Combination of Suzuki cross-coupling reaction and biocatalysis in one-pot cascade processes

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The present work was carried out during a 36-month period as part of the BIOCASCADES project, a joint collaboration among universities and their industrial partners. The first part of the project (September 2015 to February 2017) was carried out at the Chair of Industrial Organic Chemistry and Biocatalysis at the University of Bielefeld under the direction of Prof. Dr. Harald Gröger, while the secondment took place in Oviedo, Spain at EntreChem, a spin-off biotechnology company, under the supervision of Dr. Javier González-Sabín (March 2017 to September 2018). BIOCASCADE is an interdisciplinary research programme in the fields of chemistry and biological sciences that gathers eleven early-stage researchers (ESRs) to investigate the development of sustainable chemoenzymatic cascade reactions for the synthesis of optically pure amines and aminoalcohols as pharmaceutical ingredients. The aim of BIOCASCADES is not only to setup novel cascades, but also to enforce the practical, economic implementation of the synthetic routes. Thus, BIOCASCADES contributes to the development of environmentally benign technology and enhance Europe's technological leadership in the field of (chemo-) enzymatic processes.

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PUBLICATIONS

J. Paris, N. Rios-Lombardia, F. Moris, H. Gröger, J. González-Sabin

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“Always be yourself, express yourself, have faith in yourself, do not go out and look for a successful personality and duplicate it.”

Bruce Lee

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List of abbreviations

ADH	Alcohol dehydrogenase
APCI-MS	Atmospheric pressure chemical ionisation mass spectrometry
Aq.	Aqueous
ArRmut11	Mutated variant of the transaminase from *Arthrobacter sp.*
ArR-ATA	(*R*)-selective Transaminase from *Arthrobacter sp.*
ArS-ATA	(*S*)-selective Transaminase from *Arthrobacter sp.*
ATA	Amine transaminase
Boc	tert-Butyloxycarbonyl protecting group
c	Conversion
CALB	*Candida antarctica* lipase B
$CDCl_3$	Deuterated chloroform
CFE	Cell-free extract
cm	Centimeter
Cv-ATA	Transaminase from *Chromobacterium violaceum*
D-Ala	D-isomer of alanine
DCM	Dichlorometane
DES	Deep Eutectic Solvent
DKR	Dynamic Kinetic Resolution
DMSO	Dimethyl sulfoxide
ee	Enantiomeric excess
Esi-ATA	Transaminase from *Exophiala sideris*
EtOAc	Ethyl acetate
EX-5	Variant of EX-TA
EX-STA	Variant of EX-TA
EX-STA5	Variant of EX-TA

EX-ATA	Transaminase from *Exophiala Xenobiotica*
EX-wt	Wild type transaminase from *Exophiala Xenobiotica*
FAD	Flavin adenine dinucleotide
FMN	Flavin mononucleotide
G6PDH	Glucose-6-phosphate dehydrogenase
GDH	Glucose dehydrogenase
Gly	Glycerol
h	Hours
HBA	Hydrogen-bond acceptor
HBD	Hydrogen-bond donor
Hex	Hexane
HPLC	High Performance Liquid Chromatography
Hz	Hertz
i-PrOH	Isopropanol
KRED	Ketoreductase
l	Litre
L-ala	L-isomer of alanine
LDH	Lactate dehydrogenase
Lk-ADH	Alcohol dehydrogenase from *Lactobacillus kefir*
mg	Milligrams
min	Minute
mL	Millilitre
mmol	Millimole
Mp	Melting point
n.d.	Not determined
NAD^+, NADH	Nicotinamide adenine dinucleotide (oxidised and reduced form)
$NADP^+$, NADPH	Nicotinamide adenine dinucleotide phosphate (oxidised and reduced form)

NP-HPLC	Normal-phase high pressure liquid chromatography
Pac-ATA	Transaminase from *Pseudonocardia acaciae*
PLP	Pyridoxal 5'-phosphate
PMP	Pyridoxamine 5'-phosphate
ppm	Parts per million
PQQ	Pyrroloquinoline quinone
RP-HPLC	Reversed-phase high pressure liquid chromatography
Rr-ADH	Alcohol dehydrogenase from *Rhodococcus ruber*
rt	Room temperature
Shi-ATA	Transaminase from *Shinella*
Sorb	Sorbitol
SDS-PAGE	SDS-PAGE = Sodium dodecyl sulfate–polyacrylamide gel electrophoresis
THF	Tetrahydrofuran
Tja-ATA	Transaminase from *Tetrasphaera japonica*
TLC	Thin-layer chromatography
TPPTS	Triphenylphosphine-3,3',3''-trisulfonic acid trisodium salt
U	Units
U/mg	Units per milligram
U/mL	Enzymatic volumetric activity
V	Volume
Vf-ATA	Transaminase from *Vibrio fluvialis*
ω-TA	ω-transaminase

1 General introduction and scope

1.1 Biocatalysis and green chemistry

The employment of biocatalysts can provide the chemist the possibility to utilize enzymes in organic syntheses and take advantage of their high chemo-, regio-, and stereospecificity. Due to its versatility, enzymes find application in many areas which demand for enantiomerically pure compounds. Praised for high activities, high turnover rate and their renewable nature, biocatalysts have gained intense attention and application both from academia and industry.[1] Unlike chemically-catalysed reactions that often rely on harsh and intensive processes, enzymes usually favour environmentally-friendly reaction conditions performed at mild temperature and pressure in aqueous medium at physiological pH.

Biocatalysts are usually compatible with each other, making it easy to combine several enzyme classes in one reaction system. This property has been exploited to develop strategies to use enzymes to act sequentially or even in a concurrent mode. Such multienzymatic one-pot multistep processes opened the possibility to carry out reaction sequences which substantially reduce operating times by avoiding lengthy isolation steps, purifications and considerably reducing waste and cost.

The cascades of two or more catalysts face an additional challenge with chemoenzymatic combinations. Unlike biocatalysts, the combination of bio- with organo- or metallo-catalysts typically suffers from compatibility issues in regards of the reaction medium as they often encounter inactivation or instability when used in the same environments.[2] The ‘’classic’’ chemical processes are typically carried out in organic media whereas enzyme catalysis often requires water as a medium of choice. It is thus necessary to find a compatibility window to meet the requirements of each catalytic system. To circumvent these limitations a number of techniques like immobilization[3] , compartmentalization[4], and protein engineering approaches[5] have been investigated and implemented.

1.2 Biocatalytic cascades: definition and classification

The term ''cascade'' has been commonly used for one-pot reactions that proceed independently and take place concurrently or in separated timelines. Cascade reactions are more effective than the classical single-step reactions for the reason that less chemicals are used in the workup stage of the process and unstable or possibly toxic intermediates can instantly be consumed or transformed. This leads to a more effective production of compounds and higher yields.[6]

Literature examples classify multistep biocatalytic one-pot reactions into cascade (domino) processes, tandem reactions and orthogonal tandem catalysis.[7] The recent trend however tends to terminologically generalize these types of processes with the term of ''cascade reaction'' as a process that includes all concurrent or temporarily separated biocatalytic one-pot reactions irrespective of the type and number of catalysts implemented.[8] Therefore, chemoenzymatic cascade refers as a reaction system composed of at least one biocatalyst where two or more processes are carried out in the same reaction flask.

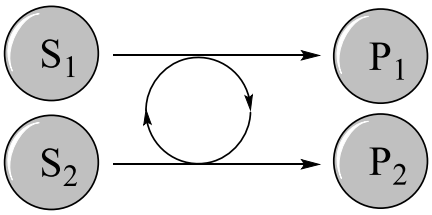
Cascades can be performed in a ''sequential mode'' when the second key catalyst is added after the completion of the first step of the reaction. On the other hand, reaction conditions in cascades carried out in a 'concurrent mode' do not change in the consecutive steps and all ingredients are added from the beginning of the process.[8] Cascade reactions performed in a sequential fashion enable to circumvent sometimes the problems associated to inhibition of enzymes or metal catalysts by a reagent or compound involved in the preceding steps. Thus, adding one reagent or modifying the reaction conditions (pH, T) at a later stage of the sequence could be a practical solution.

Incompatible catalysts can be combined in one-pot cascade process via a number of strategies and separation methods such as using a two-phase system or involving immobilization on nanoparticles or beads offer a solution to these problems.[8] Techniques such as flow chemistry[9] or compartmentalization[10] can spatially separate the two key components as well. Furthermore, cascades have their own specific characteristics and can be classified into four different designs.

i) Linear cascades consist of one-pot transformations where the starting material (or substrate) is converted to the product *via* one or more intermediates which are not isolated during the process.

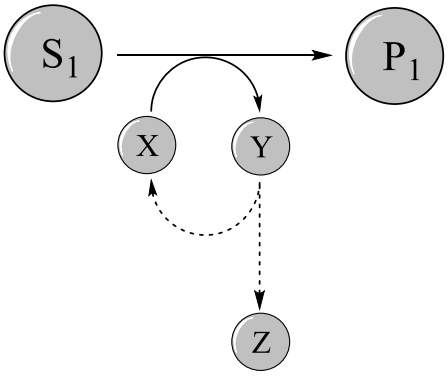
Scheme 1. Linear cascade design

ii) In the parallel cascade design two simultaneous biocatalytic reactions are carried out and both starting materials are converted into two distinct products. These types of cascades are commonly used in redox biocatalysis employing oxidoreductases.



Scheme 2. Parallel cascade design

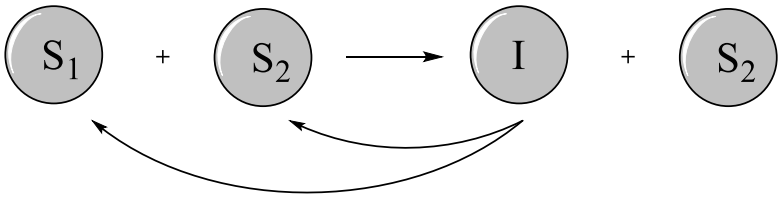
iii) Orthogonal cascades are closely related to parallel cascades with the difference that they consist of main reaction coupled with a second auxiliary reaction often used to remove the formation of undesired by-products.[8]



Scheme 3. Orthogonal cascade design

iv) In cyclic cascades the co-product is formed back into one of the starting materials. This leads to the accumulation of the desired product which is left behind in the first transformation. This system has

been often used in deracemization of amines, α-amino acids or α-hydroxy acids that consist of a redox process.[11]



Scheme 4. Cyclic cascade design

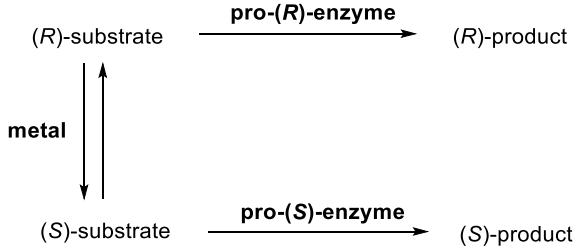
Combination of many different enzyme forms such as isolated or whole cells enzymes are likely achievable, but a process to be effective requires optimisation and appropriate operating conditions. Future development of successful chemoenzymatic combinations will most likely depend on solvent and enzyme engineering. Solvent optimisation allows successful biocatalytic reactions to be carried out in alternative reaction media meanwhile the range of available enzymes for novel synthetic applications continues to expand.

1.3 Combination of bio- and chemocatalysis

Syntheses of natural products and pharmaceuticals are mostly based on ''classical'' chemical synthetic routes with reaction intermediates often isolated and purified. Alternative processes for these ''classical'' chemical systems can be represented by multi-step one-pot reactions based on the combination of bio- and chemocatalysts. Such processes demonstrate many advantages over conventional sequential reaction schemes in terms of productivity, selectivity, and cost as well as environmental efficiency.

To overcome the limitations of challenging reaction conditions and requirements by bio- and chemocatalysis, it is necessary to develop processes equally appropriate for both reactions. Early examples of combining the chemo- and biocatalysts date from the 1980 after Van Bekkum et al. successfully developed a process based on the use of D-glucose isomerase immobilized on silica in a combination of a copper-on-silica catalyst.[12] After this pioneering breakthrough, the group of Allen and Williams developed a process that runs in water and which combines a palladium-catalysed racemisation with a lipase-catalysed hydrolysis.[13] The use of lipases in organic media has further attracted attention as it was possible to carry out metal-catalysed racemisation reactions coupled with enzymatic resolution of racemic alcohols, amines and amino acids, employing ruthenium, palladium, iridium and a range of other metal complexes.[14] Since then numerous metal-catalysed reactions have been successfully coupled with biocatalysts predominantly for DKR type reactions.[15]

Although DKR reactions can provide high yields and excellent enantiopurities, these reactions often have limitations in terms of reaction temperature and specificity of both catalysts.



Scheme 5. Example of a typical metallo-enzymatic DKR

In the last decade a number of other types of chemocatalytic reactions such as C-C bond-forming reactions have been successfully combined with biotransformations.[16] Alcohol dehydrogenases (ADH) have been successfully applied in multi-enzyme cascades with Baeyer-Villiger monooxygenases,[17] or in a combination with metal-catalysed processes, for the asymmetric synthesis

of alcohols by the stereoselective reduction of the corresponding ketones.[16],[18] A lot of these new designed chemoenzymatic processes give access to valuable building blocks for the preparation of a wide range of pharmaceutically relevant compounds. Recently the use of ω-transaminases has become an efficient biocatalytic approach for the synthesis of α-chiral primary amines. Many of these enzymes have become widely used in chemoenzymatic systems and some of these processes are currently used in the pharmaceutical industry.[19]

The combination of chemocatalytic and biocatalytic transformations in one-pot processes in aqueous medium represents an attractive research topic from both the academic as well as industrial perspective. Development of such chemoenzymatic one-pot processes in environmentally friendly solvents has emerged enormously in recent years, and numerous proof-of-concepts for the combination of metal catalysis with biotransformation has been investigated and successfully demonstrated.[20]

The first example of a one-pot process based on a combination of an asymmetric metal-catalytic transformation and an enzyme-catalysed step conducted in aqueous medium in a sequential fashion was published in 2006.[21] This study afforded the process for the enantioselective synthesis of amino acids in water and increased an interest to combine metal catalysis with biocatalysis for reactions that were previously thought to be incompatible processes.

Later in 2008, the palladium catalysed Suzuki cross-coupling reaction was combined with a further bioreduction.[16] Since then this concept was subject of intensive research and improvement with the particular aim to increase the efficiency and performance of this chemoenzymatic process.[22],[23]

The advantages of the combination of chemo- and biocatalysis enables the development of novel synthetic pathways and proof of concepts. It represents an attractive research area with a lot of industrial and academic potential towards a green and sustainable chemistry.

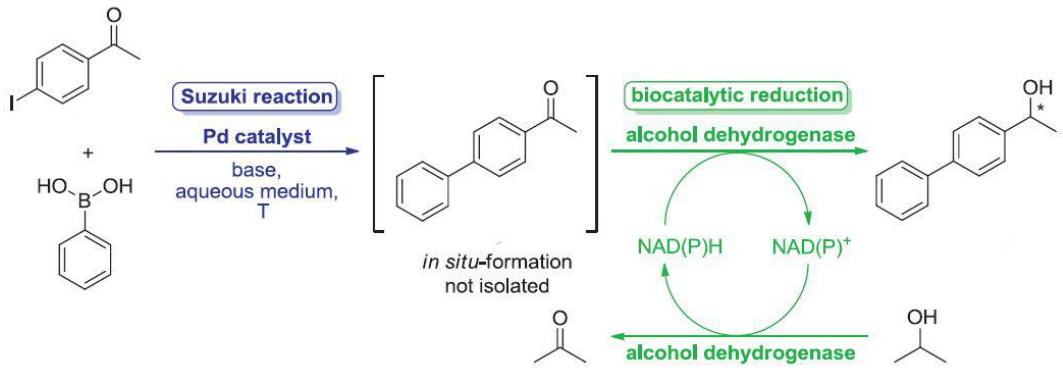
2 One-pot cascade reaction for the synthesis of enantiomerically pure biaryl alcohols in Deep Eutectic Solvents

2.1 Introduction

Due to the growing need for sustainable technologies and integration of biocatalysis in chemical cascades and one-pot processes the use of the right solvent system is of high importance. Thus, the attention is turned towards a new class of biorenewable solvents, namely the *Deep Eutectic Solvents (DES)*, which have been demonstrated as a valuable alternative to volatile organic solvents from the standpoint of sustainability.[24] Therefore the focus of this chapter is on the formation of enantiomerically pure biaryl alcohols via a one-pot sequential cascade process with particular interest on the use of these biorenewable and environmentally friendly solvents.

To this date there exists only one example of a chemoenzymatic cascade in *DESs*, which was developed in parallel to this research and consists of the combination of a ruthenium-catalysed isomerisation of allylic alcohols with an enzymatic reduction.[25] The proof that *DESs* have been successfully implemented as an efficient reaction medium for many enzymes,[26] as well as metal-catalysed reactions,[27] increased the motivation for the research in this direction.

Based upon the concept from 2012 (**Scheme 6**),[23] when the combination of a metal-catalysed reaction and enzymatic process had been carried out at room temperature in a mixture of isopropanol and water, the possibility for the cascade process in a mixture of *DESs* and aqueous buffer is investigated.



