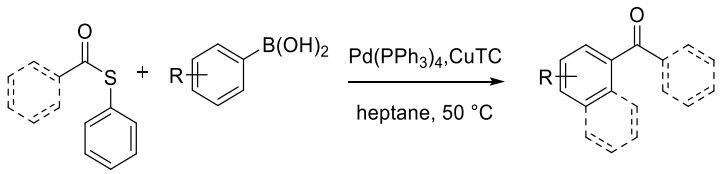


General procedure	**F.V**
Reaction scale	500 mg (3.59 mmol) (**D-3**)
Reaction time	6 h
Yield	475 mg (73 %) colorless crystals
MP[147]	146 °C (Lit. 148-150)
Sum formula, m.w.	$C_{10}H_{12}FNO$, 181.21
^{1}H-NMR (200 MHz, $CDCl_3$)	δ = 1.42 (d, J = 7.0 Hz, 3H, H1), 1.93 (s, 3H, H3´), 5.05 (p, J = 7.1 Hz, 1H, H2), 6.25 (s, 1H, NH), 6.92 – 7.05 (m, 2H, H5, H7), 7.19 – 7.30 (m, 2H, H4, H8).
^{13}C-NMR (50 MHz, $CDCl_3$)	δ = 21.7 (q, C1), 23.2 (q, C2´), 48.1 (d, C2), 115.3 (dd, 2C, J_{CF} = 21.2 Hz, C5, C7), 127.7 (d, 2C, J_{CF} = 8.0 Hz, C4, C8), 139.1(s, C3), 161.8 (d, J_{CF} = 245.4 Hz, C6), 169.2 (s, C1´).
GCMS	181 (26, M^+), 166 (24), 138 (23), 124 (100), 123 (15), 122 (35), 103 (14), 97 (17), 96 (11), 95 (12), 77 (13), 75 (14)

Analytical data was in accordance with the literature. [148]

F.VI Liebeskind Srogl cross coupling reactions

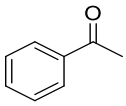
F.VI.1 Liebeskind Srogl reactions catalyzed by Tetrakis(triphenylphosphine)palladium



General procedure:

S-Phenyl thioacetate (**A-0**) or S-phenyl benzothioate (**A-2)** (0.25 mmol, 1 equiv.), boronic acid (0.28 mmole, 1.1 equiv.), CuTC (0.30 mmole, 1.2 equiv.), $Pd(PPh_3)_4$ (5 mol %) and 1 mL of heptane were charged into a screw cap vial and closed. The reaction mixture was stirred at 50 °C for 48 h. After the reaction was completed as monitored by GC-MS, it was cooled to room temperature and 4 mL of CH_2Cl_2 were added and stirred for 15 min. Then 8 mL of H_2O was added, the reaction mixture was extracted with CH_2Cl_2 (3 x 8 mL) and the combined organic layer was then dried over Na_2SO_4. After filtration the organic phase was utilized for quantification by GC or GC-MS.

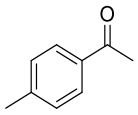
F.VI.1.1 Acetophenone (B-0)



General procedure	**F.VI.1**
Reaction scale	38 mg (0.25 mmol) (**A-0**)
Substrate concentration	250 mM
Reaction time	24 h
Conversion	99 %
Sum formula, m.w.	C_8H_8O, 120.15
GCMS:	120 (25, M^+), 105 (72), 77 (100), 74 (13), 51 (77), 50 (24)

Analytical data was in accordance with the purchased reference material.

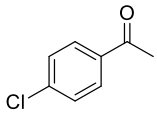
F.VI.1.2 1-(*p*-Tolyl)ethan-1-one (B-1)



General procedure	**F.VI.1**
Reaction scale	38 mg (0.25 mmol) (**A-0**)
Substrate concentration	250 mM
Reaction time	24 h
Conversion	86 %
Sum formula, m.w.	$C_9H_{10}O$, 134.18
GCMS	134 (32, M^+), 119 (100), 91 (82), 65 (22)

Analytical data was in accordance with the purchased reference material.

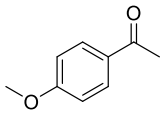
F.VI.1.3 1-(4-Chlorophenyl)ethan-1-one (B-5)



General procedure	**F.VI.1**
Reaction scale	38 mg (0.25 mmol) (**A-0**)
Substrate concentration	250 mM
Reaction time	24 h
Conversion	67 %
Sum formula, m.w.	C_8H_7ClO, 154.59
GCMS	156 (8, M^+), 154 (23), 141 (30), 139 (100), 113 (15), 111 (47), 75 (19)

Analytical data was in accordance with the purchased reference material.

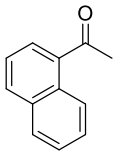
F.VI.1.4 1-(4-Methoxyphenyl)ethan-1-one (B-11)



General procedure	**F.VI.1**
Reaction scale	38 mg (0.25 mmol) (**A-0**)
Substrate concentration	250 mM
Reaction time	24 h
Conversion	99 %
Sum formula, m.w.	$C_9H_{10}O_2$, 150.18
GCMS	150 (35, M^+), 135 (100), 107 (18), 92 (15), 77 (27)

Analytical data was in accordance with the purchased reference material.

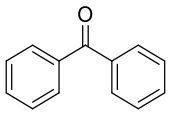
F.VI.1.5 1-(Naphtalen-1-yl)ethan-1-one (B-13)



General procedure	**F.VI.1**
Reaction scale	38 mg (0.25 mmol) (**A-0**)
Substrate concentration	250 mM
Reaction time	24 h
Conversion	89 %
Sum formula, m.w.	$C_{12}H_{10}O$, 170.21
GCMS	170 (59, M^+), 156 (12), 155 (100), 128 (12), 127 (86), 126 (17), 77 (10)

Analytical data was in accordance with the purchased reference material.

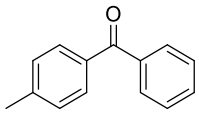
F.VI.1.6 Benzophenone (B-15)



General procedure	**F.VI.1**
Reaction scale	54 mg (0.25 mmol) (**A-2**)
Substrate concentration	250 mM
Reaction time	24 h
Conversion	99 %
Sum formula, m.w.	$C_{13}H_{10}O$, 182.22
GCMS	182 (60, M^+), 181 (10), 105 (100), 77 (44)

Analytical data was in accordance with the purchased reference material.

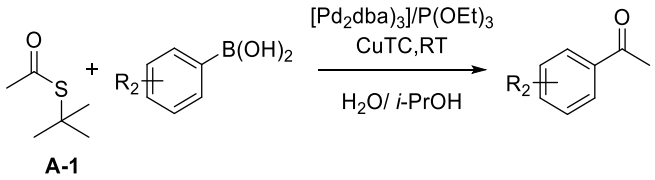
F.VI.1.7 Phenyl(*p*-tolyl)methanone (B-16)



General procedure	**F.VI.1**
Reaction scale	54 mg (0.25 mmol) (**A-2**)
Substrate concentration	250 mM
Reaction time	24 h
Conversion	99 %
Sum formula, m.w.	$C_{14}H_{12}O$, 196.25
GCMS	196 (57, M^+), 181 (13), 119 (100), 105 (26), 91 (28), 77 (19)

Analytical data was in accordance with the purchased reference material.

F.VI.2 Liebeskind Srogl reaction catalyzed by Tris(dibenzylideneacetone)dipalladium

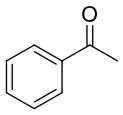


A-1

General procedure:

Thioester **A-1** (0.15 mmole, 1 equiv.), boronic acid (0.26 mmole, 1.7 equiv.), CuTC (0.24 mmole, 1.6 equiv.), $Pd_2(dba)_3$ (0.004 mmole, 2.5 mol %), $P(OEt)_3$ (0.03 mmole, 20 mol %) and 1.35 mL of H_2O and 150 µl of *i*-PrOH were charged into a screw cap vial and closed. The reaction mixture was stirred at room temperature for 24 h. After the reaction was completed as monitored by GC-MS, 4 mL of CH_2Cl_2 were added and stirred for 15 min. Then 8 mL of H_2O were added, the reaction mixture was extracted in CH_2Cl_2 and the combined organic layers were then dried over Na_2SO_4 and the organic phase was utilized for quantification by GC or GC-MS.

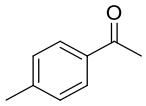
F.VI.2.1 Acetophenone (B-0)



General procedure	**F.VI.2**
Reaction scale	20 mg (0.15 mmol) (**A-1**)
Substrate concentration	100 mM
Reaction time	24 h
Conversion	78 %
Sum formula, m.w.	C_8H_8O, 120.15
GCMS	120 (25, M^+), 105 (72), 77 (100), 74 (13), 51 (77), 50 (24)

Analytical data was in accordance with the purchased reference material.

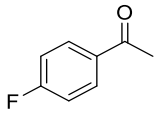
F.VI.2.2 1-(*p*-Tolyl)ethan-1-one (B-1)



General procedure	**F.VI.2**
Reaction scale	20 mg (0.15 mmol) (**A-1**)
Substrate concentration	100 mM
Reaction time	24 h
Conversion	46 %
Sum formula, m.w.	$C_9H_{10}O$, 134.18
GCMS	134 (33, M^+), 119 (100), 91 (96), 89 (10), 65 (37), 63 (12)

Analytical data was in accordance with the purchased reference material.

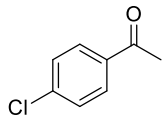
F.VI.2.3 1-(4-Fluorophenyl)ethan-1-one (B-3)



General procedure	**F.VI.2**
Reaction scale	20 mg (0.15 mmol) (**A-1**)
Substrate concentration	100 mM
Reaction time	24 h
Conversion	31 %
Sum formula, m.w.	C_8H_7FO, 138.14
GCMS	138 (24, M^+), 123 (100), 95 (73), 75 (33)

Analytical data was in accordance with the purchased reference material.

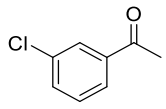
F.VI.2.4 1-(4-Chlorophenyl)ethan-1-one (B-5)



General procedure	**F.VI.2**
Reaction scale	20 mg (0.15 mmol) (**A-1**)
Substrate concentration	100 mM
Reaction time	24 h
Conversion	57 %
Sum formula, m.w.	C_8H_7ClO, 154.59
GCMS	156 (8, M^+), 154 (23), 141 (30), 139 (100), 113 (15), 111 (47), 75 (19)

Analytical data was in accordance with the purchased reference material.

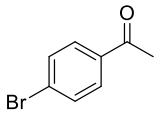
F.VI.2.5 1-(3-Chlorophenyl)ethan-1-one (B-6)



General procedure	**F.VI.2**
Reaction scale	20 mg (0.15 mmol) (**A-1**)
Substrate concentration	100 mM
Reaction time	24 h
Conversion	68 %
Sum formula, m.w.	C_8H_7ClO, 154.59
GCMS	156 (13, M^+), 154 (38), 141 (33), 139 (100), 113 (17), 111 (57)

Analytical data was in accordance with the purchased reference material.

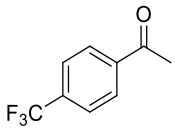
F.VI.2.6 1-(4-Bromophenyl)ethan-1-one (B-7)



General procedure	**F.VI.2**
Reaction scale	20 mg (0.15 mmol) (**A-1**)
Substrate concentration	100 mM
Reaction time	24 h
Conversion	55 %
Sum formula, m.w.	C_8H_7BrO, 199.05
GCMS	200 (25, M^+), 198 (29), 185 (95), 183 (100), 157 (39), 155 (42), 76 (41), 75 (41), 74 (25), 51 (15), 50 (39)

Analytical data was in accordance with the purchased reference material.

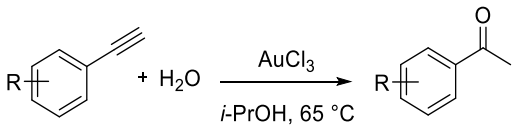
F.VI.2.7 1-(4-(Trifluoromethyl)phenyl)ethan-1-one (B-9)



General procedure	**F.VI.2**
Reaction scale	20 mg (0.15 mmol) (**A-1**)
Substrate concentration	100 mM
Reaction time	24 h
Conversion	41 %
Sum formula, m.w.	$C_9H_7F_3O$, 188.15
GCMS	188 (15, M^+), 174 (10), 173 (100), 145 (83), 95 (14), 75 (13)

Analytical data was in accordance with the purchased reference material.

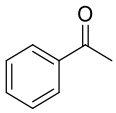
F.VII Gold (III) catalyzed hydration of alkynes



General procedure:

In a screw cap vial, equipped with a magnetic stirring bar, $AuCl_3$ (0.025-0.05 mmol) and 1mL of *i*-PrOH was added. The vial was closed and the reaction mixture was stirred for 5 minutes. Then the corresponding substrate alkyne (0.5 mmol, 1 equiv.) and 4 equiv. of H_2O were charged into the vial and closed. The resulting reaction mixture was heated for 24 h at 65 °C. After completion of the reaction, the reaction mixture was diluted (ethylacetate containing methylbenzoate as internal standard) and quantitative analysis was performed by GC.

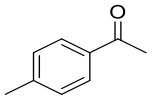
F.VII.1.1 Acetophenone (B-0)



General procedure	**F.VII**
Reaction scale	64 mg (0.5 mmol) (**G-0**); 5 mol% $AuCl_3$
Substrate concentration	500 mM
Reaction time	24 h
Conversion	98 %
Sum formula, m.w.	C_8H_8O, 120.15
GCMS	120 (25, M^+), 105 (72), 77 (100), 74 (13), 51 (77), 50 (24)

Analytical data was in accordance with the purchased reference material.

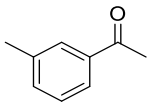
F.VII.1.2 1-(*p*-Tolyl)ethan-1-one (B-1)



General procedure	**F.VII**
Reaction scale	73 mg (0.5 mmol) (**G-1**); 5 mol% $AuCl_3$
Substrate concentration	500 mM
Reaction time	24 h
Conversion	98 %
Sum formula, m.w.	$C_9H_{10}O$, 134.18
GCMS	134 (33, M^+), 119 (100), 91 (96), 89 (10), 65 (37), 63 (12)

Analytical data was in accordance with the purchased reference material.

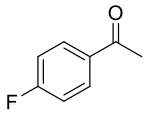
F.VII.1.3 1-(*m*-Tolyl)ethan-1-one (B-2)



General procedure	**F.VII**
Reaction scale	73 mg (0.5 mmol) (**G-2**); 5 mol % $AuCl_3$
Substrate concentration	500 mM
Reaction time	24 h
Conversion	99 %
Sum formula, m.w.	$C_9H_{10}O$, 134.18
GCMS	134 (37, M^+), 119 (98), 91 (100), 65 (26)

Analytical data was in accordance with the purchased reference material.

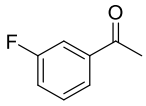
F.VII.1.4 1-(4-Fluorophenyl)ethan-1-one (B-3)



General procedure	**F.VII**
Reaction scale	60 mg (0.5 mmol) (**G-3**); 10 mol% $AuCl_3$
Substrate concentration	500 mM
Reaction time	24 h
Conversion	71 %
Sum formula, m.w.	C_8H_7FO, 138.14
GCMS	138 (24, M^+), 123 (100), 95 (73), 75 (33)

Analytical data was in accordance with the purchased reference material.

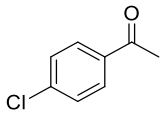
F.VII.1.5 1-(3-Fluorophenyl)ethan-1-one (B-4)



General procedure	**F.VII**
Reaction scale	60 mg (0.5 mmol) (**G-4**); 10 mol% $AuCl_3$
Substrate concentration	500 mM
Reaction time	24 h
Conversion	86 %
Sum formula, m.w.	C_8H_7FO, 138.14
GCMS	138 (34, M^+), 123 (100), 95 (83), 75 (24)

Analytical data was in accordance with the purchased reference material.

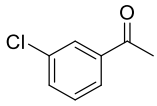
F.VII.1.6 1-(4-Chlorophenyl)ethan-1-one (B-5)



General procedure	**F.VII**
Reaction scale	68 mg (0.5 mmol) (**G-5**); 10 mol% $AuCl_3$
Substrate concentration	500 mM
Reaction time	24 h
Conversion	72 %
Sum formula, m.w.	C_8H_7ClO, 154.59
GCMS	156 (8, M^+), 154 (23), 141 (30), 139 (100), 113 (15), 111 (47), 75 (19)

Analytical data was in accordance with the purchased reference material.

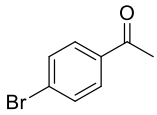
F.VII.1.7 1-(3-Chlorophenyl)ethan-1-one (B-6)



General procedure	**F.VII**
Reaction scale	68 mg (0.5 mmol) (**G-6**); 10 mol% $AuCl_3$
Substrate concentration	500 mM
Reaction time	24 h
Conversion	76 %
Sum formula, m.w.	C_8H_7ClO, 154.59
GCMS	156 (13, M^+), 154 (38), 141 (33), 139 (100), 113 (17), 111 (57)

Analytical data was in accordance with the purchased reference material.

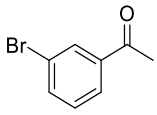
F.VII.1.8 1-(4-Bromophenyl)ethan-1-one (B-7)



General procedure	**F.VII**
Reaction scale	91 mg (0.5 mmol) (**G-7**); 10 mol% $AuCl_3$
Substrate concentration	500 mM
Reaction time	24 h
Conversion	70 %
Sum formula, m.w.	C_8H_7BrO, 199.05
GCMS	200 (25, M^+), 198 (29), 185 (95), 183 (100), 157 (39), 155 (42), 76 (41), 75 (41), 74 (25), 51 (15), 50 (39)

Analytical data was in accordance with the purchased reference material.

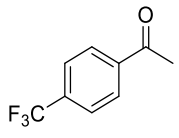
F.VII.1.9 1-(3-Bromophenyl)ethan-1-one (B-8)



General procedure	**F.VII**
Reaction scale	91 mg (0.5 mmol) (**G-8**); 10 mol% $AuCl_3$
Substrate concentration	500 mM
Reaction time	24 h
Conversion	79 %
Sum formula, m.w.	C_8H_7BrO, 199.05
GCMS	200 (32, M^+), 198 (37), 185 (89), 183 (100), 157 (54), 155 (58), 77 (15), 76 (53), 74 (22), 51 (11), 50 (22)

Analytical data was in accordance with the purchased reference material.

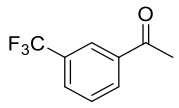
F.VII.1.101-(4-(Trifluoromethyl)phenyl)ethan-1-one (B-9)



General procedure	**F.VII**
Reaction scale	85 mg (0.5 mmol) (**G-9**); 10 mol% $AuCl_3$
Substrate concentration	500 mM
Reaction time	24 h
Conversion	49 %
Sum formula, m.w.	$C_9H_7F_3O$, 188.15
GCMS	188 (15, M^+), 174 (10), 173 (100), 145 (83), 95 (14), 75 (13)

Analytical data was in accordance with the purchased reference material.

F.VII.1.111-(3-(Trifluoromethyl)phenyl)ethan-1-one (B-10)



General procedure	**F.VII**
Reaction scale	85 mg (0.5 mmol) (**G-10**); 10 mol% $AuCl_3$
Substrate concentration	500 mM
Reaction time	24 h
Conversion	57 %
Sum formula, m.w.	$C_9H_7F_3O$, 188.15
GCMS	188 (15, M^+), 173 (100), 145 (90), 125 (10), 95 (18), 75 (17)

Analytical data was in accordance with the purchased reference material.

F.VIII Expression and preparation of alcohol dehydrogenases

F.VIII.1 ADH-A

Preparation of lyophilized cells of *E. coli*BL21(DE3)/pET22b(+)_*adh-A* (from *Rhodococcus ruber*)

E. coli BL21(DE3)/pET22b(+)_*adh-A* was stored at -80°C in LB-amp containing 25% (v/v) glycerol. Prior to use, cells were plated on LB-amp plates (100 µg·mL^{-1} final ampicillin concentration). A single colony was used to inoculate 200 mL of TB-amp (100 µg·mL^{-1} final ampicillin concentration) in a 1 L baffled shake flask. $ZnCl_2$ was added from a 100 mM stock to a final concentration of 1 mM and cells were grown at 30°C with shaking (120 rpm) for about 20 h. On the next day OD_{590} was checked ($OD5_{90}$~6.0) and 200 µL of ampicillin stock solution (50 mg·mL^{-1} stock) were added. Protein production was induced upon addition of IPTG from a 100 mM stock to a final concentration of 2 mM and cells were cultivated at 20°C with shaking (120 rpm) for 24 h. Cells were harvested by centrifugation (6 000 x g, 15 min, 4°C). The medium was discarded; cells were re-suspended in sterile water, snap frozen in liquid nitrogen and lyophilized**.** This is an optimized procedure based on the literature.[66]

F.VIII.2 LK-ADH

Preparation of lyophilized cells of *E. coli*BL21(DE3)/pET21b(+)_*LK-ADH* (from *Lactobacillus kefir*)

E. coli BL21(DE3)/pET21b(+)_*LK-ADH* was stored at -80°C in LB-amp containing 25% (v/v) glycerol. Prior to use, cells were plated on LB-amp plates (100 µg·mL^{-1} final ampicillin concentration). A single colony was used to prepare 4 mL of an overnight culture in LB medium containing 100 µg·mL^{-1} ampicillin (added from a 50 mg·mL^{-1} stock). The main culture was prepared by inoculation of 200 mL TB-amp (100 µg·mL^{-1} final ampicillin concentration) in a 1 L baffled shake flask with 2 mL of the overnight culture. Cells were grown at 37°C with shaking (120 rpm) to an OD_{590} = 0.5. Enzyme expression was induced by the addition of IPTG (1 mM final concentration) from a 100 mM IPTG stock. Cells were cultivated at 30°C with shaking (120 rpm) and harvested by centrifugation (6 000 x g, 15 min, 4°C) after 24 h. The medium was discarded; cells were resuspended in sterile water, snap frozen in liquid nitrogen and lyophilized**.**

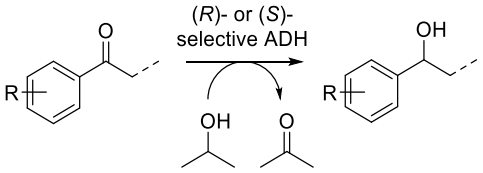
F.VIII.3 Yeast reductase

Preparation of cell free extract of *E. coli*BL21(DE3)_ *ADH* (from *Saccharomyces cerevisae*)

Gluthathione (S)-transferase (GST) fusion protein, YOL151w, pIK3, methylglyoxal reductase, 38.2 kDa

E. *coli* BL21(DE3) cells harboring the ADH-encoding pIK plasmid were grown in LB-kan (100 µg·mL^{-1} final kanamycine concentration) medium (10 mL) at 37 °C and 200 rpm for approx. 17 h. The main culture in LB-kan (25 µg·mL^{-1} final ampicillin concentration) (500 mL) was inoculated with 5% v/v and shaken at 37 °C and 200 rpm for 2.5 h (OD600 = 0.70). The culture was cooled to 24 °C, then IPTG was added to a final concentration of 0.1 mM. Shaking was continued at 24 °C and 200 rpm for 6 h. The cells were harvested by centrifugation (6 kRCF, 4 °C, 10 min), then resuspended in TrisHCl (50 mL, 50 mM, pH 7.5), and centrifuged again. The cells were stored at -20 °C o/n. The partly lysed cells were resuspended in TrisHCl (10 mL, as above) and sonicated at 4 °C (KE 76 probe, 50% amplitude, 5 s pulse, 55 s pause, 8 cycles). Cell debris was centrifuged (4 °C, 15 min with 10 kRCF, then 20 min at 15 kRCF). The supernatant was filtered (0.45 µm PES membrane), yielding 12 mL cleared crude cell extract (CCE) (Bradford assay: 18.1 ± 0.5 mg mL-1; approx. 0.4 g CCE L-1 culture). This solution was diluted with aqueous solutions of glycerol (12 mL, 50% w/v stock, 25% w/v final), DTT (24 µL, 1 M stock, 1 mM final), NaCl (240 µL, 5 M stock, 50 mM final), EDTA (192 µL, 0.25 M stock, 2 mM final) and $MgCl_2$ (48 µL, 2 M stock, 4 mM final). The CCE was then stored at -20 °C (final conc: approx. 9.0 mg mL-1). [64] For the preparation of cell free extract lyophilisate, the CCE was shock frozen in liquid nitrogen and lyophilized.

F.IX Biocatalytic reduction using whole cell lyophilistates



General procedure:

Lyophilized cells of *E. coli* (*S*)-selective ADH-A (10 mg), respective (*R*)-selective LK-ADH (20 mg) were rehydrated in Tris HCl buffer (pH 8, 320mM, 500 µl final volume) for 1 h at 30°C with 150 rpm in Eppendorf tubes (1.5 mL). Substrate (100mM) and 2-propanol (83 µl) were added and the mixture was agitated at 30°C and 200 rpm for 24 hours. The reaction was stopped by addition of ethyl acetate containing methylbenzoate as internal standard (10 µl reaction mixture extracted in 1 mL ethyl acetate). The organic layer was separated from the aqueous phase using centrifugation and dried over Na_2SO_4. Conversions and enantioselectivities were determined by GC analysis. For the production of (*S*)-alcohols the (S)-selective ADH-A from *Rhodococcus rube*r, for (*R*)-alcohols the (*R*)-selective LK-ADH from *Lactobacillus kefir* was used.