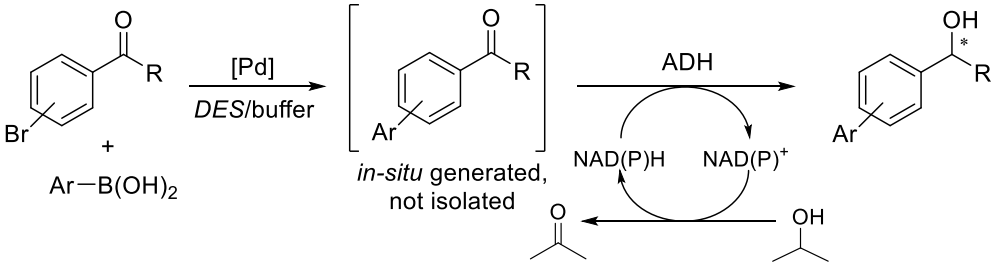
Scheme 6. Concept of the chemoenzymatic one-pot process in aqueous medium for the synthesis of the chiral biaryl alcohols[23]

Therefore, the study developed throughout this chapter investigates the advantages of *DESs* and their utilization as the reaction medium in the chemoenzymatic one-pot process for the synthesis of enantiomerically pure biaryl alcohols.

2.2 Goal and motivation

In spite of all the benefits of one-pot processes, such as the palladium-catalysed Suzuki cross-coupling reaction and a bioreduction,[23] there is still a challenge in finding an improved solvent system. One option to achieve this is the use of *DES*s due to a number of benefits and advantages.[28] Thus, this research focuses on this novel class of solvents and their contribution to an improved efficiency, while overcoming compatibility limitations and hurdles.

A mixture of aqueous buffer and *DES* is used as a solvent in the cascade reaction to synthesize biaryl prochiral ketones which are *in-situ* converted to the chiral biaryl alcohols. Choosing a suitable enzyme in the second step provides the corresponding (*R*)- or (*S*)-enantiomer in good yields and high optical purity (**Scheme 7**).



Scheme 7. Chemoenzymatic cascade towards chiral biaryl alcohols in *DES*-buffer medium

At present there are examples of Suzuki cross-coupling reactions being carried out in neat *DESs*,[27] although the feasibility of such process using the water-soluble Pd-catalyst in eutectic mixtures requires further investigation.

The combination of two reactions needs both steps to be studied and optimised separately first. Therefore, the aim in the first step of the cascade consists of finding ideal reaction conditions bearing in mind basic pH requirement, catalyst loading, substrate concentration, and reaction temperature. Considering the high price of palladium, the reduction of the amount of the metal catalysts presents benefits from an economical aspect and avoids any potential negative effect that

the metal might have on the enzyme activity. Thus, the decrease of the catalyst loading is another goal to be achieved and at the same time reach the highest limit for the substrate concentration.

The presence of high substrate loading asks for a solvent to completely solubilize highly apolar components. Thus, different preparations of eutectic mixtures have to be studied in order to find the most suitable choice while also decreasing the amount of co-substrate isopropanol as low as possible, a minimal amount necessary only for the co-factor regeneration system in the second step.

The substrate scope of the Suzuki coupling under optimised conditions has to be evaluated for the construction of substituted biaryl ketones. Furthermore, the established substrate scope has to be taken into consideration in a screening study for the second step of the cascade to ensure high activity of enzymes towards the challenging ketones in question. Once the ideal reaction conditions for the Suzuki coupling have been established, focus has to be switched on the forthcoming enzymatic reduction step.

For the reduction of ketones commercially available ketoreductases (KREDs) and overexpressed ADHs in *E. coli* are used. Being a one-pot process, it is highly important to establish reaction conditions for the enzymes to be compatible with the components of the first step of the cascade.

Once both catalytic steps are validated and optimised, the cascade reaction will be carried out. After the *in-situ* reduction of the formed ketone the final yield and enantiomeric preference of the formed biaryl alcohols will be evaluated.

2.3 State of the art

2.3.1 Palladium-catalysed Suzuki cross-coupling reaction

Palladium catalysed cross-coupling reaction of organoboron compounds was introduced in 1995 and has since then been one of the most vastly used and popular methods to create carbon-carbon bonds to obtain alkenes, styrenes, or biaryl compounds (**equation 1**).[29] Biaryl molecules are important structural components of many pharmaceuticals, herbicides and natural products.[30]

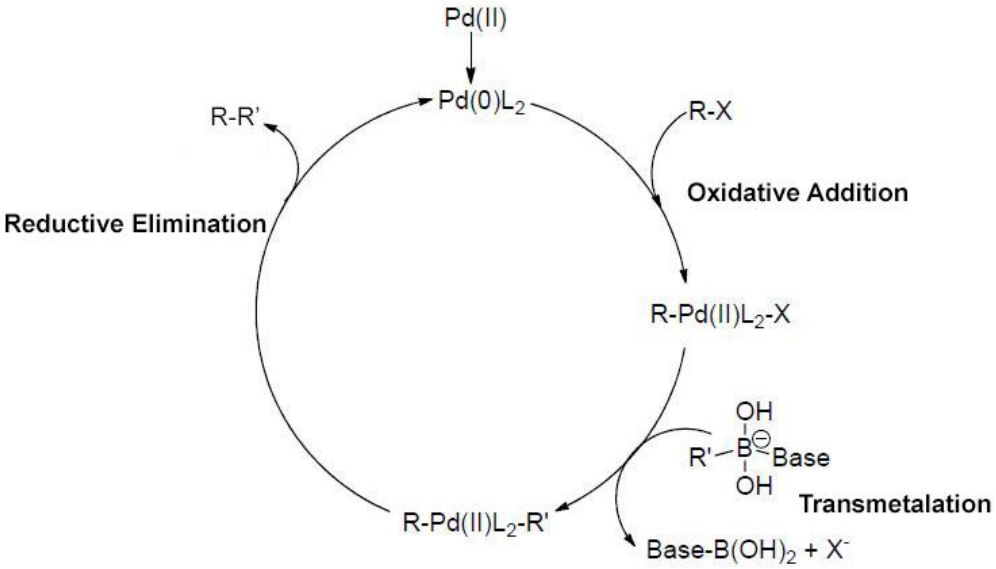
$$\text{R-M} + \text{R'-X} \xrightarrow{\text{Pd-catalyst}} \text{R-R'} \quad \textbf{(1)}$$

2.3.1.1 *Mechanism of the Suzuki cross-coupling reaction*

The cross-coupling reaction is usually driven by palladium bonded to a ligand which activates the catalyst, and a base that reacts with the boronic acid to give the desired carbon-carbon bond formation.

The Suzuki cross-coupling reaction proceeds via three fundamental steps (**Scheme 8**):[29]

1. Oxidative Addition
2. Transmetalation
3. Reductive Elimination

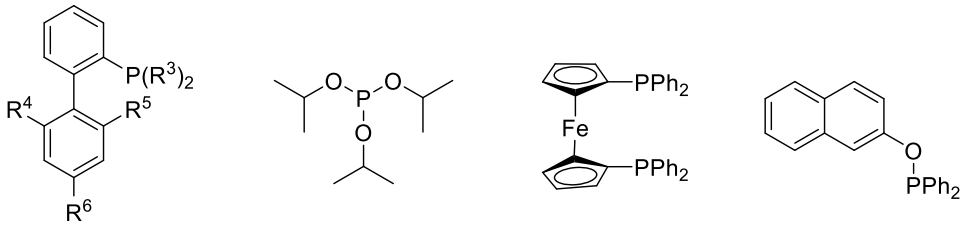


Scheme 8. Mechanism of the Suzuki cross-coupling reaction[29]

In the first step aryl halides react with the palladium(0)-complex which is oxidized to the palladium(II)-complex (Oxidative Addition). Subsequently the transfer of the organic residue (-R') from the organoborane compound to the palladium takes place in the presence of a base (transmetalation). Finally in the Reductive Elimination step the desired coupling product R-R' is cleaved and the original palladium(0)-complex is recovered.

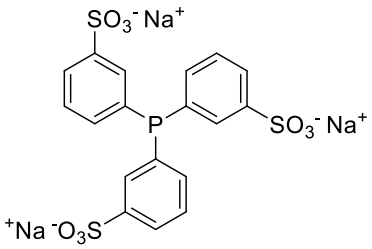
2.3.1.2 *Design of alternative phosphine-based ligands*

The Suzuki cross-coupling reaction was initially carried out in the presence of organic solvents and inorganic bases. From an industrial point of view, it was however important to further develop the process with the aim to use efficient and non-toxic catalysts and water as an inexpensive and safe solvent. Among these influencing factors the ligand plays an important role in the reaction. Therefore numerous efforts have been done in the last decade to develop efficient ligands based on triphenylphosphine which was the earliest and most widely used ligand in Suzuki cross-coupling reactions.[31] Potential ligands involve the use of electron-rich bulky phosphines[32], bulky phosphines[33] and phosphine oxides[34] (**Scheme 9**). These modifications lead to the formation of ligands that allow lower catalyst loading and improve the efficiency of the catalytic cycle by enhancing the rate of both the oxidative addition and reductive elimination processes. Different ligands furthermore allow to extend the scope of Suzuki cross-coupling reactions. The use of certain ligands overcomes solvent incompatibilities and avoids the requirement for higher reaction temperatures, and at the same time reduces environmental concerns. An improved ligand system also means the catalyst can tolerate reaction conditions suitable for one-pot processes which could also be carried out in water. The originally hardly soluble phosphines can be modified and converted to water-soluble ligands by introducing polar groups such as sulfonates and ammonium groups. This modification made it possible to carry out Suzuki cross-coupling reactions in a variety of solvents ranging from glycerol to a mixture of water with acetonitrile and isopropanol respectively.[35],[16,36]



Scheme 9. Example of alternative phosphine-based ligands for the Pd-catalysed cross-coupling reactions

Due to its solubility in water, the ligand triphenylphosphine trisulfonate (TPPTS) has been used for carbonylation reactions in aqueous media (**Scheme 10**).[16]



Scheme 10. A water-soluble (tris(3-sulfonatophenyl)phosphine) ligand (TPPTS)

Similarly, the catalytic system consisting of TPPTS/Pd(OAc) has been applied for the synthesis of Xenalipin, a potential cholesterol-reducing drug.[37] A water-soluble TPPTS/$PdCl_2$ catalytic system was employed by Hoechst for the commercial production of 2-cyano-4'-methylbiphenyl, a key intermediate in the synthesis of angiotensin II receptor antagonists used for the treatment of hypertension.[38]

2.3.1.3 *Application of Suzuki cross-coupling reactions*

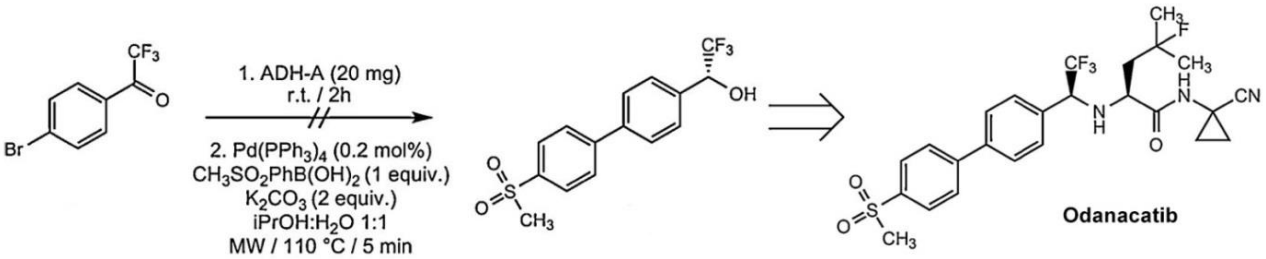
Homogeneous Suzuki cross-coupling reactions have also find application in chemical biology,[39] although high catalyst loadings are often required for the reactions to be performed in this environment.

Recovery and recycling of palladium has been an important issue from an industrial perspective due to its high costs. A number of strategies to develop solid-supported catalytic systems have been developed to facilitate the recovery of Pd-catalyst. Such heterogeneous systems are particularly interesting for the industrial synthesis of pharmaceutical to avoid any metal residues. Supported catalytic systems often include the use of polymer supports and immobilized palladium on nanoparticles or silica supports.[40]

A study reported in 2014 revealed the use of the heterogeneous Pd/C catalyst in water for Suzuki cross-coupling reactions of halophenols with boronic acids for the synthesis of phytoalexins.[41]

Carrying out a Suzuki cross-coupling reaction in aqueous media opens up the possibility of a combination with enzymatic processes. The first example of such combination was reported by Gröger and co-workers with a Pd-catalysed cross-coupling reactions followed by an enzymatic asymmetric

reduction of the transiently formed ketones.[16] This process was carried out in a mixture of isopropanol (50%) and water, and later successfully optimised to run both steps at room temperature.[23] Another noteworthy example is the asymmetric biocatalytic reduction followed by the Suzuki cross-coupling used for the synthesis of the intermediate for the cathepsin K inhibitor Odanacatib (**Scheme 11**). This enantioselective two-step synthesis was carried out to in a continuous flow process to achieve the desired product in high yields and enantiomeric excess.[42]



Scheme 11. Combination of a ketone bioreduction with a subsequent Suzuki–Miyaura coupling in a one-pot process for the synthesis of the Odanacatib precursor[42]

Recently the combination of the biocatalytic halogenation of L-tryptophan with subsequent Suzuki cross-coupling reaction has been reported. This one-pot three-steps reaction leads to the formation of aryl-substituted tryptophan derivatives which can be used for peptide or peptidomimetic synthesis.[43]

In conclusion, the Suzuki cross-coupling reaction represents one of the most straightforward methods for carbon-carbon bond formation and synthesis of biaryl and alkene derivatives. The reaction in aqueous media however often requires high temperatures, the use of co-solvents, long reaction times and is therefore generally limited by a narrow substrate scope.

2.3.2 Deep Eutectic Solvents as reaction media for chemo- and biocatalysis

2.3.2.1 *Preparation of DESs and their properties*

Many enzymatic reactions predominantly take place under mild conditions (room temperature, physiological pH and ambient pressure) in water. Water is a cheap, non-flammable, fairly abundant and safe for the environment solvent. Although exhibiting many beneficial properties one major disadvantage of using water as a solvent lies within its high polarity.

Enzymes function preferably in water, which can become a disadvantage for catalysts and organic compounds with a limited solubility in this solvent. A common strategy to overcome this limitation relies on the use of water-miscible co-solvents that not only increase the solubility of the compounds, but also act as co-substrates. This is advantageous in the case of ADH-catalysed reactions whereas isopropanol has been used as co-solvent and also as both electron donor for the co-factor regeneration system and to shift the unfavourable equilibria towards product formation.

Although the use of co-solvents usually provides benefits in terms of higher substrates loading it is necessary to consider their compatibility with enzymes as they might have unpredicted effects on the biocatalyst activity and stability. Thus, it is advisable to optimise reaction conditions each time for new processes and biocatalysts.

The methodology of using two-phase systems consisting of water and a water-immiscible organic solvent is another advantageous strategy when substrates are poorly soluble in water. In this case greater part of hydrophobic substrates and products are contained in the organic phase, while the enzyme and any water-soluble cofactors are in the aqueous phase. The product can then be easily obtained from the organic phase via distillation and extraction processes.

One of the limitations of a two-liquid phase system is a slow transfer rate between the two phases which can significantly slow down the overall reaction process.

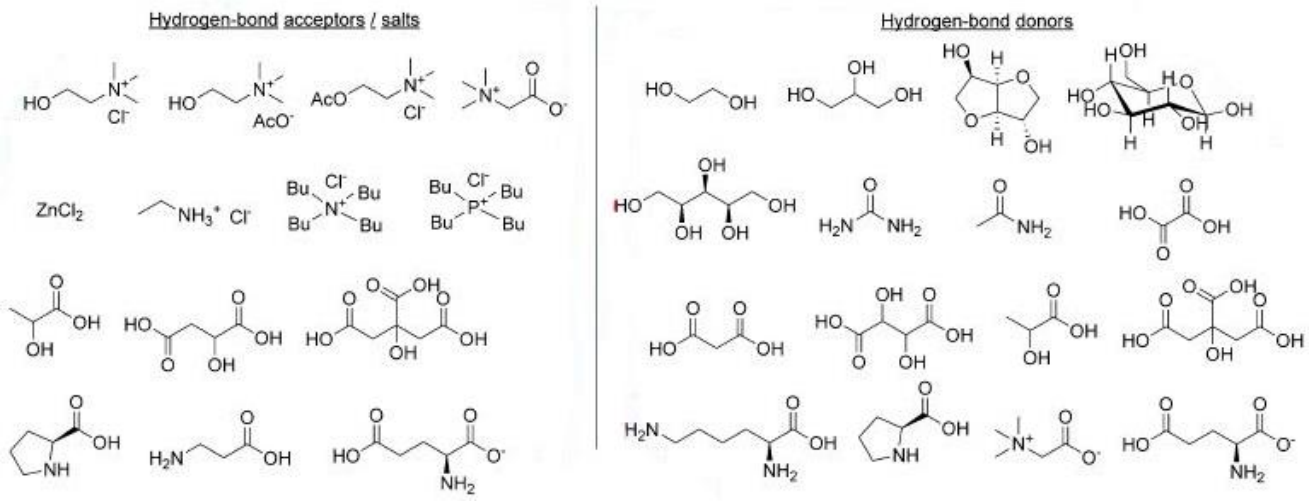
Although enzymes prefer water, numerous enzymatic processes in organic media have been described.[44] Benefits of carrying out a biocatalytic transformation in organic media includes an easier product removal and elimination of contamination. Furthermore, having an enzymatic process in organic media allows for some reactions which can hardly be performed in water due to hydrolysis such as esterification or formation of amides.[45] The drawback of such processes is often lower enzymatic activity compared to the transformation carried out in water. This has led to the

investigation of non-conventional media with the intent to perform enzymatic reactions in non-aqueous solutions.

The attempts for the search of green solvents led to the discovery of ionic liquids (ILs). In the following years a number of enzymatic transformations were reported in ILs.[28] Although having many advantages such as high thermal stability, non-flammability, high catalytic activities and many other fine-tuning properties,[46] there are a number of drawbacks that include high cost, toxicity, low biodegradability and the requirement to use organic solvent at the end of the reaction. These findings have increased the interest to another attractive class of alternative solvents, the so-called *Deep Eutectic Solvents (DESs).*

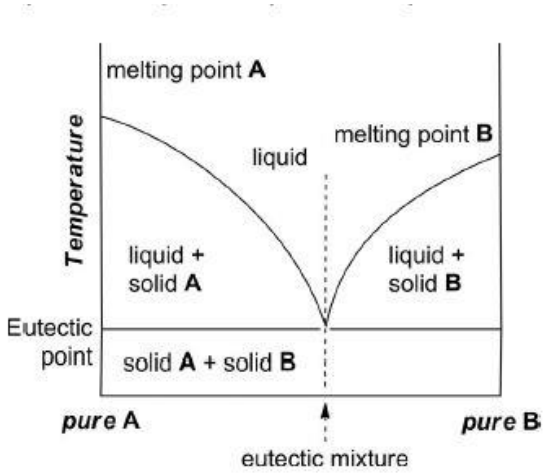
DESs offer similar properties to those of ILs in terms of low volatility, non-flammability, low vapour pressure, high thermal stability and solubility of organic compounds. They have become a subject of intense research due to a number of other advantages such as low cost and high availability of their starting materials, with minimal toxicity and extremely low environmental disposal issues.

DESs are readily available, inexpensive, biodegradable and come from renewable resources. They are often prepared from choline chloride (ChCl) and a variety of inexpensive raw materials such as sugar polyols, D-glucose, itaconic acid and carboxylic acid.[45] Thus, they consist of a hydrogen-bond acceptor (HBA) such as quaternary ammonium salts (e. g., choline chloride) and uncharged hydrogen-bond donors (HBD) such as urea, carboxylic acids or polyols. The interaction between HBA and HBD relies mostly on hydrogen bonds, van der Waals interactions and electrostatic forces which stabilise liquid configurations and results in lower melting points than those of their individual components (**Scheme 12**).[45] The atom efficiency of the final formulation is the highest possible, as all the initial components are included in the final formulation as well.



Scheme 12. Examples of HBAs and HBDs used to form *DES*[45]

The eutectic mixture is a composition of two or more phase-immiscible solid components that completely change their solid phase to become liquids at a certain temperature point called the eutectic point (**Scheme 13**).



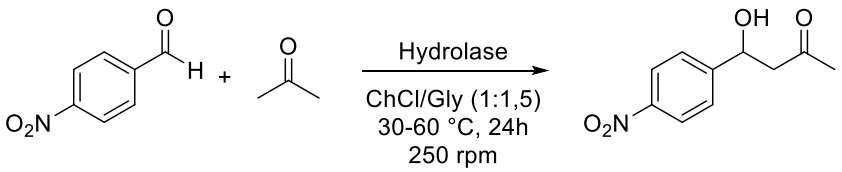
Scheme 13. Phase diagram of a *DES* mixture[47]

The use of *DESs* involves advantages in the work-up stage as high solubility of water in *DES* allows further addition of water followed by the precipitation of organic products. This strategy avoids the use of organic solvents for the extraction and the initial *DESs* to be recycled by simple evaporation of water from the aqueous layer.[48] The drawbacks of *DESs* include reports about high densities and viscosities of conventional *DESs* mixtures which could suggest a problem in continuous flow and

industrial processes. These issues can be solved by a slight increase of temperature or formation of ternary mixtures by addition of different components such as water, carboxylic acid or an organic or inorganic halide.[49]

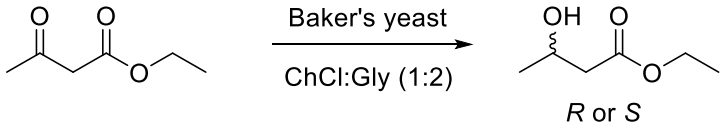
2.3.2.2 *Application of DESs*

Over the past decade *DESs* have found applications in several chemical sciences and technologies such as electrochemistry and metal processing, material chemistry, nanotechnology, photosynthesis and energy technology, separations processes, and stabilisation of DNA. With respect to synthetic purposes, *DESs* have provided examples of improved activity and selectivity in: i) organometallic-mediated stoichiometric transformations,[50] and ii) metal-,[51,52]enzyme-,[53] or organo-catalysed reactions.[54,55] *DESs* attracted attention from a biocatalysis prospective in 2008 in a research of Kazlauskas and co-workers who demonstrated good catalytic activity of hydrolases in mixtures based on ChCl/Urea (1:2) and ChCl/Gly (1:2). ChCl-based *DESs* have shown to be a good solvent of choice for lipases that catalyse the aldol reaction of aromatic aldehydes with ketones (**Scheme 14**).[56]



Scheme 14. Hydrolase-catalysed aldol reaction between 4-nitrobenzaldehyde and acetone in a *DES* mixture consisting of choline chloride and glycerol[56]

DESs could also be used as co-solvents for whole-cell biocatalysis as it was observed that *E. coli* cells retained their integrity in the media. Following that demonstration it was reported the use of baker's yeast in mixtures of ChCl/Gly (1:2) and aqueous buffer for the enantioselective reduction of ethyl acetoacetate to optically active alcohols at long reaction times (>200 h).[57] In that study an inversion of stereoselectivity was observed with the use of different proportions of *DESs* and water which suggests that certain KREDs present in the baker's yeast were completely inactivated while others with the opposite stereopreference retained activity (**Scheme 15**).



Scheme 15. Baker's yeast was used as a reductive catalyst in a mixture of *DES* and water

The use of *DESs* for an efficient conversion of fructose to 5-(hydroxymethyl)furfural (HMF) was reported in a biphasic mixture consisting of ChCl/citric acid.[58] Being a biomass-derived chemical, HMF has a great potential in the bio-based industry as a substitute for petroleum-based building blocks as well for the production of high-valued chemicals.

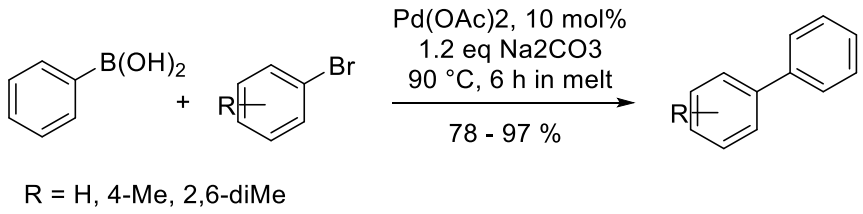
Beside their use as a solvents, preparations of choline chloride and metal chlorides have been used as catalysts in different multicomponent reactions for the preparations of β-amino ketones[59] and α-aminophosphonates.[60]

Deep eutectic mixtures have also been used in different carbon-carbon and carbon-heteroatom bond-forming processes in particular in different conjugate additions, such as the thia-Michael addition to α,β-unsaturated carbonyl compounds for the generation of *S*-alkylisothiouronium salts.[61]

Preparations of *DESs* consisting of ChCl/urea found application in a number of redox reactions at room temperature. The use of *DESs* showed substantial increase of yield and decrease of reaction time compared to the process with organic solvents.[62]

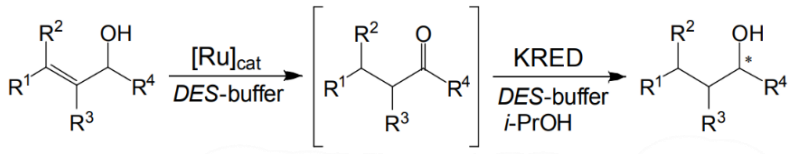
There are a few examples of organometallic reactions in *DESs* in which Grignard and organolithium reagents were added to ketones, or the oxidation of toluene to benzaldehyde with hydrogen peroxide as oxygen source.[63]

Very recently several cross-coupling reactions were successfully carried out in a variety of *DES* preparations. A mixture consisting of low-melting sugar mannitol, urea and ammonium chloride was used in Suzuki cross-coupling reactions with excellent yields after only 6 hours of reaction time (**Scheme 16**).[51] Positive results were also obtained in the Heck cross-coupling and copper-free Sonogashira coupling where D-mannose/DMU was the *DES* mixture of choice by using cationic pyridiniophosphine ligands in association with $PdCl_2$. It is important to mention that in most cases the use of *DESs* allowed to repeatedly use the catalyst and recycle the whole media.[52]



Scheme 16. Suzuki coupling in sugar–urea–salt melts[51]

The use of *DESs* has been recently successfully implemented in chemoenzymatic processes such as the tandem reaction consisting of a lipase and organocatalysts[55,64] or a ruthenium-catalysed isomeration of racemic allylic alcohols coupled with an enantioselective bioreduction (**Scheme 17**).[25]



Scheme 17. One-pot ruthenium-catalysed isomerisation of allylic alcohols combined with an enantioselective bioreduction in *DES*-buffer medium

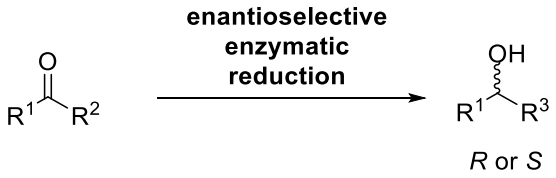
The use of solvents plays an important role in the toxicity profile of a chemical process and for a typical pharmaceutical or fine chemical batch operation it usually accounts for between 80 and 90 % of mass utilization.[65] Given their dominant role in such operations it is necessary to optimise the synthetic strategy by choosing the solvent that can provide the desired function without the undesired properties that can cause environmental, health and safety issues. For that reason, many neoteric solvents such *DESs* provide an effective alternative being identified as ''greener'' and more sustainable option. Unlike petroleum-derived solvents numerous biomass-derived solvents, such as glycerol, ethanol, limonene, γ-valerolactone, or tetrahydrofuran could be used as promising alternatives to conventional organic solvents, due to a number of beneficial attributes. However, the potential for modulation of their properties is not very high.[66]

To conclude, *DESs* have emerged as environmentally attractive reaction media for biocatalytic processes and can convincingly replace common solvents in many organic reactions. The presence of *DESs* in aqueous medium avoids problems of low solubility and low concentration of substrates. Thus, their utilisation in the fields of chemo- and biocatalysis opens up new perspective for such processes. Numerous other advantages and beneficial physical properties highlight their positive impact and allow for more sustainable organic chemistry synthesis.

2.3.3 Enzymatic reduction of ketones

2.3.3.1 *Bioreduction approaches*

Alcohol dehydrogenases (ADHs) represent a straightforward approach for the synthesis of chiral alcohols and have been widely used for the reduction of carbonyl groups (**Scheme 18**). The enantioselective reduction of prochiral ketones generates a stereocenter starting from a planar sp^2-hybridized carbon which is transformed into a tetrahedral sp^3-atom.



Scheme 18. Enantioselective biocatalytic reduction for the synthesis of chiral alcohols

Beside their practical application in organic chemistry ADHs have gained enormous industrial interest owing to the importance of the application of enantiomerically pure alcohols in the production of pharmaceuticals, natural products, flavours and agrochemicals.[67]

Enzymatic reduction reactions can be catalysed by the use of isolated enzymes or whole-cell preparations providing a green, sustainable and efficient process alongside chemical processes based on the use of synthetic catalysts such as the metal-catalysed asymmetric hydrogenation of ketones.[68]

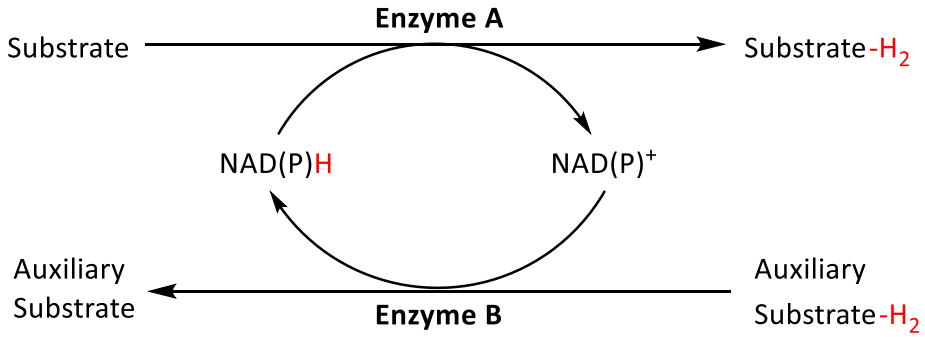
The majority of ADHs are dependent on the nicotinamide cofactors β-1,4-nicotinamide adenindinucleotide (NADH) or β-1,4-nicotinamide adenindinucleotide phosphate (NADPH) and a few on flavines (FMN, FAD) and pyrroloquinoline quinone (PQQ).[69] The reaction mechanism for the production of the alcohol involves the reduction of the carbonyl moiety followed by the concurrent oxidation of the coenzyme. For the next cycle of the substrate reduction, the coenzyme has to be reduced again.

Due to high costs of these cofactors they are not to be used stoichiometrically, thus a major task in process development is to provide an effective method for the regeneration of the consumed cofactors so that only catalytic amounts of coenzyme are required.

Many methods for the regeneration of the reduced form of the coenzyme have been developed.[70] The production of NAD(P)H can be carried out by chemical, electrochemical, photochemical and enzymatic methods, the latter one being much more efficient and representing the method of choice. Cofactor recycling is however not a major problem when whole cells are used as biocatalysts, as the organisms already possess the necessary cofactors. The most widely used organism for that purpose is baker's yeast (*Saccharomyces cerevisiae*).[71] In those cases inexpensive sugars such as sucrose and glucose can be used as auxiliary substrates to give the corresponding *(S)*-alcohols in good optical purities.

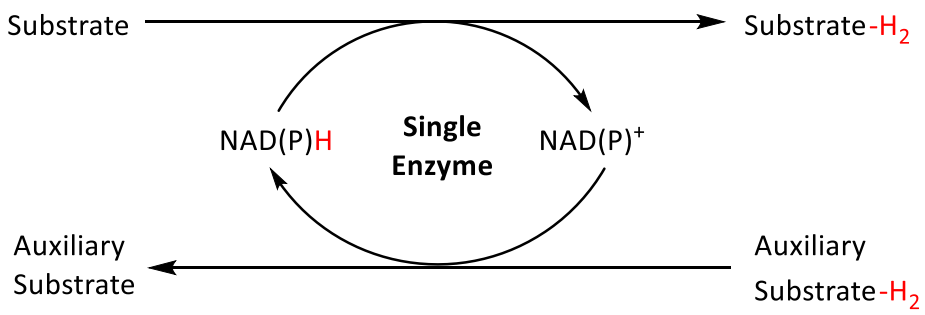
For the enzymatic method there are two different approaches, the (i) enzyme coupled and the (ii) substrate coupled process.[72]

The enzyme-coupled approach requires the application of two independent enzymes, one that reduces the main substrate, and the second one used for the cofactor recycling in the opposite redox direction (**Scheme 19**).



Scheme 19. Enzyme-coupled method for the co-factor recycling

The substrate-coupled approach requires the presence of a second auxiliary substrate for the cofactor regeneration and uses the same enzyme for both processes (**Scheme 20**).



Scheme 20. Substrate-coupled method for the co-factor recycling

One example of a two-enzyme system is the use of formate dehydrogenase (FDH) for the regeneration of NADPH by the oxidation of formate to carbon dioxide. Similarly, the method using glucose dehydrogenase (GDH) or glucose-6-phosphate dehydrogenase (G6PDH) has been widely employed for the regeneration of NADPH and NADH as well. These enzymes oxidise glucose or glucose-6-phosphate to gluconolactone or gluconolactone-6-phosphate respectively, which spontaneously hydrolyses in water to form gluconic acid.

For the substrate-coupled method isopropanol is frequently used as a co-substrate and it is oxidised to acetone. Isopropanol is used in excess to shift the equilibrium towards the direction of the desired product.

ADHs can be (*S*) and (*R*) specific, depending on the preference for the attachment of the hydride either on the *si*- or the *re*-side of the ketone. The stereochemical preference depends on the steric hindrance of the molecule and follows the model of the so called ''Prelog's rule''.[1] Most of the alcohol dehydrogenases such as yeast ADH or *Rhodococcus ruber* ADH follow the ''Prelog's rule'' and the hydride attacks the molecule from the *re*-face yielding (*S*)-configured alcohols. Anti-Prelog dehydrogenases, such as *Lactobacillus sp.* are (*R*)-selective and the hydride attacks the ketone molecule on the *si*-side giving alcohols with the opposite stereopreference.

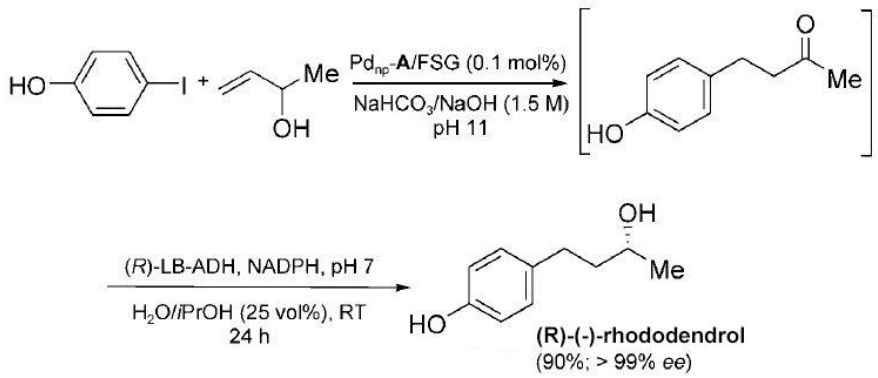
2.3.3.2 *Application of ADHs*

The use of ADHs in organic synthesis was at first limited to the substrate scope of aceto-type substrates bearing a large and small substituent like the derivatives of acetophenone, 2-alkanones and α- and β-ketoesters. Multi-substituted and hydroxyl-substituted acetophenone derivatives along with bulky ketones with large substituents that are structurally demanding remained a challenge for the enzymatic reduction.[69] In the last decade however a lot of effort has been put into the discovery of novel enzymes that are able to accept more sterically demanding ketones.