Table F-7. Biocatalytic reductions using whole cell lyophilisate of (*S*)-selective ADH-A and 100 mM substrate concentration

Cpd.No.	Product (*S*)-alcohol	Reaction scale (0.05 mmol) [mg]	Conversion (GC) [%], 4 h	ee[%]
C-0	1-Phenylethan-1-ol [128a]	6 **(B-0)**	96	>99 %
C-1	1-(4-Methylphenyl)ethan-1-ol [149]	7 **(B-1)**	90	>99 %
C-2	1-(3-Methylphenyl)ethan-1-ol [120]	7 **(B-2)**	85	>99 %
C-3	1-(4-Fluorophenyl)ethan-1-ol [a]	7 **(B-3)**	99	>99 %
C-4	1-(3-Fluorophenyl)ethan-1-ol .[150]	7 **(B-4)**	95	>99 %
C-5	1-(4-Chlorophenyl)ethan-1-ol [149]	8 **(B-5)**	98	>99 %
C-6	1-(3-Chlorophenyl)ethan-1-ol [120]	8 **(B-6)**	97	>99 %
C-7	1-(4-Bromophenyl)ethan-1-ol [149]	10 **(B-7)**	92	>99 %
C-8	1-(3-Bromophenyl)ethan-1-ol [151]	10 **(B-8)**	99	>99 %
C-9	1-(4-Trifluormethylphenyl)ethan-1-ol [a]	9 **(B-9)**	99	>99 %
C-10	1-(3-Trifluormethylphenyl)ethan-1-ol [150]	9 **(B-10)**	99	>99 %
C-11	1-(4-Methoxyphenyl)ethan-1-ol [149]	8 **(B-11)**	70	>99 %
C-12	1-Phenylpropan-1-ol [149]	7 **(B-12)**	91	>99 %
C-14	1-(2-Naphtyl)ethan-1-ol [b]	9 **(B-14)**	77	>99 %
C-15	Diphenylmethanol	9 **(B-15)**	0	na
C-17	2-Methyl-1-phenylpropan-1-ol	8 **(B-17)**	0	na
C-18	(4-Chloroyphenyl)(cyclopropyl)methanol	9 **(B-18)**	0	na

[a] Analytical data was in accordance with the isolated alcohol generated in the preparative scale experiment (F.X.1)/ (F.X.2).
[b] Analytical data was in accordance with the purchased reference material.

Table F-8. Biocatalytic reductions using whole cell lyophilisate of (*R*)-selective LK-ADH and 100 mM substrate concentration

Cpd.No.	Product (*R*)-alcohol	Reaction scale (0.05 mmol) [mg]	Conversion (GC) [%], 24 h	ee[%]
C-0	1-Phenylethan-1-ol [128a]	6 **(B-0)**	80	>99 %
C-1	1-(4-Methylphenyl)ethan-1-ol [149]	7 **(B-1)**	83	>99 %
C-2	1-(3-Methylphenyl)ethan-1-ol [120]	7 **(B-2)**	17	>99 %
C-3	1-(4-Fluorophenyl)ethan-1-ol [a]	7 **(B-3)**	83	>99 %
C-4	1-(3-Fluorophenyl)ethan-1-ol .[150]	7 **(B-4)**	91	>99 %
C-5	1-(4-Chlorophenyl)ethan-1-ol [149]	8 **(B-5)**	93	>99 %
C-6	1-(3-Chlorophenyl)ethan-1-ol [120]	8 **(B-6)**	88	>99 %
C-7	1-(4-Bromophenyl)ethan-1-ol [149]	10 **(B-7)**	71	>99 %
C-8	1-(3-Bromophenyl)ethan-1-ol [151]	10 **(B-8)**	45	>99 %
C-9	1-(4-Trifluormethylphenyl)ethan-1-ol [a]	9 **(B-9)**	99	>99 %
C-10	1-(3-Trifluormethylphenyl)ethan-1-ol [150]	9 **(B-10)**	0	na
C-11	1-(4-Methoxyphenyl)ethan-1-ol [149]	8 **(B-11)**	40	>99 %
C-12	1-Phenylpropan-1-ol [149]	7 **(B-12)**	0	na
C-14	1-(2-Naphtyl)ethan-1-ol [b]	9 **(B-14)**	77	>99 %

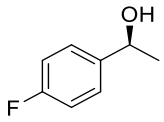
[a] Analytical data was in accordance with the isolated alcohol generated in the preparative scale experiment (F.X.1)/ (F.X.2).
[b] Analytical data was in accordance with the purchased reference material.

F.XBiocatalytic reduction: Preparative scale

General procedure:

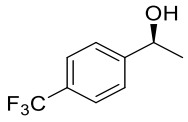
Lyophilized cells of *E. coli* ADH-A (100 mg) were rehydrated in Tris HCl buffer (pH 8, 320mM, 5 mL) and were agitated for 1 h at 30°C with 200 rpm in Greiner tubes (50 mL). Substrate (100 mmol) and 16 % v/v of *i*-PrOH (800 µl) were added and the mixture was agitated at 30°C and 200 rpm for 24 hours. The reaction mixture was extracted with Et_2O (3 × 10 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was removed to afford the desired enantiopure alcohol.

F.X.1 *(S)*-1-(4-Fluorophenyl)ethan-1-ol (C-3)



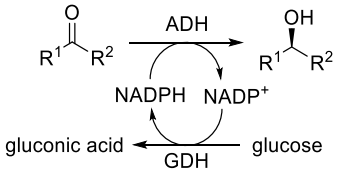
General procedure	**F.X**
Reaction scale	69 mg (0.5 mmol) (**B-3**)
Substrate concentration	100 mM
Reaction time	24 h
Yield	64 mg (91 %) colorless oil
Sum formula, m.w.	C_8H_9FO, 140.16
GCMS	140 (22, M^+), 125 (100), 123 (11), 97 (80), 96 (20), 95 (24), 77 (38), 75 (20), 51 (15)
ee (GC)	>99 %
Specific rotation[152]	α_D^{20} = –47 (*c* 1, $CHCl_3$) (Lit. –42.5 (*c* 0.91, $CHCl_3$)

F.X.2 *(S)*-1-(4-Trifluormethylphenyl)ethan-1-ol (C-9)



General procedure	**F.X**
Reaction scale	94 mg (0.5 mmol) (**B-9**)
Substrate concentration	100 mM
Reaction time	24 h
Yield	95 mg (64 %) colorless oil
Sum formula, m.w.	$C_9H_9F_3O$, 190.17
GCMS	190 (9, M^+), 176 (9), 175 (100), 147 (23), 145 (16), 127 (92), 77 (16), 75 (12), 51 (13)
ee (GC)	>99 %
Specific rotation[153]	α_D^{20} = –33 (*c* 1, $CHCl_3$) (Lit. –30.2 (*c* 1, $CHCl_3$))

F.XI Biocatalytic reduction applying ADH from *Saccharomyces cerevisae*



General procedure:

The (*S*)-selective yeast reductase (10 mg cell free extract lyophilisate) was rehydrated in Tris HCl buffer (pH 7.5, 50mM, 1 mL final volume) in Eppendorf tubes (1.5 mL). Then 8 µl of the substrate stock solution (in EtOH) was added (final concentration 4 mM), 9 µl of glucose stock solution was added (final concentration 10 mM), 0.7 µl glucose dehydrogenase stock solution was added (10 U/mL final concentration), 8 µl $NADP^+$ stock solution was added (0.8 mM final concentration) and 10 % of *i*-PrOH (100 µl) were added and the mixture was agitated at 30°C and 200 rpm for 24 hours. The reaction was stopped by addition of ethyl acetate containing methylbenzoate as internal standard (10 µl reaction mixture extracted in 1 mL ethyl acetate). The organic layer was separated from the aqueous phase using centrifugation and dried over Na_2SO_4. Conversions and enantioselectivities were determined by GC analysis.

Table F-9. Biocatalytic reductions using lyophilized cell free extract of (*S*)-selective ADH from *Saccharomyces cerevisae* (YOL151w, pIK3) and 4 mM substrate concentration

Cpd.No.	Product (*S*)-alcohol	Conversion (GC) [%], 24 h	ee[%]
C-0	1-Phenylethan-1-ol	99	>99 %
C-1	1-(4-Methylphenyl)ethan-1-ol	88	>99 %
C-5	1-(4-Chlorophenyl)ethan-1-ol	100	>99 %
C-11	1-(4-Methoxyphenyl)ethan-1-ol	78	>99 %
C-13	1-(1-Naphtyl)ethan-1-ol	99	>99 %
C-15	Diphenylmethanol	100	na
C-16	Phenyl(p-tolyl)methanol	61	na*

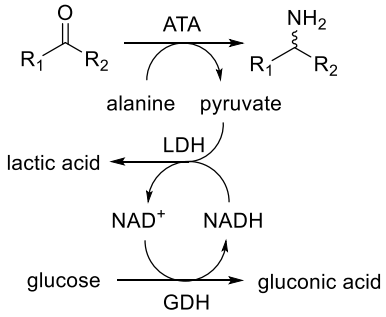
*no peak separation of the reference alcohol obtained

Analytical data was in accordance with the isolated alcohols generated in.F.IV

F.XII Expression and preparation of ω-transaminases

E. coli BL21(DE3)/pET22b transaminases were stored at -80°C in LB-amp/ LB-kan (in case of VflH6) containing 25% (v/v) glycerol. Prior to use, cells were plated on LB-amp plates or LB-kan plates. A single colony was used to prepare 4 mL of an overnight culture in LB medium containing 100 $\mu g \cdot mL^{-1}$ ampicillin or 50 $\mu g \cdot mL^{-1}$ kanamycine. The main culture was prepared by inoculation of 200 mL TB-amp (100 $\mu g \cdot mL^{-1}$ final ampicillin concentration) or TB-kan (50 $\mu g \cdot mL^{-1}$ final ampicillin concentration) in a 1 L baffled shake flask with 2 mL of the overnight culture. Cells were grown at 37°C with shaking (120 rpm) to an OD_{590} = 0.7. Enzyme expression was induced by the addition of IPTG (0.1 mM final concentration) from a 100 mM IPTG stock or 0.2% (w/v) rhamnose (pGaston). Cells were cultivated at 20°C with shaking (120 rpm) and harvested by centrifugation (6 000 x g, 15 min, 4°C) after 20 h.The medium was discarded; cells were resuspended in an aqueous PLP solution (1 mM), snap frozen in liquid nitrogen and lyophilized.[154]

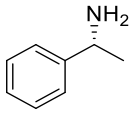
F.XIII Biocatalytic transamination applying ω-ATA from *Aspergillus Fumigatus*



General procedure:

Whole cell lyophilisate of the (*R*)-selective ATA from *Aspergillus Fumigatus* AspFum (20 mg) were rehydrated in sodium phosphate buffer (pH 7.5, 100mM, 500 µl final volume) in Eppendorf tubes (1.5 mL). Then 84 µl of D-alanine stock solution (250 mM final concentration), 34 µl of D-glucose stock solution (final concentration 150 mM), 10 µl NADH stock solution (2 mM final concentration), 100 µl PLP stock solution (in H_2O) were added (0.2 mM final concentration), 7 µl lactate-dehydrogenase stock solution were added (90 U/mL final concentration), 15 µl glucose-dehydrogenase stock solution were added (15 U/mL final concentration) and the substrate was added to a final concentration of 50 mM. After the reaction was quenched with 2 N NaOH, ethyl acetate containing methylbenzoate as internal standard (10 µl reaction mixture extracted in 400 µl ethyl acetate) was added. The organic layer was separated from the aqueous phase using centrifugation and dried over Na_2SO_4. Conversions and enantioselectivities were determined by GC analysis.

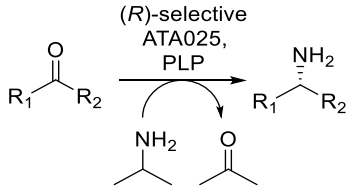
F.XIII.1 (*R*)-1-Phenylethan-1-amine (D-1)



General procedure	**F.XIII**
Substrate concentration	50 mM (**B-0**)
Reaction time	24 h
Conversion	92 %
Sum formula, m.w.	$C_8H_{11}N$, 121.18
GCMS	121 (1, M^+), 106 (100), 79 (37), 77 (20)
ee (GC)	>99 %

Analaytical data was in accordance with the purchased reference material.

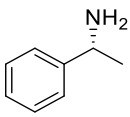
F.XIV Biocatalytic transamination applying Codexis-transaminases



General procedure:

The (*R*)-selective ATA 025 from the codex screening kit (2 mg) was rehydrated in Eppendorf tubes (1.5 mL) in a mastermix (180 µl) containing 1 M isopropylamine, 0.1 M triethanolamine and 2 mM PLP, pH 9. The substrate was added from a stock solution of the ketone in DMSO (c = 100 mM) (20 µl, final concentration 10 mM) and the mixture was agitated at 30°C and 200 rpm for 24 hours. Afterwards the reaction was stopped by addition of NaOH (pH >11) and extracted in ethyl acetate (10 µl reaction mixture extracted in 200 µl ethyl acetate). The organic layer was separated from the aqueous phase using centrifugation and dried over Na_2SO_4. Conversions and enantioselectivities were determined by GC analysis. For the chiral GC analysis acetyl chloride (10 µl) was added to 10 µl of the reaction mixture and the Eppendorf tube was agitated for 30 min. After the reaction was quenched with 2 N NaOH, it was extracted in 200 µl ethyl acetate. The organic layer was separated from the aqueous phase using centrifugation and dried over Na_2SO_4.