A highly enantioselective reduction of bulky-bulky ketones could be carried out using a recombinant ADH from *Ralstonia sp*.to give the corresponding optically active alcohols in high purity.[73] The reduction of bulky aryl alkyl ketones was reported using an isolated ADH from *Sporobolomyces salmonicolor* to obtain the corresponding alcohols in excellent optical purity. The enantioselective synthesis of chiral biaryl alcohols was also reported in that period.[74] Whole cells of *Candida chilensis* were also successfully applied for the enantioselective 1,2-reduction of prochiral α,β-unsaturated ketone to (*R*) allylic alcohols.[75]

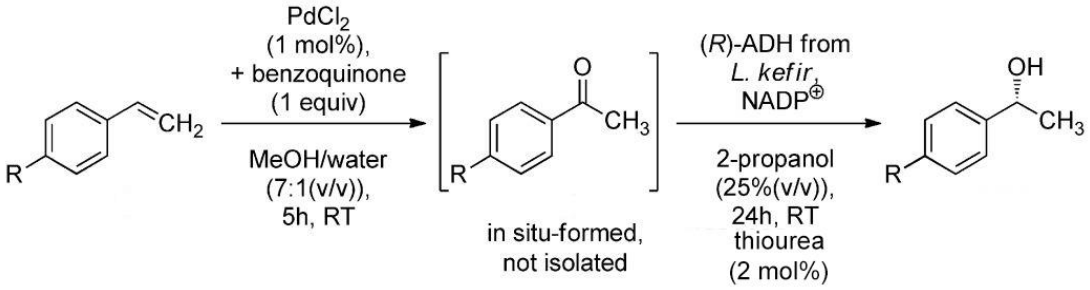
ADHs received much attention for their broad substrate tolerance and are very useful biocatalysts for the production of important building block for many APIs. The discovery of a new CgKR2 reductase is attractive for its ability to efficiently reduce chiral precursors necessary for synthesis of Angiotensin-Converting Enzyme (ACE) Inhibitors.[76] Likewise, KREDs from *Streptomyces nodosus* can be used for the reduction of protected amino acid derived α-haloketone to give intermediates used in the synthesis of an HIV protease inhibitor.[77]

ADHs were successfully combined with a number of metal-catalysed transformations. The combination of a Pd-catalysed Heck reaction with an enzymatic reduction towards a one-pot process in aqueous medium was successfully carried out for the synthesis of (*R*)-(-)-Rhododendrol (**Scheme 21**).



Scheme 21. One-pot chemoenzymatic synthesis of (*R*)-(-)-Rhododendrol

Another important transformation is the combination of a Wacker oxidation starting from a prochiral alkene which is successfully coupled with an enzymatic reduction in a one-pot process in aqueous media for the synthesis of 1-phenylethanol (**Scheme 22**).[78]



Scheme 22. Chemoenzymatic one-pot process for the enantioselective synthesis of 1-phenylethanol

In summary, enzymatic reductions of prochiral ketones into the corresponding optically active alcohols generally proceed with high enantioselectivity and yields. The use of ADHs has therefore be proven to be a highly efficient method for the reduction of C=O bonds and can be successfully used as catalysts in preparative organic chemistry and on an industrial scale for the synthesis of chiral alcohols.

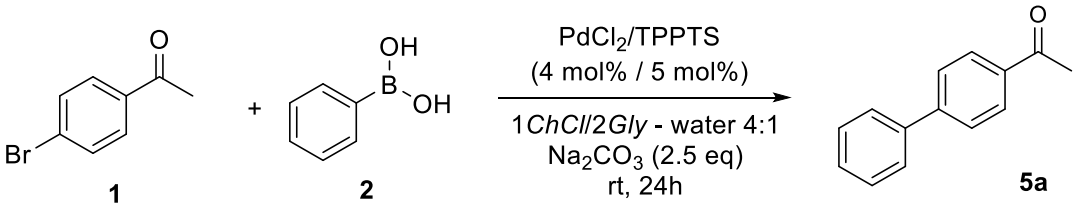
2.4 Results and discussion

As previously discussed in chapter **2.2** to investigate the viability of the one-pot cascade process a study of each separate step has to be carried out first. The Pd-catalysed Suzuki cross-coupling reaction is based on the method described in the literature.[23] The catalyst used is the previously studied water-soluble $PdCl_2$/TPPTS whose activity and stability need yet to be tested in eutectic mixtures. The subsequent enantioselective bioreduction step is carried out to obtain the corresponding alcohol after the *in-situ* formation of the desired ketone. In addition, reaching complete conversion in the first step of the cascade is highly advisable, since the unreacted ketone is also a competitive substrate for the enzymatic reduction.

2.4.1 Development of the Suzuki cross-coupling reaction in a mixture of *DES* and buffer for the synthesis biarylketones

2.4.1.1 *Suzuki cross-coupling reaction in a mixture of DES-buffer (4:1)*

According to the reported Suzuki cross-coupling reaction in water,[23] the process was carried out with the following reaction conditions: 40 mM substrate concentration, catalyst loading of 4 mol% $PdCl_2$ and 5 mol% TPPTS at room temperature for 24 hours. Rather than using a mixture of water and isopropanol the aim was to design a comparable process with the reaction medium consisting of a *DES*-water mixture. For this, a choline chloride (ChCl) and glycerol-based (Gly) eutectic mixture was prepared (*ChCl*/*Gly* 1:2 w/w), and the reaction medium also contained 20 % v/v of water (**Scheme 23).** Likewise, a slight excess of sodium carbonate was added to ensure the basic pH required for the cross-coupling. As a result, the Suzuki cross-coupling reaction proceeded smoothly at room temperature in the presence of the homogeneously dissolved catalysts system. These preliminary attempts led to the formation of the desired biaryl ketone (**5a**) with high conversion (92%). This satisfying result was on par with the quantitative conversion obtained in the originally reported process with water and isopropanol.

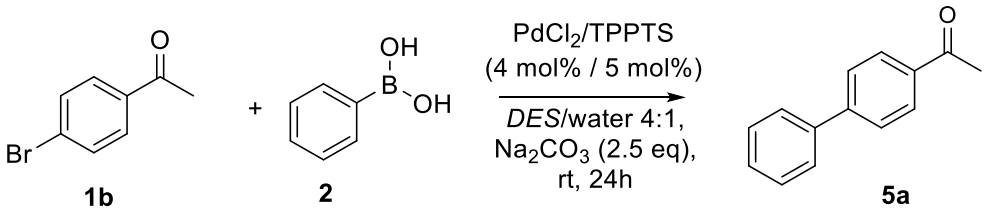


Scheme 23. Suzuki cross-coupling reaction in a mixture of 1*ChCl*/2*Gly* and water at room temperature

2.4.1.2 *Screening of different mixtures of DES and buffer*

Finding suitable the *DES*-water mixture as the reaction medium for the Suzuki cross-coupling reaction, the coupling between 4'-bromoacetophenone (**1b**) and phenylboronic acid (**2**) to yield 4'-acetylbiphenyl (**5a**) was selected as a benchmark reaction. Three additional choline chloride-based eutectic mixtures, namely 1*ChCl*/2H_2O, 1*ChCl*/1*Sorbitol* and 1*ChCl*/2*Urea* were prepared by combining choline chloride with the opportune amount (w/w) of the second component (see experimental section for more details) generating the appropriate *DES* solution.

Table 1. Suzuki cross-coupling reaction of 4'-bromoacetophenone (**1b**) and phenylboronic acid (**2**) in *DES*-water mixtures at room temperature



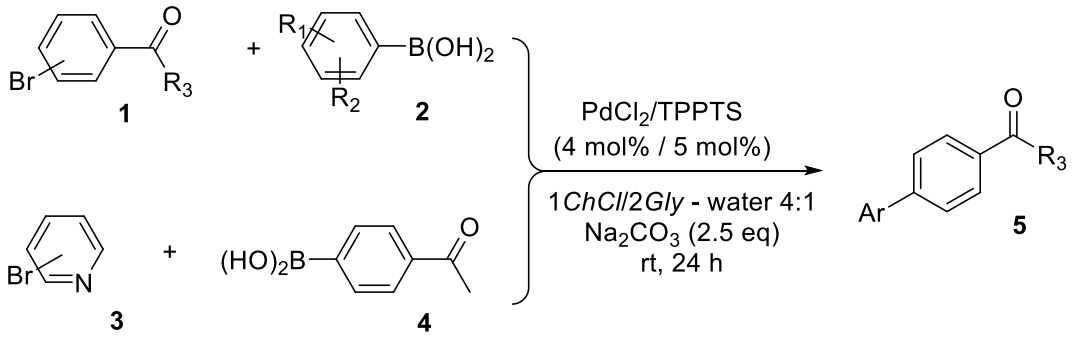
Entry	*DES*	T (℃)	*c* (%)
1	1*ChCl*/2*Gly*	rt	92
2	1*ChCl*/1*Sorb*	rt	82
3	1*ChCl*/2*Urea*	rt	0
4	1*ChCl*/2H_2O	rt	80

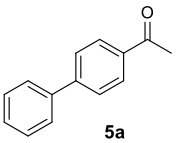
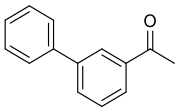
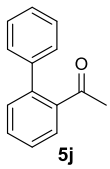
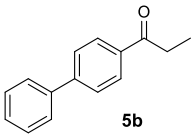
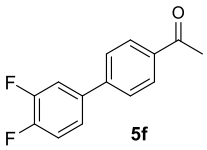
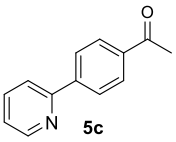
The result clearly unveiled 1*ChCl/*2*Gly* as the optimal *DES* mixture giving high conversion of 92% with respect to the formation of the ketone (**Table 1,** entry 1). On the other hand, the reaction did not work in *1ChCl/2Urea* (entry 3) while *1ChCl/2*H_2O and *1ChCl/1Sorb* displayed conversions higher than 80% (entries 4 and 2). An explanation for this unusual effect could be the potential inactivation of the palladium catalyst by urea. Similarly, sorbitol and water have a slightly negative impact on the reaction in comparison to the glycerol-containing eutectic mixture. From these results it can be concluded that hydroxy-groups are well tolerated while amide bonds might have a negative influence on the catalytic transformation.

2.4.1.3 *Substrate scope of the cross-coupling reaction*

Next, the attention was aimed at the use of 1*ChCl/*2*Gly* and the substrate scope for the Suzuki cross-coupling reaction was studied. Accordingly, *ortho-, meta-, para*-biaryl and arylpyridine ketones were devised and arylbromides and arylboronic acids containing fluorine groups were also used to produce fluorinated biaryl analogues. As a result, the resulting ketones were classified into three groups according to the reactivity exhibited by their precursor reagents (**Table 2**): i) fluorinated biaryl ketones (entry 5), ii) unsubstituted biaryl ketones (entry 1-4) and iii) arylpyridine ketones (entry 6).

Table 2. Substrate scope for the Suzuki cross-coupling reaction



Entry	Product	[Pd]/ligand [mol%]	T (℃)	Substrate concentration (mM)	*c* (%)
1	 **5a**	4/5	rt	40	92
2	 **5i**	4/5	rt	40	84
3	 **5j**	4/5	rt	40	63
4	 **5b**	4/5	rt	40	83
5	 **5f**	4/5	rt	40	95
6	 **5c**	4/5	rt	40	50

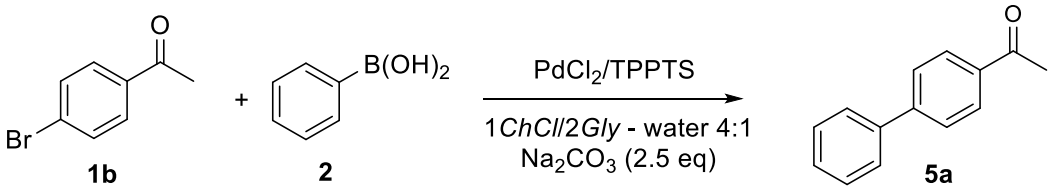
The fluorinated derivative (**5f**) reached a conversion of 95% due to high reactivity of the boronic acid bearing such electron-withdrawing groups (entry 5). On the other hand, the conversion of the pyridine-substituted biaryl ketone (**5c**) was lower than 60 % (entry 6). The nitrogen moiety again appears to have a negative impact on the conversion which was a further motivation to investigate thoroughly the process and improve the results.

2.4.1.4 *Effect of higher temperature and lower catalyst loading*

To explore the effect of parameters such as temperature and catalyst loading, the coupling between 4'-bromoacetophenone (**1b**) and phenylboronic acid (**2**) was taken as a model reaction taking place in 1*ChCl/*2*Gly*, demonstrated to be the most efficient *DES* mixture.

An increase of reaction temperature to 70°C enabled quantitative conversion towards the compound **5a** (>99%, entry 2). Remarkably, an identical result was obtained at the same temperature with a decreased catalyst loading set at only 1 mol% $PdCl_2$ and 3 mol% TPPTS (entry 3).

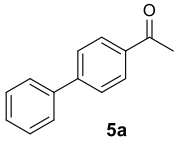
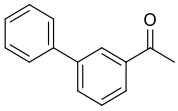
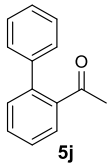
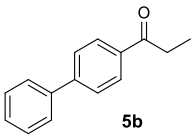
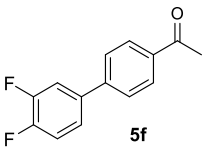
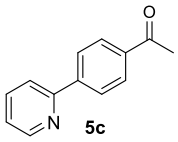
Table 3. Suzuki cross-coupling with different amounts of catalyst loading and higher reaction temperature



Entry	Product	[Pd]/ligand [mol%]	T (℃)	Substrate concentration (mM)	*c* (%)
1	1*ChCl/*2*Gly*	4/5	rt	40	92
2	1*ChCl/*2*Gly*	4/5	70	40	>99
3	1*ChCl/*2*Gly*	1/3	70	40	>99

The optimised reactions conditions based on increased temperature were extended for the preparation of the previously described biaryl ketones and the conversion dramatically improved in all cases. Likewise, the challenging pyridine compound **5c** required heating up to 100°C for a complete conversion (**Table 4**, entry 6). Since the pyridine compound had a lower tendency to be converted, these new results evidently confirmed that an increase of temperature could overcome such limitations of nitrogen-containing compounds.

Table 4. Suzuki cross-coupling reaction with reduced catalyst loading and higher reaction temperature

Entry	Product	[Pd]/ligand [mol%]	T (℃)	Substrate concentration (mM)	*c* (%)
1	 **5a**	1/3	70	40	>99
2	 **5i**	1/3	70	40	>99
3	 **5j**	1/3	70	40	>99
4	 **5b**	1/3	70	40	>99
5	 **5f**	1/3	70	40	>99
6	 **5c**	1/3	100	40	90

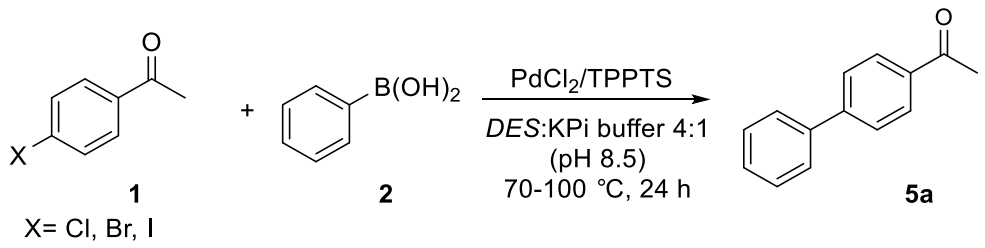
2.4.1.5 *Study of the substrate concentration*

After reaching excellent conversions in the previous study, higher substrate concentrations were investigated (**Table 5**, entries 7–11). Thus, it was found that concentrations of 100 mM or higher demanded heating to 100°C in the 1*ChCl/*2*Gly*-water mixture to reach complete conversion, with the upper limit being 200 mM. On the contrary, upon these conditions the analogue mixtures based on 1*ChCl/*1*Sorb* and 1*ChCl/*2H_2O led to poor conversions (<40%, entries 9–10) which resulted in discarding these *DES*s for further optimisation. Considering the structure of these mixtures, the unsatisfying effect caused by the use of water and sorbitol as second components could be explained by a different special structure and formation of hydrogen bonds. Physical properties such as viscosity, density and polarity, along with solubilising capacities are slightly different, a peculiarity that could present a disadvantage in their application as extraction and reaction media in this type of reactions.[79]

To ensure an effective homogeneous mixture following an increased substrate concentration the decision was made to use an aqueous phosphate buffer at fixed pH 8.5 in place of sodium carbonate. A phosphate buffer ensured an invariable pH through the cross-coupling reaction, while a rather weak Na_2CO_3 base is not strong enough to keep the pH unchanged.

Finally, the parametrisation was also extended to other aryl halides. Thus, the aryl chloride turned out to be less reactive (65% conversion, **Table 5**, entry 7) meanwhile the iodine derivative enabled complete conversion at 200 mM and 100°C (entry 8). Following the satisfactory results, the reactions with bromine and iodine reagents were essayed with a catalyst load reduced tenfold (entries 9–10). In the case of the aryl iodide the process worked efficiently (entry 9), and despite a slightly decreased conversion, the required low catalyst loading could be interesting from an economic point of view for large-scale reactions.