F.XIV.1 (*R*)-1-Phenylethan-1-amine (D-1)

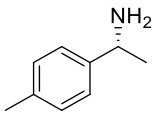


General procedure	**F.XIV**
Substrate concentration	10 mM (**B-0**)
Reaction time	24 h
Conversion	86 %
Sum formula, m.w.	$C_8H_{11}N$, 121.18
GCMS	121 (1, M^+), 106 (100), 79 (37), 77 (20)

ee (GC)	>99 %

Analaytical data was in accordance with the purchased reference material.

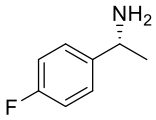
F.XIV.2 (R)-1-(p-Tolyl)ethan-1-amine (D-2)



General procedure	**F.XIV**
Substrate concentration	10 mM (**B-1**)
Reaction time	24 h
Conversion	83 %
Sum formula, m.w.	$C_9H_{13}N$, 135.21
GCMS	135 (3, M^+), 120 (100), 93 (39), 91 (29), 77 (15)
ee (GC)	>99 %

Analaytical data was in accordance with the purchased reference material.

F.XIV.3 (*R*)-1-(4-Fluorophenyl)ethan-1-amine (D-3)

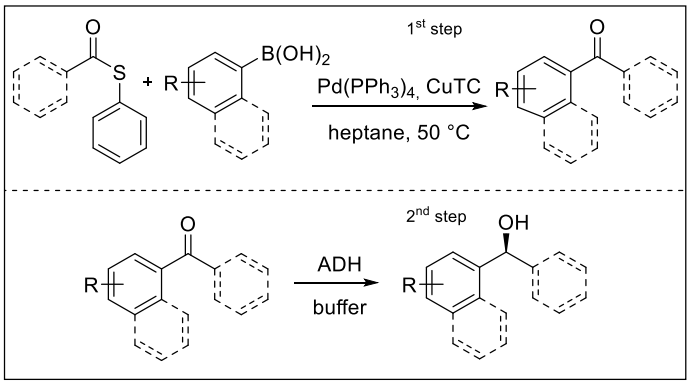


General procedure	**F.XIV**
Substrate concentration	10 mM (**B-3**)
Reaction time	24 h
Conversion	90 %
Sum formula, m.w.	$C_8H_{10}FN$, 139.17
GCMS	139 (2, M^+), 124 (100), 97 (26)
ee (GC)	>99

Analaytical data was in accordance with the purchased reference material.

F.XV Chemoenzymatic one pot reactions

F.XV.1 Liquid-liquid biphasic system- Synthesis of chiral alcohols



General procedure:

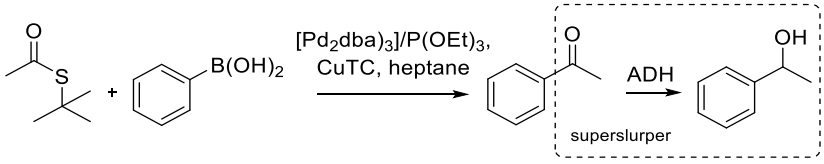
The (*S*)-selective yeast reductase (10 mg cell free extract lyophilisate) was rehydrated in Tris HCl buffer (pH 7.5, 50mM, 1 mL final volume) in Eppendorf tubes (1.5 mL). Then 14 µl of glucose stock solution were added (final concentration 15 mM) together with 0.7 µl glucose dehydrogenase stock solution (1 U/mL final concentration), 8 µl $NADP^+$ stock solution (0.8 mM final concentration), 10 % of *i*-PrOH (100 µl) and 100 µl of centrifuged Liebeskind-Srogl coupling reaction (50 mM ketone concentration, final concentration 5 mM) were added and the mixture was agitated at 30°C and 200 rpm for 24 hours. The reaction was stopped by addition of ethyl acetate containing methylbenzoate as internal standard. The organic layer was separated from the aqueous phase using centrifugation and dried over Na_2SO_4. Conversions and enantioselectivities were determined by GC or GCMS analysis.

Table F-10. Sequential chemoenzymatic one-pot reactions using lyophilized cell free extract of (*S*)-selective ADH from *Saccharomyces cerevisae* (YOL151w, pIK3) and 5 mM substrate concentration (Ketones generated by Liebeskind-Srogl coupling reaction)

Cpd.No.	Product (*S*)-alcohol	Applied coupling reaction	Conversion (GC) [%],24 h	ee[%]
C-0	1-Phenylethan-1-ol	F.VI.1.1	57	>99 %
C-1	1-(4-Methylphenyl)ethan-1-ol	F.VI.1.2	44	>99 %
C-5	1-(4-Chlorophenyl)ethan-1-ol	F.VI.1.3	91	>99 %
C-11	1-(4-Methoxyphenyl)ethan-1-ol	F.VI.1.4	63	>99 %
C-13	1-(1-Naphtyl)ethan-1-ol	F.VI.1.5	49	>99 %
C-15	Diphenylmethanol	F.VI.1.6	80	na
C-16	Phenyl(p-tolyl)methanol	F.VI.1.7	5	na*

*no peak separation of the reference alcohol obtained
Analytical data was in accordance with the isolated alcohols generated in.F.IV

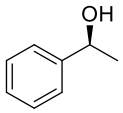
F.XV.2 Liquid-solid superslurper system: Alcohol dehydrogenase embedded in sodium polyacrylate



General procedure:

The (*S*)-selective yeast reductase (40 mg cell free extract lyophilisate) was rehydrated in 348 µl of Tris HCl buffer (pH 7.5, 50mM) in a glass vial (8 mL). Then 34 µl of glucose stock solution (final concentration 38 mM), 2.64 µl glucose dehydrogenase stock solution (4 U/mL final concentration) and 16 µl $NADP^+$ stock solution were added (1.2 mM final concentration). The resulting mixture was vortexed and 5 mg of sodium polyacrylate was added into the glas vial. After vortexing again to ensure a proper distribution within the superslurper phase, the mixture was incubated at room temperature for 30 min. Finally, 600 µl of centrifuged Liebeskind-Srogl coupling reaction conducted in heptane (25 mM initial concentration of ketone **B-0**) was added to the vial containing the super slurper phase (15 mM final concentration). The biphasic reaction mixture was agitated at 37 °C and 200 rpm for 24 hours. The overall reaction was extracted by 3 x 1 mL of EtOAc containing methylbenzoate as internal standard and dried over Na_2SO_4. Conversions and enantioselectivities were determined by GC or GCMS analysis.

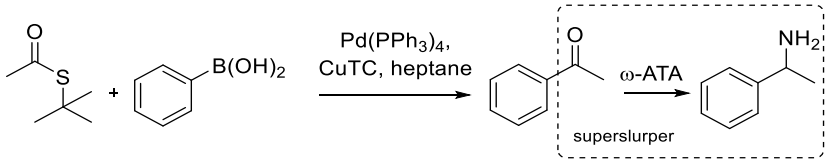
F.XV.2.1 (S)-1-(Phenyl)ethan-1-ol (C-0)



General procedure	**F.XV.2**
Coupling reaction	**F.VI.1.1**
Substrate concentration	15 mM (**B-0**)
Reaction time	24 h
Conversion	84 %
Sum formula, m.w.	$C_8H_{10}O$, 122.17
ee (GC)	>99 %

Analytical data was in accordance with the purchased reference compound.

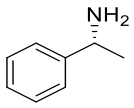
F.XV.3 Liquid-solid superslurper system: ω-Transaminase embedded in sodium polyacrylate



General procedure:

The (*R*)-selective codexis ATA025 (40 mg) was rehydrated in 400 µl of codexis mastermix (see 0) in a glass vial (8 mL). The resulting mixture was vortexed and 7 mg of sodium polyacrylate were added into the glas vial. After vortexing again to ensure a proper distribution within the superslurper phase, the mixture was incubated at room temperature for 30 min. Finally, 600 µl of centrifuged **(A)** or initial **(B)** Liebeskind-Srogl coupling reaction conducted in heptane (25 mM initial concentration of ketone **B-0**) was added to the vial containing the super slurper phase (15 mM final concentration). The biphasic reaction mixture was agitated at 37 °C and 200 rpm for 48 hours. The reaction was stopped by addition of 100 µl of 5M NaOH and vortexed. The overall reaction mixture was then extracted by 4 x 1 mL of EtOAc containing methylbenzoate as internal standard and dried over Na_2SO_4. Conversions and enantioselectivities were determined by GC or GCMS analysis.

F.XV.3.1 (*R*)-1-Phenylethan-1-amine (D-1)



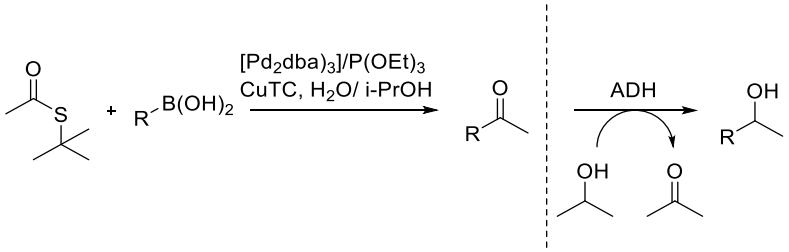
General procedure	Fehler! Verweisquelle konnte nicht gefunden werden.
Coupling reaction	**F.VI.1.1**
Substrate concentration	15 mM (**B-0**)
Reaction time	48 h
Conversion	51 % **(A)**, 27 % **(B)***
Sum formula, m.w.	$C_8H_{11}N$, 121.18
ee (GC)	>99 %

*By addition of a second super slurper phase after 24 hours, a conversion of 47 % was observed in reaction **(B)**.

Analytical data was in accordance with the purchased reference compound.

F.XV.4 Membrane based system

F.XV.4.1 Synthesis of chiral alcohols



The freshly prepared PDMS sheet was placed on one of the glass parts of the reactor, the sealing ring was applied and both reactor parts were screwed together holding the PDMS membrane between the two reaction chambers.

Chamber A, Enzymatic chamber: Lyophilized cells of *E. coli* ADH-A (150 mg) respectively LK-ADH (200 mg) were rehydrated in Tris HCl buffer (pH 8, 320mM, 1.7 mL) in a Greiner tube (50 mL) and agitated for 1 h at 30°C with 200 rpm. Before reaction chambers A were filled, 2-propanol (800 µl) was added into the Greiner tube and vortexed.

Chamber B, Liebeskind-Srogl coupling chamber: Boronic acid (0.85 mmole, 1.7 equiv), CuTC (0.8 mmole, 1.6 equiv) and $Pd_2(dba)_3$ (2.5 mol %) were added to chamber B. Additionally, 2.161 mL of H_2O, 250 µl 2-propanol, thioester (**A-1**) (0.5 mmole, 1 equiv.), and $P(OEt)_3$ (20 mol %) was placed in this chamber.

The enzymatic mixture was transferred into chamber A and the membrane reactor was agitated at 30°C and 200 rpm for 40 h.

The reaction mixture (20 µl of both chambers separately) were added to 180 µl acetonitrile containing an internal standard, vortexed and treated with 50 mg of SiliaMetS (DMT) metal scavenger. Conversions were determined by UPC^2.

For product isolation, the combined chambers were extracted with DCM (4 × 10 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and the solvent was removed. Purification was performed applying standard manual glass columns using silica gel from Merck (40-63 µm) and DCM (raw product/ SiO_2; 1:70).

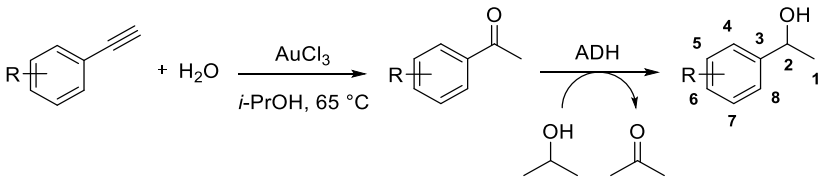
Table F-11. Simultaneous one pot reaction: synthesis of enantiopure alcohols

Cpd.No.	Product	Conversion [%] (*S*)-alcohol ADH-A	Conversion [%] (*R*)-alcohol LK-ADH	Isolated yield [%] (*R*)-alcohol LK-ADH	ee[%]
C-0	1-Phenylethan-1-ol	65	81	-	>99 %
C-3	1-(4-Fluorophenyl)ethan-1-ol	75	64	-	>99 %
C-5	1-(4-Chlorophenyl)ethan-1-ol	60	57	51	>99 %
C-6	1-(3-Chlorophenyl)ethan-1-ol	47	53	41	>99 %
C-7	1-(4-Bromophenyl)ethan-1-ol	99	50	42	>99 %
C-9	1-(4-Trifluormethylphenyl)ethan-1-ol	61	53	-	>99 %

E. coli ADH-A (150 mg) or LK-ADH (200 mg), boronic acid (0.85 mmole, 1.7 equiv), CuTC (0.8 mmole, 1.6 equiv), $Pd_2(dba)_3$ (2.5 mol %), thioester (**A-1**) (0.5 mmole, 1 equiv.), $P(OEt)_3$ (20 mol %);
Quantification by UPC[2] after dilution with AcCN containing internal standard

Analytical data were in accordance with the reference compounds in F.IV

F.XVI Sequential Gold (III) catalyzed chemoenzymatic one pot reactions



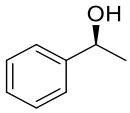
General procedure:

In a screw cap vial, equipped with a magnetic stirring bar $AuCl_3$ (0.025 - 0.05 mmol, 5 -10 mol%) and *i*-PrOH (1 mL final reaction volume) was added. The reaction mixture was stirred for 5 min, and then starting material (0.5 mmol, 1 equiv.) and 4 equiv. of H_2O were added. The reaction mixture was heated for 24 h at 65 °C.