Table 5. Parametrisation of the Suzuki cross-coupling reaction of **1** and **2** in *DES*-buffer (4 :1) medium catalysed by $PdCl_2$/TPPTS



1 X= Cl, Br, I

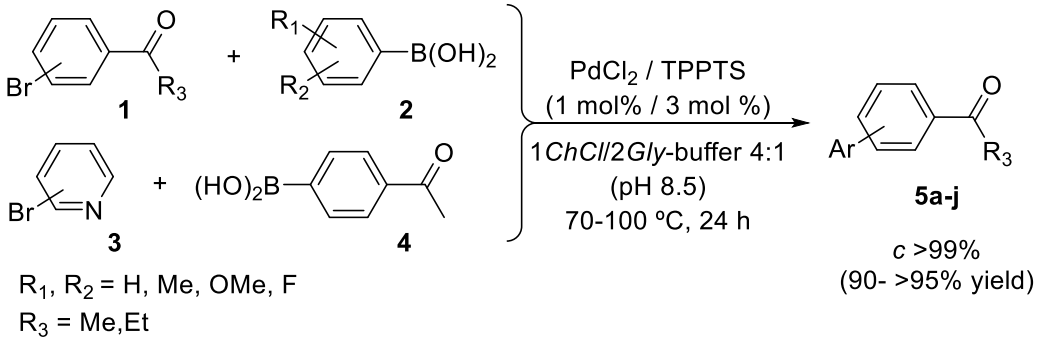
2

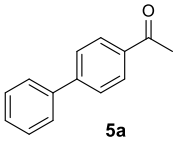
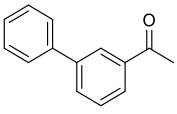
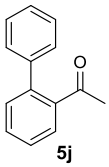
5a

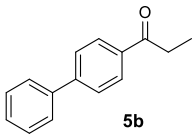
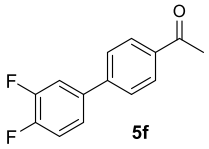
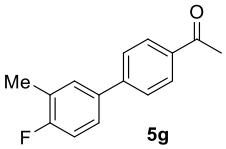
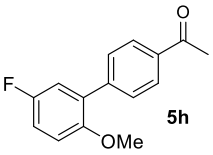
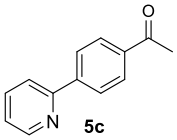
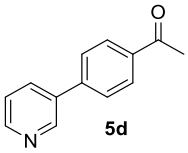
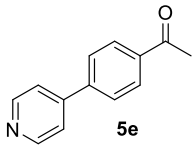
Entry	X	*DES*	[Pd]/ligand [mol%]	T (°C)	Substrate concentration (mM)	*c* (%)
1	Br	1*ChCl*/2*Gly*	1/3	70	40	>99
2	Br	1*ChCl*/2*Gly*	1/3	70	100	99
3	Br	1*ChCl*/2*Gly*	1/3	100	200	>99
4	Br	*1ChCl/1Sorb*	1/3	100	200	40
5	Br	*1ChCl/2*H_2O	1/3	100	200	35
6	Br	1*ChCl*/2*Gly*	1/3	100	300	60
7	Cl	1*ChCl*/2*Gly*	1/3	100	200	65
8	I	1*ChCl*/2*Gly*	1/3	100	200	>99
9	I	1*ChCl*/2*Gly*	0.1/0.3	100	200	92
10	Br	1*ChCl*/2*Gly*	0.1/0.3	100	200	40

As depicted in **Table 6** the substrate scope of the Suzuki coupling under optimised conditions was extended for the total of 10 biaryl and arylpyridine ketones, some of them exhibiting different patterns of substitution. Thus, a set of 10 compounds was prepared by reacting appropriate aryl bromides and arylboronic acids.

Table 6. Scope of the Suzuki cross-coupling reaction in *DES*-buffer medium under the optimised reaction conditions [200 mM substrate concentration, 1*ChCl:*2*Gly*-KPi buffer pH 8.5 (4:1), $PdCl_2$ (1 mol%), TPPTS (3 mol%), 70 °C or 100 °C, 24 h]



Entry	Product	[Pd]/ligand [mol%]	T (℃)	Substrate concentration (mM)	*c* (%)
1[a]	 5a	1/3	70	200	>99
2	 5i	1/3	70	200	>99
3	 5j	1/3	70	200	>99

Entry	Product	[Pd]/ligand [mol%]	T (°C)	Substrate concentration (mM)	*c* (%)
4	 **5b**	1/3	70	200	>99
5	 **5f**	1/3	70	200	>99
6[a]	 **5g**	1/3	70	200	>99
7	 **5h**	1/3	70	200	>99
8	 **5c**	1/3	100	200	>99
9[a]	 **5d**	1/3	100	200	>99
10	 **5e**	1/3	100	200	>99

[a] In these cases (entry 1, 6 and 9) biarylketones were synthesised at a preparative scale. After the reaction and following a simple workup (see experimental section for more information) no further column chromatography purification was required and the resulting products were isolated in high yields ranging from 90 to 95%.

The conversion to the biaryl ketones was measured by HPLC and product was quantitatively afforded in all cases. The resulting products were easily recovered from the reaction medium by adding saturated aqueous NH_4Cl followed by an extraction with cyclopentylmethyl ether. The organic phases were then combined, dried over Na_2SO_4, filtered and concentrated under vacuum providing the crude product.

2.4.2 Bioreduction of biarylketones in a mixture of *DES* and buffer for the synthesis of biaryl alcohols

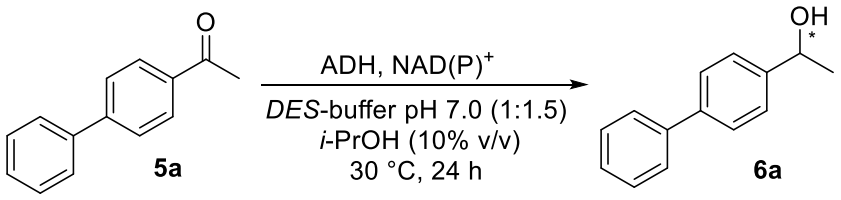
Once assessed the conditions for the Suzuki cross-coupling in the first step of the cascade the aim was directed at the enzymatic reduction process by using a Codex® commercial kit of KREDs and two ADHs overexpressed in *E. coli,* namely the *(R)*-selective ADH from *Lactobacillus kefir* DSM 20587[80] and the ADH from *Rhodococcus ruber* DSM 44541*[81]* with opposite enantioselectivity.

2.4.2.1 *Screening of alcohol dehydrogenases for the synthesis of the biaryl alcohol in a DES-buffer mixture*

The biaryl ketone **5a** was initially tested with a set of engineered KREDs from Codexis, the (*R*)-selective ADH from *L. kefir* and the (*S*)-selective ADH from *R. ruber*. In a typical experiment, **5a** (5 mM) was incubated in the presence of a KRED (200% w/w) at 30°C and 250 rpm in a mixture of 1*ChCl*/2*Gly* buffer 1: 1.5 (containing 1.25mM $MgSO_4$ and 1 mM $NADP^+$) supplemented with isopropanol (*i*-PrOH, 10% v/v) for cofactor recycling. The choice of this reaction medium composition, which contains about 35% (v/v) of *DES*, was based on the decision preserve the stability of the enzymes.

From the series of KREDs of the kit, most of the biocatalysts rendered biphenylethan-1-ol (**6a**) in quantitative conversion and perfect enantioselectivity (>99% *ee*, entries 1–17).

Table 7. ADH-catalysed reduction of 4'-acetylbiphenyl (**5a**) in *DES*-buffer medium



Entry	KRED	*c* (%)	*ee* (%)
1	P1-A04	>99	>99 (*R*)
2	P1-B02	>99	99 (*R*)
3	P1-B05	>99	>99 (*R*)
4	P1-B10	>99	>99 (*R*)
5	P1-H08	>99	98 (*R*)
6	P2-G09	>99	>99 (*S*)
7	P2-B02	>99	63 (*S*)
8	P2-C02	>99	90 (*S*)
9	P2-C11	>99	>99 (*R*)
10	P2-D03	>99	93 (*R*)
11	P2-D11	35	99 (*R*)
12	P2-D12	95	95 (*R*)
13	P2-G03	>99	>99 (*R*)
14	P2-H07	>99	>99 (*R*)
15	P3-B03	>99	>99 (*S*)
16	P1-A12	>99	>99 (*R*)
17	P3-H12	95	98 (*S*)
18	*L. kefir* DSM 20587	>99	>99 (*R*)
19	*R. ruber* DSM 44541	>99	>99 (*S*)

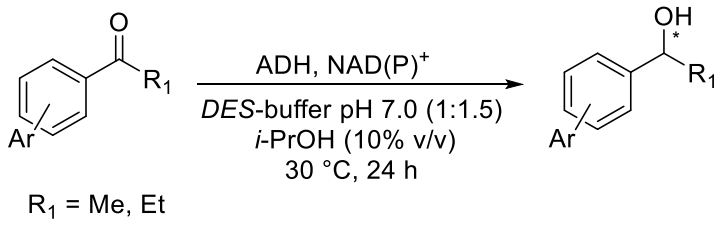
Complementary to the Codex® kit screening, the reactions with *R. ruber* and *L. kefir* (entries 18–19) were carried out in a 15 mL falcon tube and 10 U of enzyme was added to a mixture of 1*ChCl*:2*Gly* in phosphate buffer set at pH 7.0 containing 10% v/v isopropanol and the appropriate co-

factor. At the end of the reaction the mixture was treated with saturated aqueous NH_4Cl and extracted with ethyl acetate. The organic layers were then combined, filtered and concentrated under vacuum to provide the crude product with the yield in both cases higher than 95%. The conversion was measured on the achiral reverse-phase HPLC and it was demonstrated that the reaction worked efficiently in this reaction medium, forming both enantiomers of **6a** in enantiomerically pure form (>99% *ee*).

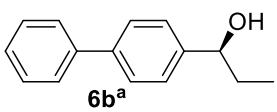
2.4.2.2 *Substrate scope of the enzymatic reduction*

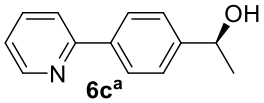
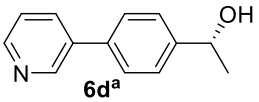
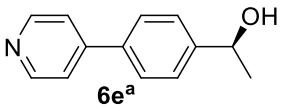
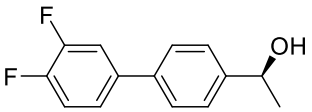
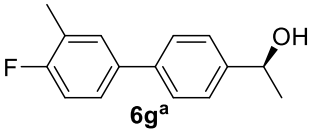
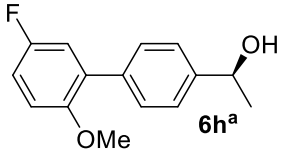
Next, on the basis of the established enzymatic conditions in **Table 7**, the reduction of remaining ketones **5b–j** was screened with the overexpressed ADHs from *L. kefir* and *R. ruber* along with four purified enzymes from the Codex® kit, in particular the *(R)*-selective KRED_P1-A04 and KRED_P2-H07 and the *(S)*-selective KRED_P2-G09 and KRED_P3-H12 (**Table 8**). The choice for these four biocatalysts was based on the results afforded in **Table 7** with **5a**, and also on the grounds of their excellent activity recently reported for the bioreduction of propiophenone in *DES*-buffer 1:1 mixtures.[25]

Table 8. ADH-catalysed reduction of biaryl ketones in *DES*-buffer medium



R_1 = Me, Et

Entry	Ketone	Enzyme	Alcohol	*c* (%)	*ee* (%)
1	**5b**	P1-A04	 **6b**[a]	>99	>99 (*R*)
2	**5b**	P2-G09		>99	>99 (*S*)
3	**5b**	P2-H07		>99	>99 (*R*)
4	**5b**	P3-H12		>99	>99 (*S*)
5	**5b**	*L. kefir*		23	n.d.

Entry	Ketone	Enzyme	Alcohol	*c* (%)	*ee* (%)
6[a]	**5b**	*R. ruber*		95	>99 (*S*)
7	**5c**	P1-A04		>99	>99 (*R*)
8	**5c**	P2-G09		>99	>99 (*S*)
9	**5c**	P2-H07	 **6c**[a]	>99	>99 (*R*)
10	**5c**	P3-H12		>99	>99 (*S*)
11	**5c**	*L. kefir*		>99	84 (*R*)
12[a]	**5c**	*R. ruber*		>99	>99 (*S*)
13	**5d**	P1-A04		>99	>99 (*R*)
14	**5d**	P2-G09		>99	92 (*S*)
15	**5d**	P2-H07	 **6d**[a]	>99	>99 (*R*)
16	**5d**	P3-H12		>99	>99 (*S*)
17[a]	**5d**	*L. kefir*		>99	>99 (*R*)
18	**5d**	*R. ruber*		>99	>99 (*S*)
19	**5e**	P1-A04		>99	>99 (*R*)
20	**5e**	P2-G09		>99	>99 (*S*)
21	**5e**	P2-H07	 **6e**[a]	>99	>99 (*R*)
22	**5e**	P3-H12		>99	>99 (*S*)
23	**5e**	*L. kefir*		>99	>99 (*R*)
24[a]	**5e**	*R. ruber*		>99	>99 (*S*)
25	**5f**	P1-A04		>99	>99 (*R*)
26	**5f**	P2-G09	 **6f**[a]	>99	67 (*S*)
27	**5f**	P2-H07		>99	>99 (*R*)
28	**5f**	P3-H12		>99	95 (*S*)
29	**5f**	*L. kefir*		>99	>99 (*R*)
30[a]	**5f**	*R. ruber*		>99	>99 (*S*)
31	**5g**	P1-A04		>99	>99 (*R*)
32	**5g**	P2-G09		>99	94 (*S*)
33	**5g**	P2-H07	 **6g**[a]	>99	>99 (*R*)
34	**5g**	P3-H12		>99	>99 (*S*)
35	**5g**	*L. kefir*		>99	>99 (*R*)
36[a]	**5g**	*R. ruber*		>99	>99 (*S*)
37	**5h**	P1-A04		>99	>99 (*R*)
38	**5h**	P2-G09		>99	>99 (*S*)
39	**5h**	P2-H07	 **6h**[a]	>99	>99 (*R*)

Entry	Ketone	Enzyme	Alcohol	*c* (%)	*ee* (%)
40	**5h**	P3-H12		>99	>99 (*S*)
41	**5h**	*L. kefir*		>99	>99 (*R*)
42[a]	**5h**	*R. ruber*		>99	>99 (*S*)
43	**5i**	P1-A04		>99	>99 (*R*)
44	**5i**	P2-G09		95	>99 (*S*)
45	**5i**	P2-H07		>99	97 (*R*)
46	**5i**	P3-H12	**6i**[a]	>99	97 (*S*)
47	**5i**	*L. kefir*		94	96 (*R*)
48[a]	**5i**	*R. ruber*		>99	>99 (*S*)
49	**5j**	P1-A04		7	n.d.
50	**5j**	P2-G09		32	n.d.
51	**5j**	P2-H07		26	>99 (*R*)
52	**5j**	P3-H12		79	16 (*S*)
53[a]	**5j**	P1-B02		>99	>99 (*S*)
54	**5j**	P1-B05	**6j**[a]	>99	>99 (*S*)
55	**5j**	*L. kefir*		0	n.d.
56	**5j**	*R. ruber*		75	>99 (*S*)

[a] These selected enantiomerically pure biaryl alcohols were synthesised at a preparative scale. At the end of the reaction the solution was quenched with NH_4Cl and extracted with ethyl acetate. The organic layers were then combined, filtered and concentrated under vacuum to provide the crude product in high yields (>95%).

All examples described that it was possible to access both enantiomers of the target alcohols after 24 hours with very high conversion (>99%) and enantioselectivity (99% *ee*). The only exception was the sterically hindered *ortho*-biaryl ketone **5j**, which led to (*R*)-**6j,** however with lower conversion compared to the other substrates. Exceptionally, the biaryl ketone **5j** was also screened with a different KRED from the kit, namely P1-B02, which was recently found to be very active towards **5j** in aqueous medium.[82] Pleasantly, this biocatalyst made it possible to obtain (*S*)-**6j** with complete conversion and enantioselectivity (**Table 8**, entry 53). Interestingly, both *L. kefir* and *R. ruber* ADHs overexpressed in *E. coli* were very efficient in terms of reactivity and selectivity towards some of the substrates (c>99%, *ee*>99%). These results make these biocatalysts especially attractive for a hypothetical gram-scale process in comparison with the commercial purified ones limited by their high cost.

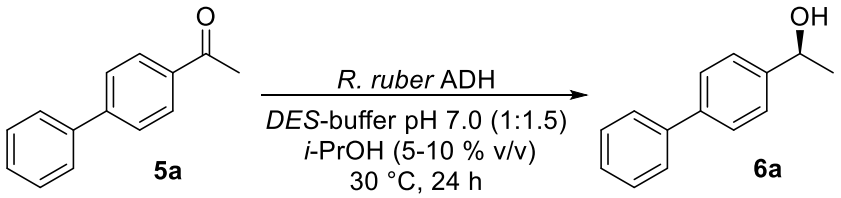
2.4.2.3 *Reducing the amount of isopropanol for the bioreduction process*

Isopropanol is critical for the bioreduction process as a co-substrate necessary for the regeneration of cofactors $NADP^+$ or NAD^+. In the previous reported cascade system the overall one-pot process consisted of a mixture of equal amounts of water and isopropanol (v/v).[23] In that particular case isopropanol did not only act as a co-substrate in the cofactor regeneration system, but also as a co-solvent in the first step used to help dissolve the hydrophobic biaryl molecules.

The current system consisting of a mixture of *DES* and water benefits from the excellent dissolving properties of the *DES*, which helps in the case of hardly water-soluble substrates, and can also be beneficial in particular in cases of high *Km* (Michaelis constant) values. In such examples it is not necessary to supplement additional amounts of isopropanol to the reaction medium.