When the hydration step was completed as monitored by GC-MS, 50 mg of the (*R*)- or (*S*)-selective alcohol dehydrogenase whole cell lyophilisate was resuspended in 700 µl of 350 mM Tris-HCl buffer pH 8 in a separate vial and shaken 1 h at 30 °C and 200 rpm. Then 300 µl of the hydration reaction mixture was combined with the resuspended enzyme, which results in an overall *i*-PrOH content of 30 %. After 4 hours the chemoenzymatic one pot reaction was completed as monitored by GC-MS and extracted with EtOAc containing methylbenzoate as internal standard and the conversion was determined by GC or GC-MS.

In case of a preparative scale experiment 170 mg of the (*R*)- or (*S*)-selective alcohol dehydrogenase whole cell lyophilisate was resuspended in 2.333 mL of 350 mM Tris-HCl buffer pH 8 in a separate vial and shaken 1 h at 30 °C and 200 rpm. Then the complete hydration reaction mixture (1 mL) was combined with the resuspended enzyme. After 4 hours the chemoenzymatic one pot reaction was completed as monitored by GC-MS and the overall reaction mixture was extracted with DCM (3 × 10 mL), washed with H_2O (2 × 10 mL), and dried over Na_2SO_4. The solvent was evaporated, and the product was purified applying standard manual glass columns using silica gel from Merck (40-63 µm) and DCM (raw product/ SiO_2; 1:40).

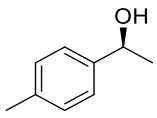
F.XVI.1 (*S*)-1-(Phenyl)ethan-1-ol (C-0)



General procedure	**F.XVI**
Hydration reaction	F.VII.1.1
Substrate concentration	147 mM (**B-0**)
Reaction time	4 h
Conversion	93 %
Conversion over two steps	91 %
Sum formula, m.w.	$C_8H_{10}O$, 122.17
ee (GC)	>99 %

Analytical data was in accordance with the reference compound in F.IV.

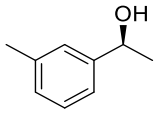
F.XVI.2 (*S*)-1-(4-Methylphenyl)ethan-1-ol (C-1)



General procedure	**F.XVI**
Hydration reaction	F.VII.1.2
Substrate concentration	147 mM (**B-1**)
Reaction time	4 h
Conversion	78 %
Conversion over two steps	76 %
Sum formula, m.w.	$C_9H_{12}O$, 136.19
ee (GC)	>99 %

Analytical data was in accordance with the reference compound in F.IV.

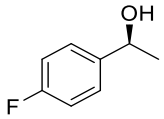
F.XVI.3 (*S*)-1-(3-Methylphenyl)ethan-1-ol (C-2)



General procedure	**F.XVI**
Hydration reaction	F.VII.1.3
Substrate concentration	149 mM (**B-2**)
Reaction time	4 h
Conversion	100 %
Conversion over two steps	99 %
Sum formula, m.w.	$C_9H_{12}O$, 136.19
ee (GC)	>99 %

Analytical data was in accordance with the reference compound in F.IV.

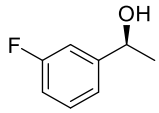
F.XVI.4 *(S)*-1-(4-Fluorophenyl)ethan-1-ol (C-3)



General procedure	**F.XVI**
Hydration reaction	F.VII.1.4
Substrate concentration	107 mM (**B-3**)
Reaction time	4 h
Conversion	100 %
Conversion over two steps	71 %
Yield over two steps	50 mg (71 %) colorless oil
Sum formula, m.w.	C_8H_9FO, 140.16
ee (GC)	>99 %

Analytical data was in accordance with the reference compound in F.IV.

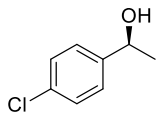
F.XVI.5 *(S)*-1-(3-Fluorophenyl)ethan-1-ol (C-4)



General procedure	**F.XVI**
Hydration reaction	F.VII.1.5
Substrate concentration	129 mM (**B-4**)
Reaction time	4 h
Conversion	100 %
Conversion over two steps	86 %
Sum formula, m.w.	C_8H_9FO, 140.16
ee (GC)	>99 %

Analytical data was in accordance with the reference compound in F.IV.

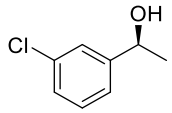
F.XVI.6 (*S*)-1-(4-Chlorophenyl)ethan-1-ol (C-5)



General procedure	**F.XVI**
Hydration reaction	F.VII.1.6
Substrate concentration	108 mM (**B-5**)
Reaction time	4 h
Conversion	99 %
Conversion over two steps	71 %
Sum formula, m.w.	C_8H_9ClO, 156.61
ee (GC)	>99 %

Analytical data was in accordance with the reference compound in F.IV.

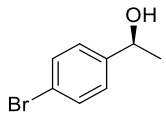
F.XVI.7 (*S*)-1-(3-Chlorophenyl)ethan-1-ol (C-6)



General procedure	**F.XVI**
Hydration reaction	F.VII.1.7
Substrate concentration	114 mM (**B-6**)
Reaction time	4 h
Conversion	100 %
Conversion over two steps	76 %
Yield over two steps	50 mg (64 %) colorless oil
Sum formula, m.w.	C_8H_9ClO, 156.61
ee (GC)	>99 %

Analytical data was in accordance with the reference compound in F.IV.

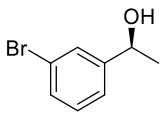
F.XVI.8 *(S)*-1-(4-Bromophenyl)ethan-1-ol (C-7)



General procedure	**F.XVI**
Hydration reaction	F.VII.1.8
Substrate concentration	105 mM (**B-7**)
Reaction time	4 h
Conversion	95 %
Conversion over two steps	67 %
Sum formula, m.w.	C_8H_9BrO, 201.06
ee (GC)	>99 %

Analytical data was in accordance with the reference compound in F.IV.

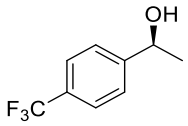
F.XVI.9 *(S)*-1-(3-Bromophenyl)ethan-1-ol (C-8)



General procedure	**F.XVI**
Hydration reaction	F.VII.1.9
Substrate concentration	119 mM (**B-8**)
Reaction time	4 h
Conversion	67 %
Conversion over two steps	53 %
Sum formula, m.w.	C_8H_9BrO, 201.06
ee (GC)	>99 %

Analytical data was in accordance with the reference compound in F.IV.

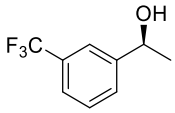
F.XVI.10 *(S)*-1-(4-Trifluormethylphenyl)ethan-1-ol (C-9)



General procedure	**F.XVI**
Hydration reaction	F.VII.1.10
Substrate concentration	74 mM (**B-9**)
Reaction time	4 h
Conversion	100 %
Conversion over two steps	49 %
Sum formula, m.w.	$C_9H_9F_3O$, 190.17
ee (GC)	>99 %

Analytical data was in accordance with the reference compound in F.IV.

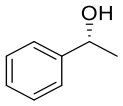
F.XVI.11 *(S)*-1-(3-Trifluormethylphenyl)ethan-1-ol (C-10)



General procedure	**F.XVI**
Hydration reaction	F.VII.1.11
Substrate concentration	86 mM (**B-10**)
Reaction time	4 h
Conversion	100 %
Conversion over two steps	57 %
Sum formula, m.w.	$C_9H_9F_3O$, 190.17
ee (GC)	>99 %

Analytical data was in accordance with the reference compound in F.IV.

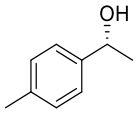
F.XVI.12 *(R)*-1-(Phenyl)ethan-1-ol (C-0)



Gneeral procedure	**F.XVI**
Hydration reaction	F.VII.1.1
Substrate concentration	147 mM (**B-0**)
Reaction time	4 h
Conversion	95 %
Conversion over two steps	93 %
Sum formula, m.w.	$C_8H_{10}O$, 122.17
ee (GC)	>99 %

Analytical data was in accordance with the reference compound in F.IV.

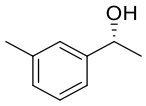
F.XVI.13 (*R*)-1-(4-Methylphenyl)ethan-1-ol (C-1)



General procedure	**F.XVI**
Hydration reaction	F.VII.1.2
Substrate concentration	147 mM (**B-1**)
Reaction time	4 h
Conversion	74 %
Conversion over two steps	73 %
Sum formula, m.w.	$C_9H_{12}O$, 136.19
ee (GC)	>99 %

Analytical data was in accordance with the reference compound in F.IV.

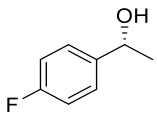
F.XVI.14 (*R*)-1-(3-Methylphenyl)ethan-1-ol (C-2)



General procedure	**F.XVI**
Hydration reaction	F.VII.1.3
Substrate concentration	149 mM (**B-2**)
Reaction time	4 h
Conversion	15 %
Conversion over two steps	15 %
Sum formula, m.w.	$C_9H_{12}O$, 136.19
ee (GC)	>99 %

Analytical data was in accordance with the reference compound in F.IV.

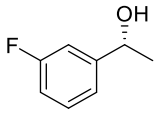
F.XVI.15 *(R)*-1-(4-Fluorophenyl)ethan-1-ol (C-3)



General procedure	**F.XVI**
Hydration reaction	F.VII.1.4
Substrate concentration	107 mM (**B-3**)
Reaction time	4 h
Conversion	92 %
Conversion over two steps	65 %
Sum formula, m.w.	C_8H_9FO, 140.16
ee (GC)	>99 %

Analytical data was in accordance with the reference compound in F.IV.

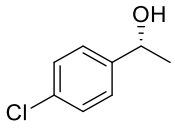
F.XVI.16 *(R)*-1-(3-Fluorophenyl)ethan-1-ol (C-4)



General procedure	**F.XVI**
Hydration reaction	F.VII.1.5
Substrate concentration	129 mM (**B-4**)
Reaction time	4 h
Conversion	86 %
Conversion over two steps	74 %
Sum formula, m.w.	C_8H_9FO, 140.16
ee (GC)	>99 %

Analytical data was in accordance with the reference compound in F.IV.

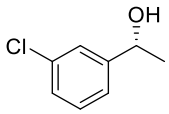
F.XVI.17 (*R*)-1-(4-Chlorophenyl)ethan-1-ol (C-5)



General procedure	**F.XVI**
Hydration reaction	F.VII.1.6
Substrate concentration	108 mM (**B-5**)
Reaction time	4 h
Conversion	96 %
Conversion over two steps	69 %
Sum formula, m.w.	C_8H_9ClO, 156.61
ee (GC)	>99 %

Analytical data was in accordance with the reference compound in F.IV.

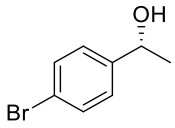
F.XVI.18 (*R*)-1-(3-Chlorophenyl)ethan-1-ol (C-6)



General procedure	**F.XVI**
Hydration reaction	F.VII.1.7
Substrate concentration	114 mM (**B-6**)
Reaction time	4 h
Conversion	38 %
Conversion over two steps	29 %
Sum formula, m.w.	C_8H_9ClO, 156.61
ee (GC)	>99 %

Analytical data was in accordance with the reference compound in F.IV.

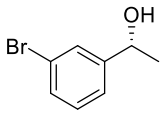
F.XVI.19 *(R)*-1-(4-Bromophenyl)ethan-1-ol (C-7)



General procedure	**F.XVI**
Hydration reaction	F.VII.1.8
Substrate concentration	105 mM (**B-7**)
Reaction time	4 h
Conversion	95 %
Conversion over two steps	67 %
Sum formula, m.w.	C_8H_9BrO, 201.06
ee (GC)	>99 %

Analytical data was in accordance with the reference compound in F.IV.

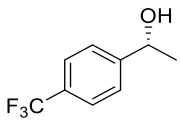
F.XVI.20 *(R)*-1-(3-Bromophenyl)ethan-1-ol (C-8)



General procedure	**F.XVI**
Hydration reaction	F.VII.1.9
Substrate concentration	119 mM (**B-8**)
Reaction time	4 h
Conversion	21 %
Conversion over two steps	17 %
Sum formula, m.w.	C_8H_9BrO, 201.06
ee (GC)	>99 %

Analytical data was in accordance with the reference compound in F.IV.

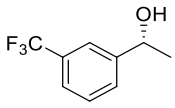
F.XVI.21 *(R)*-1-(4-Trifluormethylphenyl)ethan-1-ol (C-9)



General procedure	**F.XVI**
Hydration reaction	F.VII.1.10
Substrate concentration	74 mM (**B-9**)
Reaction time	4 h
Conversion	100 %
Conversion over two steps	49 %
Sum formula, m.w.	$C_9H_9F_3O$, 190.17
ee (GC)	>99 %

Analytical data was in accordance with the reference compound in F.IV.