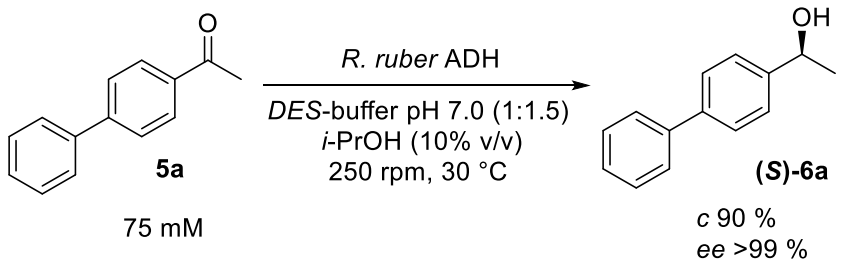
To outperform the generally suggested concentration of isopropanol in the medium, capped between 10 and 15 % v/v, the aim was to carry out the bioreduction reducing its amount to only 5 % v/v. The decrease in conversion was unfortunately observed (**Table 9**) as the medium certainly required substantial amounts of the co-substrate to push the equilibrium towards the product formation. Thus, it was concluded that isopropanol is not only necessary from a kinetic point of view, but considerable amounts are required to thermodynamically drive the reaction to completion and avoid the reduction of the formed acetone.

Table 9. ADH-catalysed reduction of 4'-acetylbiphenyl (**5a**) with a decreased amount of isopropanol



Entry	Ketone	Enzyme	*i*-PrOH (%v/v)	*Conv.* (%)
1	**5a**	*R. ruber* DSM 44541	10	>99
2	**5a**	*R. ruber* DSM 44541	5	43

2.4.2.4 *Increasing the concentration of the substrate for the bioreduction*



Scheme24. ADH-catalysed reduction of the biaryl ketone **5a** at 75 mM concentration

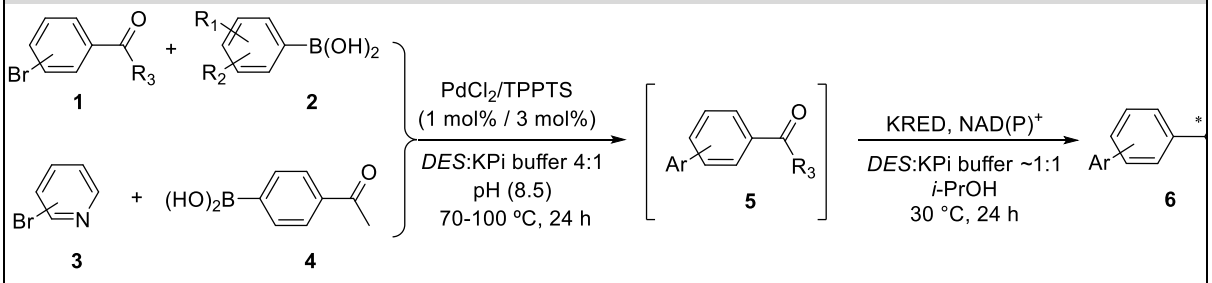
An increase of the ketone concentration in the biocatalytic step leads to preferable reaction parameters for a manufacturing setting and for that reason the biotransformation was carried out with an increased substrate loading. Excellent solubilising properties of *DESs*, used at 35% v/v, therefore ensured a homogeneous reaction mixture for the selected bioreduction with the enzyme from *R. ruber* at 75 mM substrate concentration (**Scheme 24**), enabling comparable results to those obtained in the initial screenings at 5 mM.

2.4.3 Synthesis of biaryl alcohols in a one-pot cascade process

With both catalytic steps validated and optimised in terms of reaction parameters, substrate concentration and composition of the reaction medium, the combination in a one-pot fashion with sequential steps was thoroughly monitored. At first, a Suzuki cross-coupling reaction was conducted at 200 mM substrate concentration in a *DES*-buffer 4:1 medium, followed by the *in-situ* enzymatic reduction of the transiently formed ketone with previous dilution to 75 mM with the *DES*-buffer 1:1 medium containing isopropanol, enzyme and cofactor. Accordingly, and based on a recent study about the effect of water in the nanostructure of *DES*,[83] the coupling step was considered to be accomplished in a choline chloride/glycerol/water deep eutectic solvent mixture. Respectively, the medium for the bioreduction, containing approximately 50% H_2O, should be considered an aqueous solution of *DES* components.

A selection of four target alcohols was made to show the general applicability of the process, including examples of unsubstituted (**6a**), fluorinated (**6g**) and pyridyl derivatives (**6d**, **e**). The reductions were carried out utilising the two recombinant ADHs from *L. kefir* DSM 20587 and *R. ruber* DSM 44541, overexpressed in *E. coli*, which demonstrated to be promising biocatalysts in the initial screening.

Table 10. One-pot synthesis of biaryl alcohols by palladium-catalysed Suzuki cross-coupling followed by enzymatic reduction



R_1, R_2 = H, Me, OMe, F
R_3 = Me,Et

Entry	Cross-coupling T (°C)	Enzyme	Product	Overall conversion (%)	Isolated Yield (%)	*ee* (%)	Absolute configuration
1	100	*L. kefir*	**6a**	78	70	>99	(*R*)
2	100	*R. ruber*	**6a**	86	80	>99	(*S*)
3	100	*L. kefir*	**6d**	85	80	>99	(*R*)
4	100	*R. ruber*	**6d**	90	85	>99	(*S*)
5	100	*L. kefir*	**6e**	91	86	>99	(*R*)
6	100	*R. ruber*	**6e**	92	84	>99	(*S*)
7	70	*R. ruber*	**6g**	90	83	>99	(*S*)

The bioreduction of the formed ketone intermediates generated both enantiomers of **6a** with good conversion and >99% *ee* (**Table 10**, entry 1 and 2). Similar cascades were established to produce the biaryl alcohols **6d**, **6e** and **6g**. The ADH from *L. kefir* DSM 20587 led to the formation of the corresponding *(R)*-enantiomer with >99% *ee* and conversions of up to 91% (entries 3 and 5). Meanwhile, the ADH from *R. ruber* DSM 44541 gave access to the *(S)*-counterparts with conversions over 90% (entries 4, 6 and 7).

In all cases, the workup was identical to the single-step process previously described, and although having an additional step it was simple and straightforward. Specifically, after 48 hours of overall reaction time the mixture was supplemented with saturated NH_4Cl and extracted with ethyl

acetate. The combined organic layers were dried over Na_2SO_4, filtered and concentrated under vacuum to provide the crude product. Further purification by flash chromatography (silica gel 60A˚, hexane-ethyl acetate 1: 1) provided the target biaryl alcohols in high yields (>80% with the exception of entry 1).

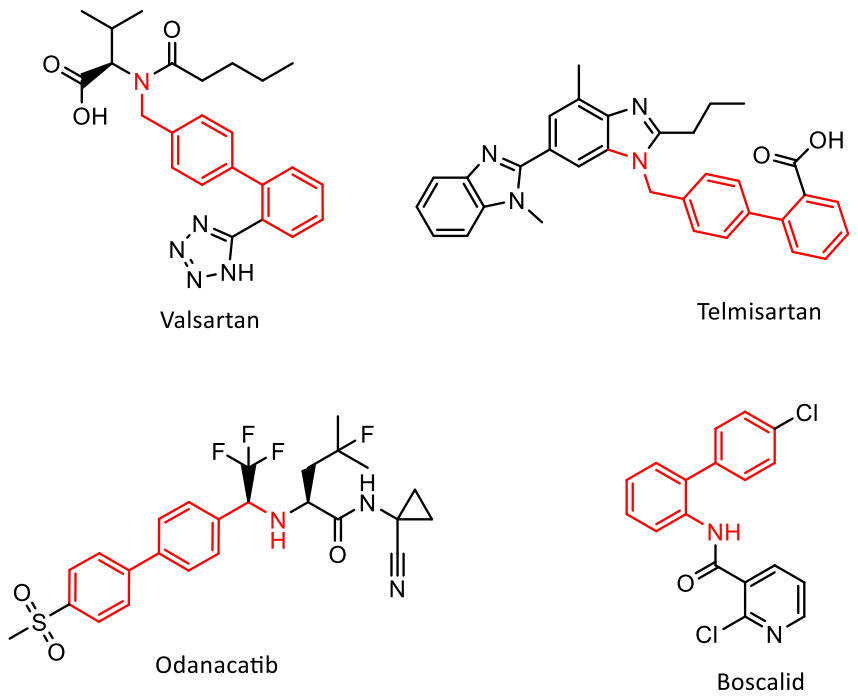
2.5 Conclusion

A chemoenzymatic cascade consisting on a palladium-catalysed Suzuki cross-coupling followed by an enzymatic reduction mediated by alcohol dehydrogenases has been efficiently implemented in *ad hoc* mixtures of *DES*s and aqueous media. The two catalytic steps took place efficiently and the excellent enantioselectivity displayed by the biocatalysts enabled the preparation of both enantiomers of several chiral biaryl alcohols in enantiomerically pure form. Likewise, the presence of the neoteric solvent in the medium enabled to tackle the solubility hurdles of substrates aiding the biotransformation to be carried out at 75 mM concentration. Furthermore, the advantages of *DESs* being utilised as a reaction media are found to be of fundamental importance contributing to excellent catalytic performances of both reaction steps of the cascade.

3 Process design for enantioselective syntheses of bulky amines based on the use of chemocatalysts and transaminases

3.1 Introduction

The construction of chiral amine structures has raised interest in the field of drug development, as the related biaryl amines are key intermediates for the synthesis of a broad range of pharmaceuticals and natural products.[84] The presence of the biaryl amine moiety can be seen in a number of biologically active compounds such as Valsartan (hypertension), Telmisartan (hypertension), Boscalid (fungicide) and Odanacatib (osteoporosis).

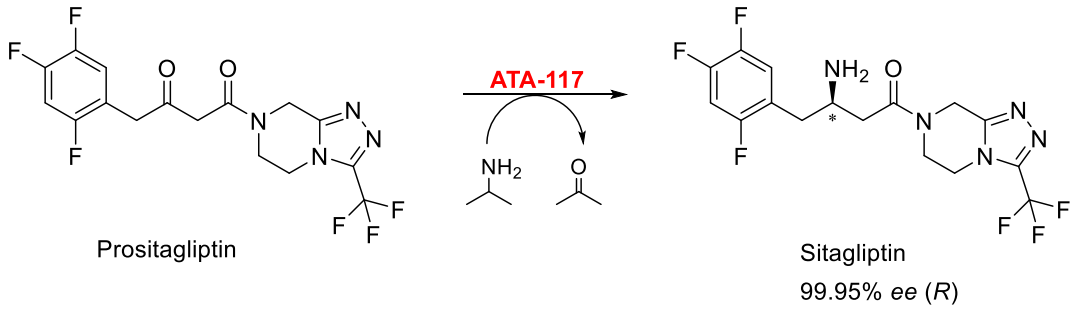


Scheme 25. Examples of drugs containing the biaryl amine moiety

The enantiomeric synthesis of chiral biaryl amine compounds can be carried out in a variety of chemical pathways which often involve the use of toxic materials, harsh reaction conditions, tedious work-ups and various purification steps. On the other hand, amine transaminases (ATAs) emerged as a highly valuable alternative catalytic “tool” for the formation of chiral amines, thus enabling novel perspectives for a green and sustainable synthetic access to this structural motif. For that reason, the biocatalytic process employing these enzymes has recently become of greater interests as an efficient method to produce compounds with the amine moiety by reversibly catalysing the transfer of an amine group from an amino donor to a ketone or aldehyde.

The active site of the majority of wild-type ATAs consists of a large and a small binding pocket and in almost all cases the small binding pocket can accommodate only a methyl group.[85] For that reason biaryl ketones are not commonly used in enzymatic transamination reactions due to their size and inability to access the small binding pocket of most transaminases. To address this challenge, recent advances in protein engineering have enabled the conversion of a variety of sterically demanding ketones such as the rationally engineered (*S*)-selective ATA from *V. fluvialis* for the synthesis of the enantiomerically pure *ortho*-substituted biaryl amine.[5]

ATAs found in the environment were at first all (*S*)-specific until the discovery of *Aspergillus terreus* (Ate) and *Arthrobacter (*Ar*)* ATAs displaying the opposite (*R*)-enantiopreference.[86] Although attractive for their enantioselectivity, the (*R*)-selective ATAs have a less versatile substrate spectrum than their counterpart and so far, only the engineered enzymes have been able to accept highly bulky ketones.[87,88] The synthetic potential of (*R*)-selective ATAs has been investigated by the groups from Codexis and Merck & Co who successfully engineered the *Arthrobacter* ATA-117 applied for the large-scale production of the antidiabetic drug Sitagliptin (**Scheme 26**).[19] This biocatalytic process is an excellent example of the implementation of biocatalysis in the pharmaceutical industry and has triggered enormous attention since its development.



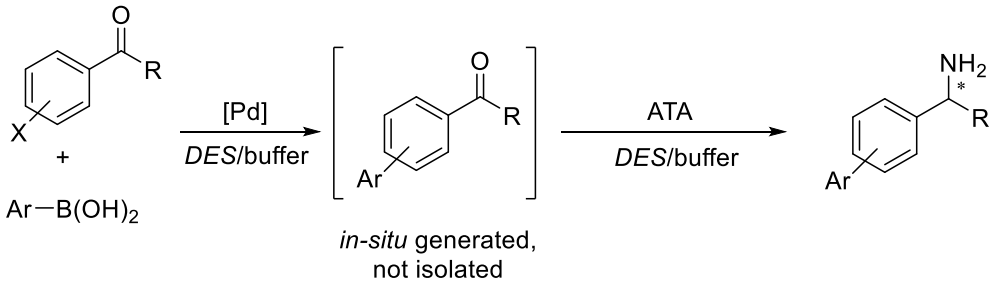
Scheme 26. Biocatalytic synthetic production of Sitagliptin reported by Savile et al[19]

The potential pharmaceutical applications for these chiral biaryl units was a motivation to search for new (*R*)-selective ATAs and expand the substrate scope within bulky prochiral substrates. Having in mind numerous advantages of biocatalytic one-pot reactions the idea was to include a reaction system consisting of a chemocatalysed synthesis of biaryl ketones followed by the biocatalytic transformation. At first, the biaryl unit could be constructed through a palladium-catalysed Suzuki-cross coupling reaction of monoaromatic ketones and boronic compounds.[89] Combining this process with the asymmetric reductive amination into a two steps reaction represents an elegant approach for the synthesis of these target biaryl amine molecules. A similar application of ATAs in a chemocatalytic process has been investigated by Bornscheuer's group for the formation of biaryl amines.[90] Taking into account the importance on the environmental impact, the use of a right solvent

system is of crucial significance. Thus, having in mind the results from the previous chapter and the significance of *Deep Eutectic Solvents*, the aim was to investigate the use of this medium in combination with amine transaminases for the synthesis of enantiomerically pure biaryl amines.

3.2 Goals and motivation

Driven by the awareness of the use of bio-renewable and non-toxic solvents the interest increased to develop a chemoenzymatic multi-step one-pot process towards biaryl-substituted amines by combining a Suzuki cross-coupling reaction with an enzymatic transamination in *DESs* (**Scheme 27**).



Scheme 27. Chemoenzymatic cascade towards chiral biaryl amines in a *DES*-buffer medium

The goal is to demonstrate the usefulness of the newly discovered ATAs in a process not very common with this class of enzymes due to their sensible nature and inefficient operational stability at high substrate concentrations, along with their incompatibility with metal catalysts and harsh conditions usually required for the cross-coupling reactions.[91] The palladium-catalysed Suzuki cross-coupling promotes a C-C bond formation between halides and boronic acids, and although originally performed in organic solvents, recent research demonstrated its applicability in aqueous systems (see chapter 2). This discovery is unquestionably beneficial for amine transaminases that function ideally in aqueous systems. Thus, the *DES*-buffer mixtures projected for this one-pot process might be well-tolerated by ATAs. Nevertheless, their activity and stability in *DESs* has yet to be determined. For the reason of being designed as a one-pot process particular attention has to be given to the reaction media to be highly compatible with both steps of the reaction.

Initially the two reactions need to be established separately to optimise the conditions suitable for both steps. The optimisation will not only include reaction parameters such as temperature and pH, but also the choice of the ideal amine donor, co-solvent and biocatalyst

formulation with the emphasis on the substrate concentration. Additionally, to access the target chiral biaryl-substituted amine molecules the biocatalysts will be improved by means of protein engineering. Thus, its effect on enzymatic stability and activity towards certain bulky substrates will be evaluated. After successfully optimising the two steps individually, both reactions will be studied in a one-pot process stabilising the settings for a concurrent process.

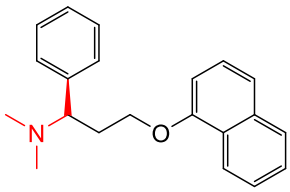
Among a few literature examples that cover biotransformations in *DES* and *DES*-buffer mixtures,[53] this is the first study that examines the application of amine transaminases in these neoteric solvents.

3.3 State of the art

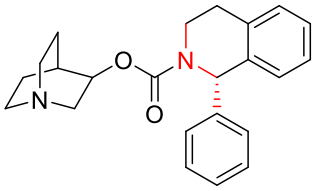
3.3.1 Biocatalysis employing transaminases

3.3.1.1 *Introduction to transaminases*

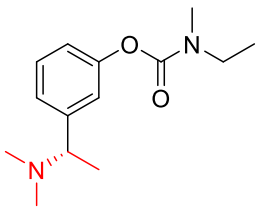
Amino acids and amines are essential for living organisms as they play a key role in the nitrogen metabolism and are involved in a variety of other important complex functions.[92] These compounds are highly important in the food industry and various pharmaceutical applications are based on the structure of optically pure amines. They can differ structurally and account for important building blocks for the synthesis of drugs like antibiotics, antidiabetics, antihypertensives and many others (**Scheme 28**).



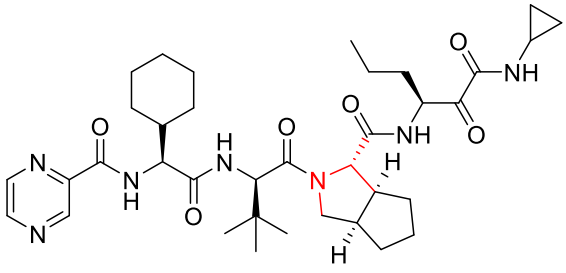
Dapoxetine



Solfenacin



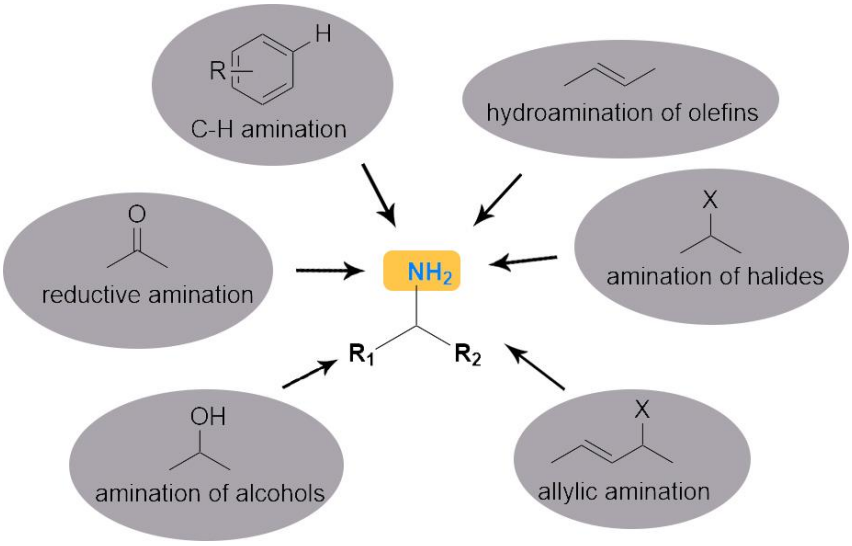
Rivastigmine



Telaprevir

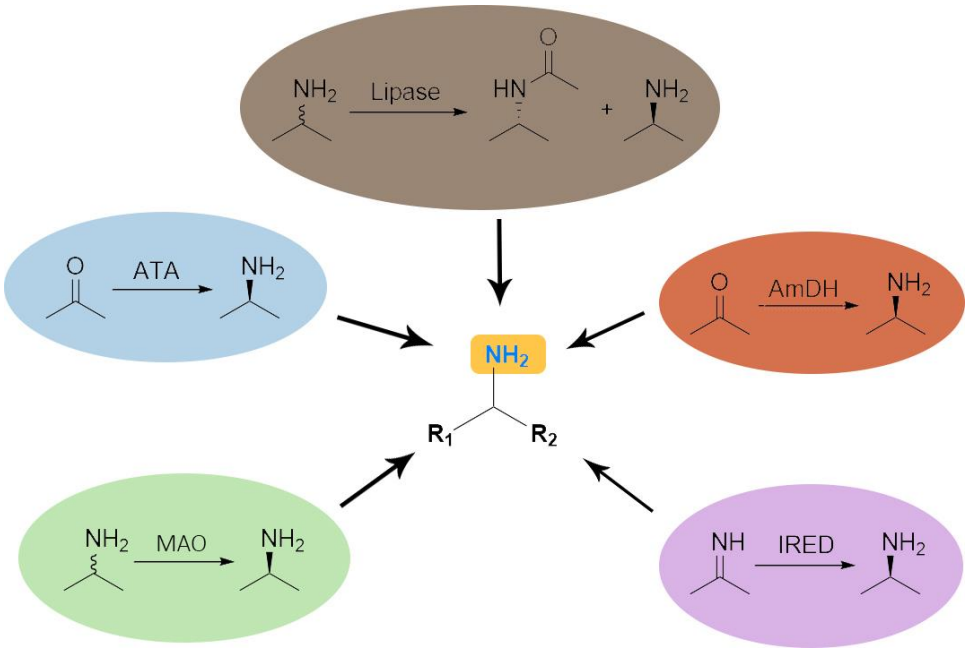
Scheme 28. Examples of pharmaceuticals containing chiral amine building blocks

Optically active amines can be prepared through a variety of classical methods (**Scheme 29**)[93] and the introduction of the amino group often results in the incorporation of its protected form, followed by subsequent deprotection steps. Numerous methodologies to access the amino group include chemical reactions such as asymmetric hydrogenation of acetamides and imines through the employment of transition metal catalysts,[94] or the asymmetric addition of nucleophiles to activated imines.[95] Chemical methods also include processes such as the asymmetric hydrogenation of imines[96] or the reductive amination of ketones.[97] Most commonly amines are prepared from alcohols by converting them into the corresponding halides or sulfonates.[98],[99] This is then followed by a nucleophilic introduction of the azide group and a subsequent reduction to yield the amine product. To introduce enantioselectivity some reactions generally include the use of chiral ligands, chiral auxiliaries or metal complexes.[100] Furthermore, chiral amines can be isolated from the racemic mixture by crystallising with diastereomeric salts of chiral carboxylic acids. Other methods for the synthesis of chiral amines include C-H amination of hydrocarbons and intramolecular hydroamination of aminoalkenes.[101]



Scheme 29. Chemical methods for the synthesis of amines

Compared to chemical processes, enzymatic methods certainly represent an elegant alternative for the synthesis of chiral amines. Many enzymes can be used for their preparation, such as transaminases (ATAs), hydrolases, amine oxidases (AO), amino acid dehydrogenases (AmDHs), imine reductases (IREDs) (**Scheme 30**).[102]



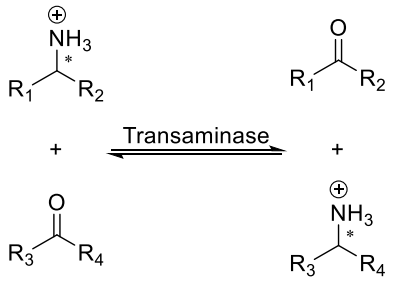
Scheme 30. Biocatalytic routes to optically active amines

Amine transaminases (EC 2.6.1.18) were first identified more than 50 years ago[103] as highly versatile biocatalysts for the synthesis of optically active amines or α-amino acids. They can be classified based on sequence similarities and a three-dimensional structure into six subgroups belonging to the fold class I and fold class IV. Another classification is based on their substrate specificity as they differ in their natural amine donor with respect to the transferred amino group. α-transaminases require the presence of a carboxylic acid group in the position to the carbonyl functionality whether ω-transaminases accept any ketone or amine and therefore are much more useful and generally applied. [102]

Today more than 60 microbial transaminases have been discovered,[104] and approximately 20 new transaminases have been recently identified mostly by means of genome mining and metagenomics.[105] The vast majority of ATAs exhibit (*S*)-selectivity with the enzyme originating from *Vibrio fluvialis* as the most intensively studied transaminase.[106] Recent technological advancements resulted in the identification of new enzymes with higher thermostability, broader substrate scope, and in particular those that exhibit (*R*)-selectivity.[88,107,108]

3.3.1.2 *Reaction mechanism*

ATAs reversibly catalyse the transfer of an amino group from the amino donor to the carbonyl moiety of an amino acceptor (**Scheme 31**).

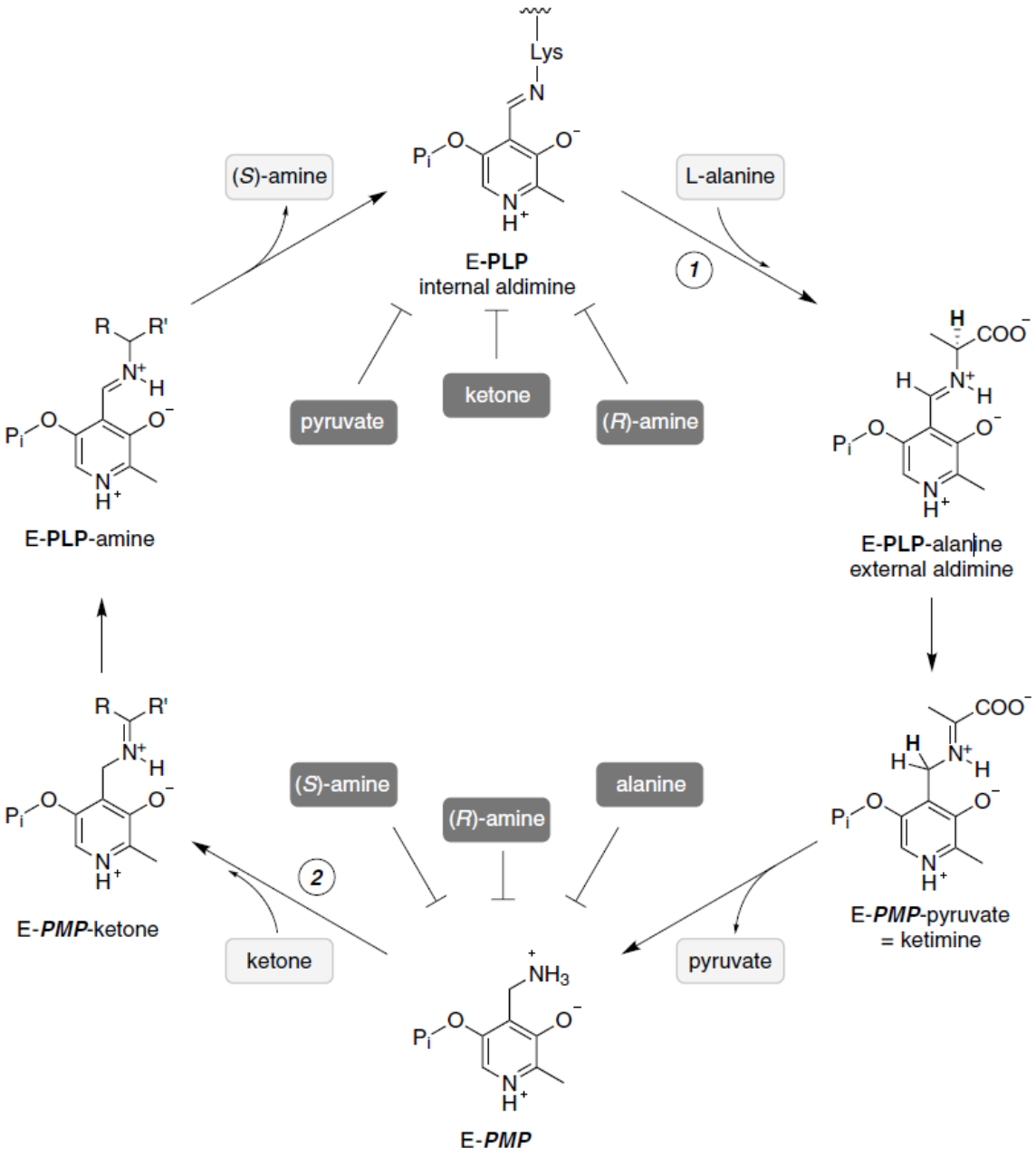


Scheme 31. ATA-catalysed transamination reaction

In 1944 It was proposed that pyridoxal might be part of a coenzyme needed for the transamination reaction and that transaminases acted via two half-way reactions that interconverted pyridoxal and pyridoxamine.[109] Soon the co-enzyme was identified as pyridoxal 5'-phospate (PLP) or pyridoxamine 5'-phosphate (PMP). Later in 1955 the vitamin B_6-based cofactor PLP was synthesised and it was found out to be an essential coenzyme for a variety of enzymes.[110]

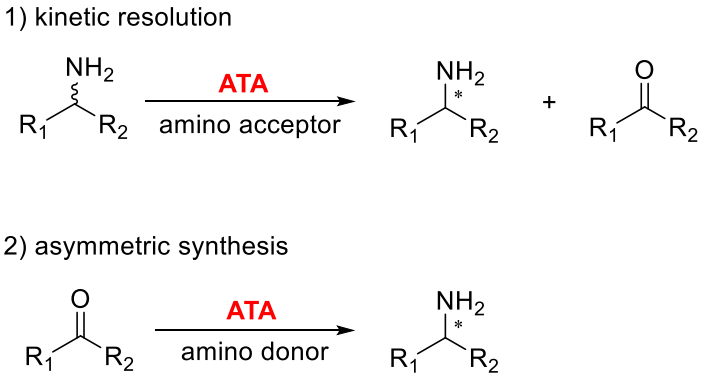
The group transfer is mediated by PLP which plays a crucial role by forming a Schiff base with the active site lysine of the enzyme and helps transfer electron and ammonia between the amine donor and amino acceptor (**Scheme 32**).[111]

The reaction mechanism initially involves the transfer of the amino group to the PLP (1), disconnection from lysine and formation of an enzyme-bound PMP intermediate with a corresponding keto by-product. The subsequent step involves the transfer of the amine group from PMP to the amino acceptor (2), producing the corresponding amine and regenerating the cofactor to its initial state for another catalytic cycle.



Scheme 32. Reaction cycle of PLP-dependant transaminases for the asymmetric synthesis of amines[69]

The production of chiral amines using ATAs can be carried out through mainly two different approaches, namely the **kinetic resolution** and **asymmetric synthesis** (**Scheme 33**). Compared to the resolution approach that yields a maximum of 50% yield, the synthetic approach is more convenient and can generate a theoretical yield of 100%.



Scheme 33. Possible enzymatic routes to optically active amines using ATAs

By means of **kinetic resolution** one enantiomer is removed from the racemate with the enantiospecific transaminase leaving the other enantiomer, which can be extracted, in enantiomerically pure form. This method strongly favours product formation when α-ketoacids such as pyruvate are used as amino acceptors. Kinetic resolution is interesting to obtain the enantiomer of the opposite configuration and since most amine-TA show (*S*)-enantiopreference, it has also been an attractive method to prepare (*R*)-amines.

One of the limitations of the applications of ATAs is the substrate and product inhibition resulting from its reaciton mechanism (**Scheme 32**). Since two forms of the free enzyme are formed (E-PLP and E-PMP) the inhibition may be caused by the binding of substrates and products to the 'incorrect' form of the enzyme (e.g. pyruvate and E-PLP) forming dead-end complexes.[112] A number of techniques have been investigated to remove the ketone from the reaction media to increase the product yield. Biphasic (aqueous/organic) reaction systems for the kinetic resolution demonstrated to be an efficient stategy for the removal of inhibiting ketones.[112] Alternatively, processes combining an enzyme-membrane reactor were developed to physically separate the enzymes from the organic solvent and simultaneously facilitate the removal of from the aqueous solution.[113] In the case of the formation of volatile ketone by-prodacts their removal could be performed using reduced pressure.[114]

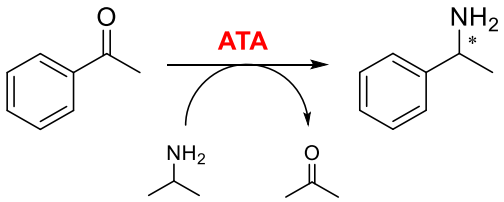
In the **asymmetric synthesis** approach the enantiomerically pure amine is synthesised by transferring the amino group from the amine donor to the prochiral ketone. This reaction has been of

great interest as it can generate 100% theoretical yield, and particularly for that reason the applicative potential of ATAs has been thoroughly studied.[115]

Along with the substrate/product inhibition and their restricted substrate scope, a major hurdle in the asymmetric synthesis of chiral amines is the unfavourable reaction equilibrium since all steps in the mechanism are reversible.[69] As a result of these obstacles, the application of ATAs in organic chemistry has certain limitations and for their use in stereoselective synthesis several efficient strategies have been developed[104] which will be discussed in the following paragraphs.

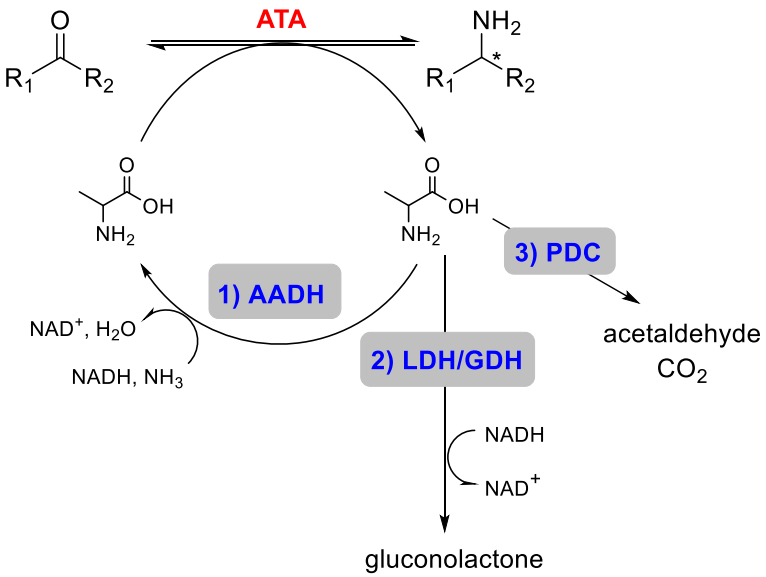
3.3.1.3 *Strategies for ATA-catalysed processes*

A conventional approach in order to push the transamination reaction towards completion is to use an excess of co-substrate. This method has been developed for the transamination of acetophenone involving large excess of the inexpensive amine donor isopropylamine (1M) for the synthesis of 1-phenylethylamine (**Scheme 34**).[116] Although simple and rapid, this approach may be limited by substrate insolubility, enzyme inhibition and often incompatibility of this amine donor with certain ATAs.