F.XVI.22 *(R)*-1-(3-Trifluormethylphenyl)ethan-1-ol (C-10)



General procedure	**F.XVI**
Hydration reaction	F.VII.1.11
Substrate concentration	86 mM (**B-10**)
Reaction time	4 h
Conversion	0 %
Conversion over two steps	0 %
Sum formula, m.w.	$C_9H_9F_3O$, 190.17

Analytical data was in accordance with the reference compound in F.IV.

F.XVII Calculation and reporting of analytical screening data/ ee

All enantiomeric excess values were calculated from peak areas of uncalibrated GC-FID measurements (achiral or chiral, as applicable) after automatic uniform integration unless noted otherwise. The following equations were used for the calculation:

Measurement Unit	Equation	
Relative conversion (Rel. Conv.)	$\% \, Rel.Conv. = \frac{\sum GC \; area \; product(s)}{\sum GC \; area \; starting \; material(s) + \sum GC \; area \; product(s)} \cdot 100$	(Eq. F-1)
ee	$\% \, ee = \frac{GC \; area \; enantiomer \; A - GC \; area \; enantiomer \; B}{\sum GC \; areas \; of \; enantiomers} \cdot 100$	(Eq. F-2)

ee_S: enantiomeric excess of starting material, ee_P: enantiomeric excess of product.

Conversion values were calculated from peak areas of calibrated GC-FID or GCMS measurements after integration and refer to product formation. In the case of preliminary substrate screenings conversions are given as relative conversions (see Eq. F-1).

Table F-12 GC methods for chiral analytics and quantification

GC Methods	
Method A (Standard-Biotrans_33min)	80°C-2min→ 80-160°C, 5°C/min, 160°C-1min→ 160-220°C, 10°C/min, 220°C-8min
Method B (PSC_Biotrans_75min_80-220_2ramp)	80°C-1min→80-220°C, 2°C/min, 220°C-5min
Method C (PSC_Biotrans_150min_80-220_1ramp)	80°C-1min→ 80-220°C, 1°C/min, 220°C-5min
Method D (PSC_Biotrans_35minSEP_iso)	80°C-1min→ 80-90°C, 5°C/min, 90°C-12min→ 90-220°C, 10°C/min, 220°C-5min
Method E $_{\text{achiral analysis}}$ (STD_Achiral_PSC65-290_11min)	65°C-0.5min→ 65-220°C, 25°C/min, 220°C-0min→ 220-290°C, 80°C/min, 290°C-3min

G Appendix

G.I Publications resulting from this thesis

P. Schaaf, V. Gojic, T. Bayer, F. Rudroff, M. Schnürch, M. D. Mihovilovic: Easy access to enantiopure (*S*)- and (*R*)-alcohols via combination of Au(III) catalyzed alkyne hydration and enzymatic reduction*, submitted.*

P. Schaaf, M. Schnürch, U. T. Bornscheuer, F. Rudroff, M. D. Mihovilovic: Simultaneous chemoenzymatic one-pot reaction cascade for the production of chiral alcohols, *in preparation.*

B. Gröll, P. Schaaf, M. D. Mihovilovic, M. Schnürch: Cu(I)-catalyzed one-pot decarboxylation-alkynylation reactions on 1,2,3,4-tetrahydroisoquinolines and one-pot synthesis of triazolyl-1,2,3,4-tetrahydroisoquinolines, *J. Mol. Catal. A: Chem.*, **2017**, 426, 398-406.

N. Oberleitner, C. Peters, J. Muschiol, M. Kadow, S. Saß, T. Bayer, P. Schaaf, N. Iqbal, F. Rudroff, M. D. Mihovilovic, U. T. Bornscheuer: An Enzymatic Toolbox for Cascade Reactions: A Showcase for an In Vivo Redox Sequence in Asymmetric Synthesis, *ChemCatChem*, **2013**, 5, 3524-3528.

G.II Curriculum vitae

PATRICIA SCHAAF

Date of birth: *June 07, 1985*
Place of birth: *Gräfelfing, Munich*
Nationality: *Germany*

Oct 2012 – Sep 2016	PhD position at the Institute of Applied Synthetic Chemistry, Technische Universität Wien supervised by Prof. Marko D. Mihovilovic "Chemoenzymatic one-pot reaction sequence for the production of chiral alcohols and amines"
Sep 2012	Graduation with distinction at Universität Wien (MSc) Master thesis: "Synthesis of substituted 1H-pyrazolo[4,3-*C*] pyridines as potential inhibitors of cyclin-dependent kinases" Supervised by Prof. Wolfgang Holzer, Department of Pharmaceutical Chemistry, Division of Drug Synthesis
Sep 2011 – Jan 2012	University of Gothenburg and Chalmers University of Technology, Gothenburg, Sweden
Oct 2006 – Sep 2012	Master studies & Bachelor studies in Chemistry at Universität Wien

Professional experience

Oct 2016 – present	Pharmacovigilance Manager, Drehm Pharma, Vienna
Sep 2009	Internships at Merck Serono, Medical Affairs, (Regulatory Affairs, QA Comlaint Management, Drug Safety), Darmstadt, Germany
Apr 2006 – Jul 2006	Internships at peadiatric hospital "Dritter Orden", surgery ward, Munich, Germany

Language skills

German	Mother tongue
English	Fluent in written and spoken
French, Swedish	Basic knowledge

Further skills

Profound knowledge of MS office and chemistry related databases and software (Scifinder, Reaxys, ChemDraw)

Intensive experience with diverse analytical techniques (GC, GC-MS, HPLC, spectrometry, chromatography)

Additional work experience

Jul 2014 – Jul 2015	Voluntary extra tuition for unaccompanied under-age refugees, Caritas, Vienna
Aug 2009 – Sep 2009, Aug 2010 – Sep 2010	Au pair, Kilkenny, Ireland

Leisure activities

Sports (running, cycling, riding, hiking, snowboarding), travelling

Publications

B. Gröll, P. Schaaf, M. Schnürch: Improved simplicity and practicability in copper-catalyzed alkynylation of tetrahydroisochinoline, *Monatsh Chem*, **2016.**

B. Gröll, P. Schaaf, M. D. Mihovilovic, M. Schnürch: Cu(I)-catalyzed one-pot decarboxyla-tion-alkynylation reactions on 1,2,3,4-tetrahydroisoquinolines and one-pot synthesis of triazolyl-1,2,3,4-tetrahydroisoquinolines, *J. Mol. Catal. A: Chem*., **2016.**

N. Oberleitner, C. Peters, J. Muschiol, M. Kadow, S. Saß, T. Bayer, P. Schaaf, N. Iqbal, F. Rudroff, M. D. Mihovilovic, U. T. Bornscheuer: An Enzymatic Toolbox for Cascade Reactions: A Showcase for an In Vivo Redox Sequence in Asymmetric Synthesis, *ChemCatChem*, **2013**, 5, 3524-3528.

G. Vilkauskaite, P. Schaaf, A. Sackus, V. Krystof, W. Holzer: Synthesis of pyridyl substituted pyrazolo[4,3-*c*] pyridines as potential inhibitors of pro-tein kinases, *ARCHIVOC*, **2014**, 135-149.

M. Hanif, P.Schaaf, W. Kandioller, M. Hejl, M. A. Jakupec, A. Roller, B. K. Keppler, C. G. Hartinger: Influence of the Arene Ligand and the Leaving Group on the Anticancer Activity of (Thio)maltol Ruthenium (II)–(η6-Arene) Complexes, *Aust. J. Chem*., **2010**, 63, 1521–1528.

Poster presentation

P. Schaaf, T, Bayer, M. Schnürch, U. T. Bornscheuer, F. Rudroff, M. D. Mihovilovic: Chemoenzymatic One Pot Reaction Sequence for the Production of Chiral Alcohols and Amines, *BIOTRANS,* **2015**

P. Schaaf, T, Bayer, M. Schnürch, U. T. Bornscheuer, F. Rudroff, M. D. Mihovilovic: Chemoenzymatic One Pot Reaction Cascade; Combination of metal and enzyme assisted transformations, *COST Traning School Systems Biocatalysis,* **2016**

G.III List of abbreviation

Abbreviation	
ADH	alcohol dehydrogenase
amp	ampicillin
ATA	transaminase
a_w	water activity
CFE	cell free extract
CuTC	copper(I)-thiophene-2-carboxylate
CuMeSal	copper(I) 3-methylsalicylate
DCM	dichloromethane
DMF	diemthylformamide
DMPU	1,3-dimethyltetrahydropyrimidin-2(1*H*)-one
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
E. coli	*Escherichia coli*
ee	enantiomeric excess
FDH	formate dehydrogenase
FID	flame ionization detector
G6P	glucose-6-phosphate
G6PDH	glucose-6-phosphate dehydrogenase
GC	gas chromatography
GDH	glucose dehydrogenase
IPTG	isopropyl-β-D-thiogalactopyranoside
kan	kanamycin
LB	lysogeny broth
LDH	lactate dehydrogenase
LG	leaving group
MM	master mix
MS	mass spectrometry
NAD^+	nicotinamide adenine dinucleotide
NADH	nicotinamide adenine dinucleotide (reduced)
$NADP^+$	nicotinamide adenine dinucleotide phosphate
NADPH	nicotinamide adenine dinucleotide phosphate (reduced)
o/n	overnight
OD	optical density
PDC	pyruvate decarboxylase
PDMS	polydimethylsiloxane
PLE	pig liver esterase
PMSF	phenylmethylsulfonyl fluoride
rpm	rounds per minute
RT	room temperature
THF	tetrahydrofuran
TLC	thin layer chromatography
Tris	tris(hydroxymethyl)aminomethane
v/v	volume per volume
WT	wild type
YADH	yeast alcohol dehydrogenase

G.IV References

[1] F. Diederich, P. J. Stang, Editors, *Metal-catalyzed Cross-coupling Reactions*, Wiley-VCH, **1998**.
[2] M. S. Kharasch, E. K. Fields, *J. Am. Chem. Soc.* **1941**, *63*, 2316-2320.
[3] K. Tamao, K. Sumitani, M. Kumada, *J. Amer. Chem. Soc.* **1972**, *94*, 4374-4376.
[4] R. J. P. Corriu, J. P. Masse, *J. Chem. Soc., Chem. Commun.* **1972**, 144.
[5] M. Yamamura, I. Moritani, S.-I. Murahashi, *J. Organomet. Chem.* **1975**, *91*, C39-C42.
[6] S. Baba, E. Negishi, *J. Am. Chem. Soc.* **1976**, *98*, 6729-6731.
[7] J. F. Fauvarque, A. Jutand, *Bull. Soc. Chim. Fr.* **1976**, 765-770.
[8] E. Negishi, A. O. King, N. Okukado, *J. Org. Chem.* **1977**, *42*, 1821-1823.
[9] D. Milstein, J. K. Stille, *J. Am. Chem. Soc.* **1979**, *101*, 4992-4998.
[10] N. Miyaura, K. Yamada, A. Suzuki, *Tetrahedron Lett.* **1979**, 3437-3440.
[11] Y. Hatanaka, S. Fukushima, T. Hiyama, *Heterocycles* **1990**, *30*, 303-306.
[12] aS.-M. T. Toguem, A. Villinger, P. Langer, *Synlett* **2009**, 3311-3314; bS.-M. T. Toguem, A. Villinger, P. Langer, *Synlett* **2010**, 909-912.
[13] aM. Sharif, S. Reimann, A. Villinger, P. Langer, *Synlett* **2010**, 913-916; bM. Sharif, M. Zeeshan, S. Reimann, A. Villinger, P. Langer, *Tetrahedron Lett.* **2010**, *51*, 2810-2812.
[14] K. Tamao, Y. Kiso, K. Sumitani, M. Kumada, *J. Amer. Chem. Soc.* **1972**, *94*, 9268-9269.
[15] A. L. Casado, P. Espinet, *J. Am. Chem. Soc.* **1998**, *120*, 8978-8985.
[16] H. Prokopcova, C. O. Kappe, *Angew. Chem., Int. Ed.* **2008**, *47*, 3674-3676.
[17] L. Wang, W. He, Z. Yu, *Chem Soc Rev* **2013**, *42*, 599-621.
[18] L. S. Liebeskind, J. Srogl, *J. Am. Chem. Soc.* **2000**, *122*, 11260-11261.
[19] H. Prokopcova, C. O. Kappe, *Angew. Chem., Int. Ed.* **2009**, *48*, 2276-2286.
[20] Y. Yu, L. S. Liebeskind, *J. Org. Chem.* **2004**, *69*, 3554-3557.
[21] H. Yang, H. Li, R. Wittenberg, M. Egi, W. Huang, L. S. Liebeskind, *J. Am. Chem. Soc.* **2007**, *129*, 1132-1140.
[22] aM. Egi, L. S. Liebeskind, *Org. Lett.* **2003**, *5*, 801-802; bN. Leconte, L. Pellegatti, A. Tatibouet, F. Suzenet, P. Rollin, G. Guillaumet, *Synthesis* **2007**, 857-864; cF.-A. Alphonse, F. Suzenet, A. Keromnes, B. Lebret, G. Guillaumet, *Synlett* **2002**, 447-450; dN. Leconte, A. Keromnes-Wuillaume, F. Suzenet, G. Guillaumet, *Synlett* **2007**, 204-210; eS. Oumouch, M. Bourotte, M. Schmitt, J.-J. Bourguignon, *Synthesis* **2005**, 25-27.
[23] Z. Zhang, L. S. Liebeskind, *Org. Lett.* **2006**, *8*, 4331-4333.
[24] C. Savarin, J. Srogl, L. S. Liebeskind, *Org. Lett.* **2001**, *3*, 91-93.
[25] A. Aguilar-Aguilar, L. S. Liebeskind, E. Pena-Cabrera, *J. Org. Chem.* **2007**, *72*, 8539-8542.
[26] C. L. Kusturin, L. S. Liebeskind, W. L. Neumann, *Org. Lett.* **2002**, *4*, 983-985.
[27] H. Prokopcova, L. Pisani, C. O. Kappe, *Synlett* **2007**, 43-46.
[28] L. S. Liebeskind, J. Srogl, *Org. Lett.* **2002**, *4*, 979-981.
[29] H. Yang, L. S. Liebeskind, *Org. Lett.* **2007**, *9*, 2993-2995.
[30] A. Morita, H. Kiyota, S. Kuwahara, *Biosci., Biotechnol., Biochem.* **2006**, *70*, 2564-2566.
[31] J. M. Villalobos, J. Srogl, L. S. Liebeskind, *J. Am. Chem. Soc.* **2007**, *129*, 15734-15735.
[32] aJ. Chatt, L. A. Duncanson, *J. Chem. Soc.* **1953**, 2939-2947; bM. J. S. Dewar, *Bull. Soc. Chim. Fr.* **1951**, C71-79.
[33] T. de Haro, C. Nevado, *Adv. Synth. Catal.* **2010**, *352*, 2767-2772.
[34] R. K. Hocking, E. C. Wasinger, F. M. F. De Groot, K. O. Hodgson, B. Hedman, E. I. Solomon, *J. Am. Chem. Soc.* **2006**, *128*, 10442-10451.
[35] B. D. Mokar, R.-S. Liu, *Chem. Commun. (Cambridge, U. K.)* **2014**, *50*, 8966-8969.
[36] A. K. Das, S. Park, S. Muthaiah, S. H. Hong, *Synlett* **2015**, *26*, 2517-2520.
[37] Y. Fukuda, K. Utimoto, *J. Org. Chem.* **1991**, *56*, 3729-3731.
[38] A. Leyva, A. Corma, *J. Org. Chem.* **2009**, *74*, 2067-2074.
[39] P. Nun, R. S. Ramon, S. Gaillard, S. P. Nolan, *J. Organomet. Chem.* **2010**, *696*, 7-11.
[40] Y. Xu, X. Hu, S. Zhang, X. Xi, Y. Wu, *ChemCatChem* **2016**, *8*, 262-267.
[41] W. Wang, J. Jasinski, G. B. Hammond, B. Xu, *Angew. Chem., Int. Ed.* **2010**, *49*, 7247-7252, S7247/7241-S7247/7234.
[42] Y. Xu, X. Hu, J. Shao, G. Yang, Y. Wu, Z. Zhang, *Green Chem.* **2015**, *17*, 532-537.
[43] F.-X. Zhu, W. Wang, H.-X. Li, *J. Am. Chem. Soc.* **2011**, *133*, 11632-11640.
[44] Y. Fukuda, K. Utimoto, *Synthesis* **1991**, 975-978.
[45] R. Casado, M. Contel, M. Laguna, P. Romero, S. Sanz, *J Am Chem Soc* **2003**, *125*, 11925-11935.
[46] M. Lein, M. Rudolph, A. S. K. Hashmi, P. Schwerdtfeger, *Organometallics* **2010**, *29*, 2206-2210.
[47] aE. Mizushima, K. Sato, T. Hayashi, M. Tanaka, *Angew. Chem., Int. Ed.* **2002**, *41*, 4563-4565; bR. E. Ebule, D. Malhotra, G. B. Hammond, B. Xu, *Adv. Synth. Catal.* **2016**, *358*, 1478-1481.
[48] aE. Kusaka, T. Ito, K. Tanabe, S.-i. Nishimoto, *Bioconjugate Chem.* **2013**, *24*, 1435-1444; bF. Liu, Y. Cui, L. Wang, H. Wang, Y. Yuan, J. Pan, H. Chen, L. Yuan, *ACS Appl. Mater. Interfaces* **2015**, *7*, 11547-11554.
[49] E. Garcia-Junceda, I. Lavandera, D. Rother, J. H. Schrittwieser, *J. Mol. Catal. B: Enzym.* **2015**, *114*, 1-6.
[50] H. Groger, W. Hummel, *Curr Opin Chem Biol* **2014**, *19*, 171-179.
[51] J. Tao, J.-H. Xu, *Curr. Opin. Chem. Biol.* **2009**, *13*, 43-50.
[52] T. Hudlicky, J. W. Reed, *Chem. Soc. Rev.* **2009**, *38*, 3117-3132.
[53] A. Wells, *Org. Process Res. Dev.* **2006**, *10*, 678-681.
[54] A. Weckbecker, W. Hummel, *Biocatal. Biotransform.* **2006**, *24*, 380-389.
[55] A. Weckbecker, W. Hummel, *Methods Biotechnol.* **2005**, *17*, 225-237.
[56] M. Krauueer, W. Hummel, H. Groeger, *Eur. J. Org. Chem.* **2007**, 5175-5179.
[57] W. Kroutil, H. Mang, K. Edegger, K. Faber, *Curr. Opin. Chem. Biol.* **2004**, *8*, 120-126.
[58] A. Matsuyama, H. Yamamoto, Wiley-VCH Verlag GmbH & Co. KGaA, **2004**, pp. 217-231.
[59] B. Kosjek, W. Stampfer, M. Pogorevc, W. Goessler, K. Faber, W. Kroutil, *Biotechnol. Bioeng.* **2004**, *86*, 55-62.
[60] J. E. Leresche, H.-P. Meyer, *Org. Process Res. Dev.* **2006**, *10*, 572-580.
[61] J. D. Stewart, *Curr. Opin. Biotechnol.* **2000**, *11*, 363-368.
[62] T. Ema, Y. Sugiyama, M. Fukumoto, H. Moriya, J.-N. Cui, T. Sakai, M. Utaka, *J. Org. Chem.* **1998**, *63*, 4996-5000.

[63] Y. Kawai, K. Hida, D. H. Dao, A. Ohno, *Tetrahedron Lett.* **1998**, *39*, 9219-9222.
[64] I. A. Kaluzna, T. Matsuda, A. K. Sewell, J. D. Stewart, *J. Am. Chem. Soc.* **2004**, *126*, 12827-12832.
[65] W. Stampfer, B. Kosjek, W. Kroutil, K. Faber, Ciba Specialty Chemicals Holding Inc., Switz. . **2003**, p. 56 pp.
[66] K. Edegger, C. C. Gruber, T. M. Poessl, S. R. Wallner, I. Lavandera, K. Faber, F. Niehaus, J. Eck, R. Oehrlein, A. Hafner, W. Kroutil, *Chem. Commun. (Cambridge, U. K.)* **2006**, 2402-2404.
[67] M. Karabec, A. Lyskowski, K. C. Tauber, G. Steinkellner, W. Kroutil, G. Grogan, K. Gruber, *Chem. Commun. (Cambridge, U. K.)* **2010**, *46*, 6314-6316.
[68] G. De Gonzalo, I. Lavandera, K. Faber, W. Kroutil, *Org. Lett.* **2007**, *9*, 2163-2166.
[69] aF. H. Arnold, *Protein Eng.* **1988**, *2*, 21-25; bF. H. Arnold, *Trends Biotechnol.* **1990**, *8*, 244-249.
[70] Y. Korkhin, A. J. Kalb, M. Peretz, O. Bogin, Y. Burstein, F. Frolow, *J. Mol. Biol.* **1998**, *278*, 967-981.
[71] E. Krissinel, K. Henrick, *J Mol Biol* **2007**, *372*, 774-797.
[72] W. Hummel, *Appl. Microbiol. Biotechnol.* **1990**, *34*, 15-19.
[73] C. W. Bradshaw, W. Hummel, C. H. Wong, *J. Org. Chem.* **1992**, *57*, 1532-1536.
[74] J. Haberland, W. Hummel, T. Daussmann, A. Liese, *Org. Process Res. Dev.* **2002**, *6*, 458-462.
[75] aF. F. Noe, W. J. Nickerson, *J. Bacteriol.* **1958**, *75*, 674-681; bK.-H. Kim, *J. Biol. Chem.* **1964**, *239*, 783-786.
[76] M. Fuchs, J. E. Farnberger, W. Kroutil, *Eur. J. Org. Chem.* **2015**, *2015*, 6965-6982.
[77] C. M. Chwieduk, *Drugs* **2011**, *71*, 349-361.
[78] aL. S. Schneider, R. Anand, M. R. Farlow, *Int. J. Geriatr. Psychopharmacol.* **1998**, *1*, S26-S34; bC. M. Spencer, S. Noble, *Drugs Aging* **1998**, *13*, 391-411.
[79] R. G. Talbot, J. Nimmo, D. G. Julian, R. A. Clark, J. M. Neilson, L. F. Prescott, *Lancet* **1973**, *2*, 399-404.
[80] M. S. Malik, E.-S. Park, J.-S. Shin, *Appl. Microbiol. Biotechnol.* **2012**, *94*, 1163-1171.
[81] J. L. Galman, I. Slabu, N. J. Weise, C. Iglesias, F. Parmeggiani, R. C. Lloyd, N. J. Turner, *Green Chem.* **2016**, Ahead of Print.
[82] R. C. Simon, N. Richter, E. Busto, W. Kroutil, *ACS Catal.* **2014**, *4*, 129-143.
[83] W. Kroutil, E.-M. Fischereder, C. S. Fuchs, H. Lechner, F. G. Mutti, D. Pressnitz, A. Rajagopalan, J. H. Sattler, R. C. Simon, E. Siirola, *Org. Process Res. Dev.* **2013**, *17*, 751-759.
[84] I. V. Pavlidis, M. S. Weiss, M. Genz, P. Spurr, S. P. Hanlon, B. Wirz, H. Iding, U. T. Bornscheuer, *Nat. Chem.* **2016**, *8*, 1076-1082.
[85] aU. Bornscheuer, M. Genz, O. Melse, S. Schmidt, C. Vickers, T. van den Bergh, H.-J. Joosten, M. Doerr, *ChemCatChem* **2016**, Ahead of Print; bP. F. Mugford, U. G. Wagner, Y. Jiang, K. Faber, R. J. Kazlauskas, *Angew Chem Int Ed Engl* **2008**, *47*, 8782-8793.
[86] M. Fuchs, D. Koszelewski, K. Tauber, W. Kroutil, K. Faber, *Chem. Commun. (Cambridge, U. K.)* **2010**, *46*, 5500-5502.
[87] aN. Oberleitner, C. Peters, J. Muschiol, M. Kadow, S. Sass, T. Bayer, P. Schaaf, N. Iqbal, F. Rudroff, M. D. Mihovilovic, U. T. Bornscheuer, *ChemCatChem* **2013**, *5*, 3524-3528; bM. Schrewe, N. Ladkau, B. Buehler, A. Schmid, *Adv. Synth. Catal.* **2013**, *355*, 1693-1697.
[88] aE.-S. Park, J.-Y. Dong, J.-S. Shin, *Org Biomol Chem* **2013**, *11*, 6929-6933; bC. K. Savile, J. M. Janey, E. C. Mundorff, J. C. Moore, S. Tam, W. R. Jarvis, J. C. Colbeck, A. Krebber, F. J. Fleitz, J. Brands, P. N. Devine, G. W. Huisman, G. J. Hughes, *Science (Washington, DC, U. S.)* **2010**, *329*, 305-309.
[89] aK. Fesko, K. Steiner, R. Breinbauer, H. Schwab, M. Schuermann, G. A. Strohmeier, *J. Mol. Catal. B: Enzym.* **2013**, *96*, 103-110; bE.-S. Park, M. S. Malik, J.-Y. Dong, J.-S. Shin, *ChemCatChem* **2013**, *5*, 1734-1738.
[90] B. Wang, H. Land, P. Berglund, *Chem Commun (Camb)* **2013**, *49*, 161-163.
[91] J. Muschiol, C. Peters, N. Oberleitner, M. D. Mihovilovic, U. T. Bornscheuer, F. Rudroff, *Chem. Commun. (Cambridge, U. K.)* **2015**, *51*, 5798-5811.
[92] aT. Irrgang, K. Kutlescha, *Spec. Chem. Mag.* **2011**, *31*, 31-33; bJ. R. Andreatta, M. Dilip, CRC Press, **2016**, pp. 23-45; cG. Zhan, W. Du, Y.-C. Chen, *Chem. Soc. Rev.* **2017**, *46*, 1675-1692.
[93] aV. Eschenbrenner-Lux, H. Waldmann, K. Kumar, Wiley-VCH Verlag GmbH & Co. KGaA, **2014**, pp. 497-522; bH. Pellissier, Wiley-VCH Verlag GmbH & Co. KGaA, **2014**, pp. 325-418; cS. Takizawa, H. Sasai, Wiley-VCH Verlag GmbH & Co. KGaA, **2014**, pp. 419-462.
[94] G. W. Huisman, J. Liang, A. Krebber, *Curr. Opin. Chem. Biol.* **2010**, *14*, 122-129.
[95] B. N. M. Leeuwen, A. M. Wulp, I. Duijnstee, A. J. A. Maris, A. J. J. Straathof, *Appl. Microbiol. Biotechnol.* **2012**, *93*, 1377-1387.
[96] M. Makkee, A. P. G. Kieboom, H. Van Bekkum, J. A. Roels, *J. Chem. Soc., Chem. Commun.* **1980**, 930-931.
[97] aJ. V. Allen, J. M. J. Williams, *Tetrahedron Lett.* **1996**, *37*, 1859-1862; bP. M. Dinh, J. A. Howarth, A. R. Hudnott, J. M. J. Williams, W. Harris, *Tetrahedron Lett.* **1996**, *37*, 7623-7626.
[98] S.-T. Chen, W.-H. Huang, K.-T. Wang, *J. Org. Chem.* **1994**, *59*, 7580-7581.
[99] aO. Pamies, J.-E. Baeckvall, *Chem. Rev. (Washington, DC, U. S.)* **2003**, *103*, 3247-3261; bA. L. E. Larsson, B. A. Persson, J.-E. Backvall, *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 1211-1212; cJ. H. Choi, Y. H. Kim, S. H. Nam, S. T. Shin, M.-J. Kim, J. Park, *Angew. Chem., Int. Ed.* **2002**, *41*, 2373-2376; dA. Berkessel, M. L. Sebastian-Ibarz, T. N. Mueller, *Angew. Chem., Int. Ed.* **2006**, *45*, 6567-6570.
[100] M. J. Fink, M. Schoen, F. Rudroff, M. Schnuerch, M. D. Mihovilovic, *ChemCatChem* **2013**, *5*, 724-727.
[101] E. Burda, W. Hummel, H. Groeger, *Angew. Chem., Int. Ed.* **2008**, *47*, 9551-9554.
[102] A. Prastaro, P. Ceci, E. Chiancone, A. Boffi, R. Cirilli, M. Colone, G. Fabrizi, A. Stringaro, S. Cacchi, *Green Chem.* **2009**, *11*, 1929-1932.
[103] V. Gauchot, W. Kroutil, A. R. Schmitzer, *Chem. - Eur. J.* **2010**, *16*, 6748-6751, S6748/6741-S6748/6745.
[104] S. Borchert, E. Burda, J. Schatz, W. Hummel, H. Groeger, *J. Mol. Catal. B: Enzym.* **2012**, *84*, 89-93.
[105] A. Boffi, S. Cacchi, P. Ceci, R. Cirilli, G. Fabrizi, A. Prastaro, S. Niembro, A. Shafir, A. Vallribera, *ChemCatChem* **2011**, *3*, 347-353.
[106] S. Sgalla, G. Fabrizi, R. Cirilli, A. Macone, A. Bonamore, A. Boffi, S. Cacchi, *Tetrahedron: Asymmetry* **2007**, *18*, 2791-2796.
[107] I. Schnapperelle, W. Hummel, H. Groeger, *Chem. - Eur. J.* **2012**, *18*, 1073-1076, S1073/1071-S1073/1023.
[108] H. Groeger, W. Hummel, *Curr. Opin. Chem. Biol.* **2014**, *19*, 171-179.
[109] K. Tenbrink, M. Sessler, J. Schatz, H. Groeger, *Adv. Synth. Catal.* **2011**, *353*, 2363-2367.
[110] C. A. Denard, J. F. Hartwig, H. Zhao, *ACS Catal.* **2013**, *3*, 2856-2864.
[111] V. Koehler, Y. M. Wilson, M. Duerrenberger, D. Ghislieri, E. Churakova, T. Quinto, L. Knoerr, D. Haeussinger, F. Hollmann, N. J. Turner, T. R. Ward, *Nat. Chem.* **2013**, *5*, 93-99.
[112] V. Koehler, T. R. Ward, *ChemCatChem* **2014**, *6*, 2191-2193.
[113] K. Baer, M. Krausser, E. Burda, W. Hummel, A. Berkessel, H. Groeger, *Angew. Chem., Int. Ed.* **2009**, *48*, 9355-9358, S9355/9351-S9355/9322.

[114] G. Rulli, N. Duangdee, K. Baer, W. Hummel, A. Berkessel, H. Groeger, *Angew. Chem., Int. Ed.* **2011**, *50*, 7944-7947, S7944/7941-S7944/7922.
[115] G. Rulli, M. Heidlindemann, A. Berkessel, W. Hummel, H. Groeger, *J. Biotechnol.* **2013**, *168*, 271-276.
[116] G. E. Jeromin, *Biotechnol. Lett.* **2009**, *31*, 1717-1721.
[117] M. Heidlindemann, G. Rulli, A. Berkessel, W. Hummel, H. Groeger, *ACS Catal.* **2014**, *4*, 1099-1103.
[118] V. Stepankova, S. Bidmanova, T. Koudelakova, Z. Prokop, R. Chaloupkova, J. Damborsky, *ACS Catal.* **2013**, *3*, 2823-2836.
[119] M. B. Runge, M. T. Mwangi, A. L. Miller, II, M. Perring, N. B. Bowden, *Angew. Chem., Int. Ed.* **2008**, *47*, 935-939.
[120] H. Sato, W. Hummel, H. Groeger, *Angew. Chem., Int. Ed.* **2015**, *54*, 4488-4492.
[121] J. Latham, J.-M. Henry, H. H. Sharif, B. R. K. Menon, S. A. Shepherd, M. F. Greaney, J. Micklefield, *Nat. Commun.* **2016**, *7*, 11873.
[122] N. Rios-Lombardia, C. Vidal, M. Cocina, F. Moris, J. Garcia-Alvarez, J. Gonzalez-Sabin, *Chem. Commun. (Cambridge, U. K.)* **2015**, *51*, 10937-10940.
[123] N. Rios-Lombardia, E. Liardo, F. Moris, J. Gonzalez-Sabin, C. Vidal, J. Garcia-¡Ð°Alvarez, *Angew Chem Int Ed Engl* **2016**, *55*, 8691-8695.
[124] aM. I. Shkol'nik, *Uch. Zap. Petro-zavodskogo Gos. Univ.* **1964**, *12*, 126-128; bY. Cai, Q. Mu, Z. Wang, B. Zhang, B. Yan, *Weishengwuxue Tongbao* **2008**, *35*, 1171-1175; cA. Shanmuganathan, S. V. Avery, S. A. Willetts, J. E. Houghton, *FEBS Lett.* **2003**, *556*, 253-259; dJ. Ulrichova, D. Walterova, F. Brezina, V. Simanek, *Bioorg. Med. Chem. Lett.* **1992**, *2*, 1097-1100.
[125] M. Koley, L. Wimmer, M. Schnuerch, M. D. Mihovilovic, *J. Heterocycl. Chem.* **2013**, *50*, 1368-1373.
[126] aP. Nikolova, O. P. Ward, *Biotechnol. Bioeng.* **1992**, *39*, 870-876; bH. Groger, W. Hummel, C. Rollmann, F. Chamouleau, H. Husken, H. Werner, C. Wunderlich, K. Abokitse, K. Drauz, S. Buchholz, *Tetrahedron* **2004**, *60*, 633-640; cP. Mueller, B. L. Bangasser, L. Greiner, S. Na'amnieh, P. S. Baeuerlein, D. Vogt, C. Mueller, *Open Catal. J.* **2011**, *4*, 113-116; dA. Bornadel, R. Hatti-Kaul, F. Hollmann, S. Kara, *Tetrahedron* **2015**, Ahead of Print; eS. Leuchs, T. Nonnen, D. Dechambre, S. Na'amnieh, L. Greiner, *J. Mol. Catal. B: Enzym.* **2013**, *88*, 52-59.
[127] aH. Groeger, W. Hummel, S. Buchholz, K. Drauz, T. V. Nguyen, C. Rollmann, H. Huesken, K. Abokitse, *Org. Lett.* **2003**, *5*, 173-176; bS.-P. Zou, Y.-G. Zheng, E.-H. Du, Z.-C. Hu, *J. Biotechnol.* **2014**, *188*, 42-47.
[128] aK. Goldberg, A. Krueger, T. Meinhardt, W. Kroutil, B. Mautner, A. Liese, *Tetrahedron: Asymmetry* **2008**, *19*, 1171-1173; bD. Metrangolo-Ruiz De Temino, W. Hartmeier, M. B. Ansorge-Schumacher, *Enzyme Microb. Technol.* **2005**, *36*, 3-9.
[129] aA. L. Miller, II, N. B. Bowden, *Adv. Mater. (Weinheim, Ger.)* **2008**, *20*, 4195-4199; bA. L. Miller, N. B. Bowden, *J. Org. Chem.* **2009**, *74*, 4834-4840.
[130] Y. Poojari, A. S. Palsule, S. J. Clarson, R. A. Gross, *Silicon* **2009**, *1*, 37-45.
[131] J. H. Schrittwieser, F. Coccia, S. Kara, B. Grischek, W. Kroutil, N. d'Alessandro, F. Hollmann, *Green Chem.* **2013**, *15*, 3318-3331.
[132] F. Li, N. Wang, L. Lu, G. Zhu, *J Org Chem* **2015**, *80*, 3538-3546.
[133] J. L. N. Fernandes, M. Costa de Souza, E. C. S. Brenelli, J. A. Brenelli, *Synthesis* **2009**, 4058-4062.
[134] N. S. Hatzakis, I. Smonou, *Bioorg. Chem.* **2005**, *33*, 325-337.
[135] H. C. Maytum, B. Tavassoli, J. M. J. Williams, *Org. Lett.* **2007**, *9*, 4387-4389.
[136] C. Azerraf, D. Gelman, *Chem. - Eur. J.* **2008**, *14*, 10364-10368.
[137] I. P. Query, P. A. Squier, E. M. Larson, N. A. Isley, T. B. Clark, *J. Org. Chem.* **2011**, *76*, 6452-6456.
[138] L. Cao, J. Ding, M. Gao, Z. Wang, J. Li, A. Wu, *Org. Lett.* **2009**, *11*, 3810-3813.
[139] N. Froschl, J. Harlass, *Monatsh. Chem.* **1932**, *59*, 275-288.
[140] R. J. Rahaim, R. E. Maleczka, *Org. Lett.* **2011**, *13*, 584-587.
[141] J. v. Braun, E. Hahn, J. Seemann, *Ber. Dtsch. Chem. Ges. B* **1922**, *55B*, 1687-1700.
[142] H. L. Ngo, W. Lin, *J. Org. Chem.* **2005**, *70*, 1177-1187.
[143] F. Nerdel, H. Liebig, *Justus Liebigs Ann. Chem.* **1959**, *621*, 42-50.
[144] B. V. Subba Reddy, N. Sivasankar Reddy, C. Madan, J. S. Yadav, *Tetrahedron Lett.* **2010**, *51*, 4827-4829.
[145] Y. Yamamoto, H. Hasegawa, H. Yamataka, *J. Org. Chem.* **2011**, *76*, 4652-4660.
[146] K. Huang, S. Li, M. Chang, X. Zhang, *Org. Lett.* **2013**, *15*, 484-487.
[147] R. Sanz, A. Martinez, V. Guilarte, J. M. Alvarez-Gutierrez, F. Rodriguez, *Eur. J. Org. Chem.* **2007**, 4642-4645.
[148] P. Both, H. Busch, P. P. Kelly, F. G. Mutti, N. J. Turner, S. L. Flitsch, *Angew. Chem., Int. Ed.* **2016**, *55*, 1511-1513.
[149] C. E. Paul, I. Lavandera, V. Gotor-Fernandez, W. Kroutil, V. Gotor, *ChemCatChem* **2013**, *5*, 3875-3881.
[150] M. Toesch, M. Schober, R. Breinbauer, K. Faber, *Eur. J. Org. Chem.* **2014**, *2014*, 3930-3934.
[151] K. Wu, H. Wang, L. Chen, H. Fan, Z. Zhao, D. Wei, *Appl. Microbiol. Biotechnol.* **2016**, Ahead of Print.
[152] Y. Li, Y. Zhou, Q. Shi, K. Ding, R. Noyori, C. A. Sandoval, *Adv. Synth. Catal.* **2011**, *353*, 495-500.
[153] Y. Li, K. Ding, C. A. Sandoval, *Org. Lett.* **2009**, *11*, 907-910.
[154] aF. Steffen-Munsberg, C. Vickers, A. Thontowi, S. Schaetzle, T. Meinhardt, M. Svedendahl Humble, H. Land, P. Berglund, U. T. Bornscheuer, M. Hoehne, *ChemCatChem* **2013**, *5*, 154-157; bM. Hoehne, S. Schaetzle, H. Jochens, K. Robins, U. T. Bornscheuer, *Nat. Chem. Biol.* **2010**, *6*, 807-813.