Scheme 34. ATA- catalysed transamination of acetophenone with isopropylamine amine donor[116]

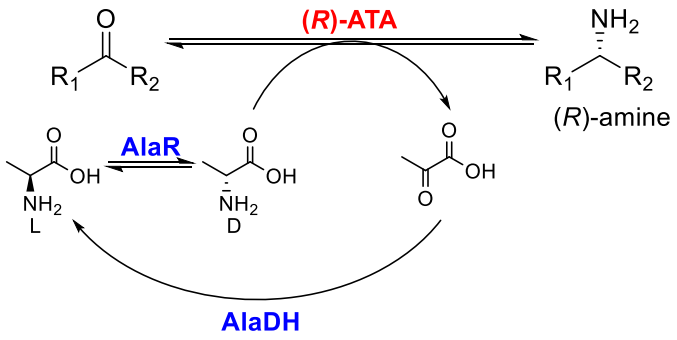
Most ATAs rely on the use of alanine as the amine donor which implies the formation of pyruvate, thus several methods have been studied to remove the formed keto-acid by-product (**Scheme 35**).[106] One of today's most widely use strategies was investigated for the first time by Kim and co-workers and involved the use of lactate dehydrogenase (LDH) for the reduction of pyruvate into lactate.[117] For an efficient process the NAD(P)H co-factor is required with the addition of a dehydrogenase enzyme such as formate dehydrogenase (FDH) or glucose dehydrogenase (GDH).[118] This thoroughly studied LDH/GDH system has been afterwards employed for the synthesis of many compounds.[115]



Scheme 35. Strategies to remove the co-product pyruvate with 1) amino acid dehydrogenase (AADH); 2) lactate dehydrogenase (LDH) in combination with glucose dehydrogenase (GDH); 3) pyruvate decarboxylase (PDC)

Pyruvate could alternatively be removed with the pyruvate decarboxylase (PCD) enzyme which does not require a co-factor recycling system and the equilibrium is shifted by the evaporation of the CO_2 formed.[119]

Another elegant strategy is to use the amino acid dehydrogenase (AADH), in particular the alanine dehydrogenase (AlaDH) to recycle the formed pyruvate back to the corresponding alanine.[120] This system requires ammonia and NAD^+ which can be easily regenerated using the previously described FDH or GDH co-factor recycling system.[121] Moreover, AlaDH was studied in the combination with alanine racemase (AlaR) as an efficient method to provide the regeneration of L-alanine and its *in-situ* racemisation to obtain the opposite D-alanine isomer (**Scheme 36**). This strategy represents an effective way to use the readily available L-alanine as a starting material for (*R*)-selective ATAs that require the far more expensive D-alanine amine donor.[120]

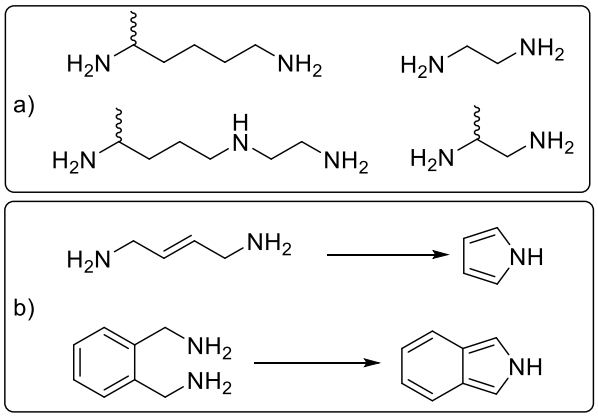


Scheme 36. Combining AlaDH and alanine racemase, the produced pyruvate is first converted into L-ala and then racemised

Furthermore, pyruvate could be removed non-enzymatically through a process that implies treatment with H_2O_2.[122] Although this strategy doesn't require any additional enzyme, it is restricted by the low stability of enzymes in the presence of hydrogen peroxide.

From an industrial point of view having isopropylamine as a low-cost amine donor is more desirable compared to alanine. This is an easily obtainable and cost-efficient chemical which is transformed into acetone during the transamination reaction. Moreover, acetone is easily removable under reduced pressure but can be also effectively *in-situ* removed by combining the process with alcohol dehydrogenases (ADH) to produce isopropanol.[123]

An alternative method to the equilibrium shifting process is the use of ''smart'' co-substrates which spontaneously transform into a stable by-product with the result of pushing the equilibrium towards the products side. For this purpose, several diamine co-substrates are used to spontaneously cyclize or dimerize in a way to prevent the reverse reaction from occurring (**Scheme 37**).[124]



Scheme 37. **a)** various different amine donor can be used to shift the reaction equilibrium. **b)** diamines such as but-2-ene-1,4-diamine and *o*-xylylenediamine can be used to displace the equilibrium by cyclisation

The use of whole cells or co-expression of multiple enzymes for the asymmetric synthesis of amines is an approach that might overcome most of the drawbacks presented by the previously mentioned methods including high costs of enzyme production and addition of high amounts of biomass in reaction mixtures.

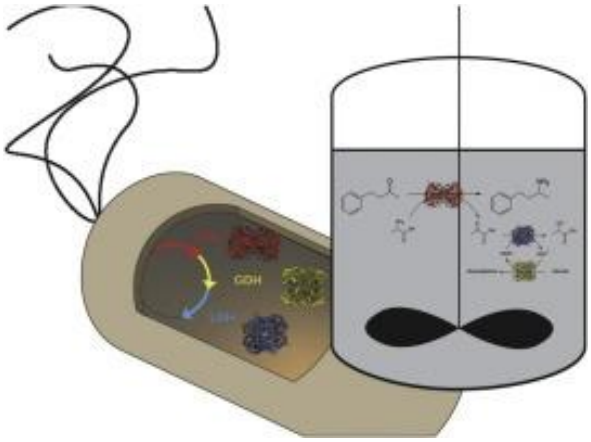


Figure 1. Illustration of a whole-cell system that co-expresses three enzymes for the amination of 4-phenyl-2-butanone[125]

An organism that co-expresses transaminases and enzymes for the pyruvate removal and co-factor regeneration is a promising strategy for that purpose.[126] In certain cases the whole-cell biocatalysis improves the stability of ATAs as it was described in the study of *Vibrio fluvialis*-ATA whose chemical tolerance was enhanced with the multi-enzyme system.[127] Very recently a group of researchers from the TU Graz co-expressed a system for the production of ATAs, GDH and LDH (**Figure 1**).[125] They demonstrated the use of *E. coli* cell-free extract for the amination of 4-phenyl-2-butanone achieving high yields and reaching high substrate loadings, comparable to industrially relevant conditions.

3.3.2 Improving ATAs performance

To expand the practical and economical application of ATAs in organic synthesis their stability issues and non-physiological conditions can be improved. Recent works on ATAs include the development of strategies to improve the thermostability, tolerance towards organic solvents and chemicals and storage stability.[104] This section focuses on different approaches that have been used to optimise the performance of ATAs.

3.3.2.1 *Generation and discovery of new enzymes*

The generation of novel biocatalysts relies on practically two strategies, namely the gene mining to identify new enzymes and protein engineering to mutate the wild-type enzymes.

Isolation of wild-type enzymes and identification in sequence databases is often a tedious and time-consuming strategy but it is advantageous due to the enormous supply of sequences and genomes in public databases. Screening of ATAs can be classified into **classical** and **computational methods**. Using classical methods a high number of microorganisms were identified containing ATA.[128] This strategy allowed for the identification of ATAs in organisms such as *Vibrio fluvialis JS17, Bacillus thuringiensis JS64, Anthrobacter sp. KNK 168, Pseudomonas sp. NCIMB 11753* and many others. With computational methods a vast number of genes have been deposited in databases which in turn allowed to identify novel enzymes such as the *Chromobacterium violaceum* and many (*R*)-selective transaminases.[85]

The *in-silico* strategies complemented with the traditional approach for identifying enzymes through tests on microorganisms positively contributed to the discovery of enzymes with higher activity, desired enantiopreference and higher stability. Recently thermostable ATAs have been identified with operational temperatures over 60°C.[129] With the help of bioinformatic research new enzymes with a broader substrate spectrum were discovered. Many of these novel enzymes showed good activity towards isopropylamine and provided access to different chiral amines and α-amino acids.[130],[131],[108]

An increasing number of crystal structures and characterization of available ATAs made it possible to structure-orientate engineering studies with the aim to obtain improved transaminases.[104] Thus, having an improved understanding of the enzyme's substrate binding allowed researchers to engineer a *Vibrio fluvialis* mutant able to convert bulky-bulky ketones through mutations of the small pocket of the active site.[132]

3.3.2.2 *Biocatalyst formulation*

Immobilization is a strategy often used to improve enzyme properties and facilitate their implementation at a larger scale. It is one of the most vastly used methods to increase their efficiency by improving their stability and recyclability, allowing for their employment in different solvents systems, at high pH and temperature and often increased substrate concentration.[133] The large-scale application of commercialized enzymes is often restricted by the use of expensive carrier materials, hence the need for effective and economical immobilization methods. Immobilization might also positively affect functional properties of enzymes as it was often demonstrated that the enantioselectivity of different enzymes could be improved.[3]

Immobilization techniques could reduce problems that transaminase encounter such as the product and substrate inhibition as a result of their reaction mechanism, or inactivation caused by

various components of the reaction medium. Early attempts of transamination immobilization include the encapsulation of both commercially available enzymes as well as cell-free extracts in sol-gel matrix or chitosan beads.[134] These strategies allowed the catalyst to be reused, resulting in a lower effective enzyme loading. Furthermore, these methods showed activity even at pH 11 and no loss of conversion or enantioselectivity was observed. Additionally enzymes often retained their initial activity even after prolonged storage periods, which could be associated with the conformal stabilization of the enzyme following the immobilization.[134,135] Beside the use of immobilized enzymes in aqueous reaction systems, these formulations were active and stable in organic solvents. This study reported a successful immobilization of transaminases on a hydrophobic SEPABEADS EXE120 support which allowed for multiple rounds of enzyme use without any significant loss of activity.[136] This immobilization method was recently studied with the transaminase from *Halomonas elongata* in a continuous flow-biocatalysis setup.[137]

Applications of immobilized (*S*) and (*R*)-selective transaminases enabled high conversions in batch, but also in continuous flow processes. For that purpose ATAs were often immobilized on cellulose matrix,[138] polymeric resins,[139] methacrylated beads,[140] and MnO_2 nanorods after being fused to an elastin-like polypeptide.[141]

An important aspect of enzyme immobilization is often their loss of activity, probably due to their structural change that leads to irreversible denaturation.[142] In order to be effective, the procedure of immobilisation should be fast, scalable and reproducible whilst maintaining the enzymatic activity. These supports should be mechanically stable, readily available and cost-effective in order to bring the process to industrial biocatalysis.

Lyophilisation is often an option employed when transamination reactions are carried out in organic solvent. This fairly straightforward strategy involves the removal of water molecules followed by rapid freezing which allows the protein structure to maintain its conformation and prevents denaturation. This is a stable biocatalyst formulation that often permits longer storage.

An alternative and certainly the most preferred biocatalyst formulation is the enzyme in its purified form. However, purification methods are often expensive and time consuming. Alternatively cell-free extracts (CFE) often show comparable activity to the purified form and are the most widely used formulation on laboratory scale due to its ease of production and low cost.[104]

3.3.2.3 *Medium engineering*

Enzymes generally exhibit lower activity in organic solvents and a lot of effort has been put into the discovery of enzymes that are naturally stable in that reaction medium.[143] One option to achieve higher stability in organic solvents is to increase their activity by means of protein engineering. Additionally, applying reaction medium engineering is another possible strategy addressed to solve stability issues of transaminases as having a suitable and well-tolerated solvent is certainly an attractive advantage to improve their application for diverse chemical processes.

Certain solvents have been reported to have positive effects on enzymatic half-life.[144] The most studied were the additions of glycerol, methanol and DMSO and it was determined that they improved storage abilities of *Chromobacterium violaceum* ATA.[145]

The synthesis of chiral amines often involves the presence of highly apolar compounds, such as ketones or aromatic compounds and since biotransformations are typically carried out in aqueous systems they generally suffer from the poor solubility of reagents and products in water. To circumvent this problem organic solvents can be supplemented as co-solvents sometimes with the expense of enzyme stability generally limited in these media. For that reason, water-miscible co-solvents are often used to increase the solubility of rather insoluble substrates such as bulky ketones or aromatic compounds.

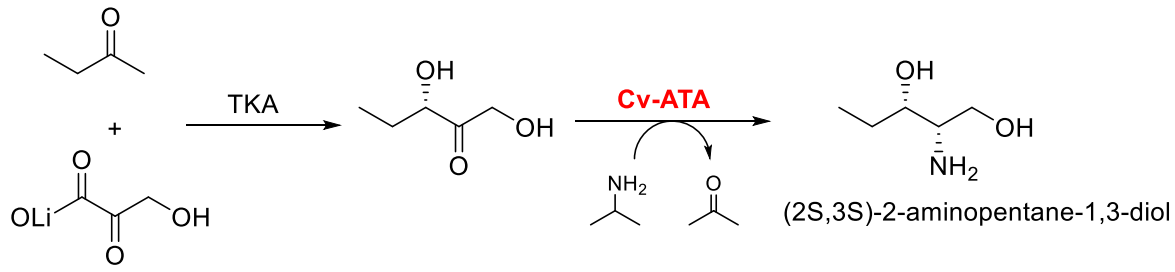
Nonetheless, certain transaminases employed as crude enzyme preparations also worked in organic media without the need of being immobilized.[146] Nine transaminases accepted isopropylamine as amine donor and worked effectively in *tert*-butyl ether (MTBE). In that study the biocatalytic transformation in organic media showed advantages such as higher reaction rates (up to 17-fold), less tedious work-up procedure (i.e. no basification and extraction required), possibility to recycle the enzyme and lack of substrate inhibition.

Efficient medium engineering evaluates the effect of co-solvents on the enzyme's reaction rate as well as stability. In 2010 the group of N. J. Turner developed a convenient process development strategy as they evaluated various co-solvents and their effect on the transamination reaction. [147] Their effect was thoroughly studied with (*R*)- and (*S*)-stereospecific ATAs and it was demonstrated that the enzymes achieved higher activities with little amounts of DMSO (2.5-5% v/v) compared to the reaction with only buffer as the reaction medium.[148] The effect of various water-soluble and immiscible co-solvents was tested in the transamination reaction of 4-phenyl-2-butanone.[149] The presence of 15% of solvents such as DMSO and methanol significantly decreased the conversion, although the enantiomeric excess remained unchanged. However, low tolerance of wild-type enzymes

towards co-solvents was observed, a flaw which could be additionally improved by means of protein engineering.

3.3.2.4 *Application of ATAs in bio- and chemoenzymatic cascade reactions*

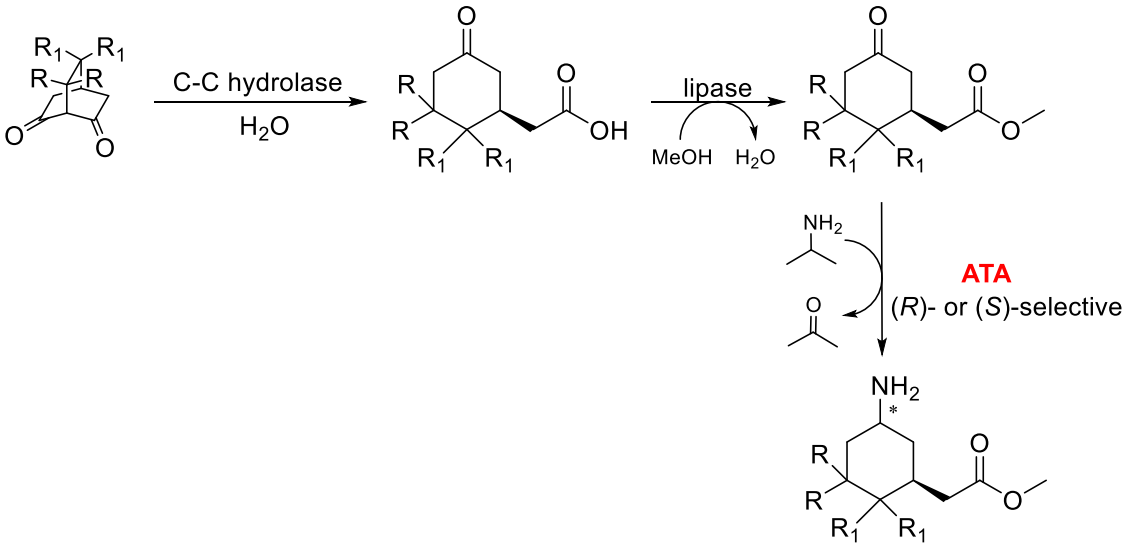
Having many properties in common with other enzymes ATAs often being employed in multiple-enzyme cascade reactions and combined with other biocatalysts. For that purpose ATAs are often present in associations with other enzymes such as lyases, transketolases and hydrolases. In particular, Smith et al describe an efficient biocatalytic route to chiral amino alcohols consisting of the combination of a transketolase with the transaminase from *Chromobacterium violaceum* for the synthesis of this important class of compounds (**Scheme 38**).[150]



Scheme 38. Two-step biocatalytic synthesis of 2-amino-1,3-diols using transketolase (TKA) and amine transaminase from *Chromobacterium violaceum*

As described in section 3.3.1.1 amines are frequently synthesised from alcohols. Similarly, an artificial multienzyme network approach has been designed in the asymmetric amination of secondary alcohols to the corresponding α-chiral primary amines through the use of a multistep enzymatic system consisting of alcohol dehydrogenases (ADHs) and ATAs.[151]

Another prominent example of the combination of biocatalysts is the synthesis of 3'-substituted cyclohexylamine derivatives (**Scheme 39**).[152] This asymmetric three-steps cascade starts with the formation of chiral centres with a C-C 6-oxocamphor hydrolase, followed by the esterification catalysed by a CAL-B lipase. Finally, the ketone is transformed into the corresponding amine using an (*R*)- or (*S*)-selective amine transaminase to obtain *cis*- and *trans*- diastereomers in optically pure forms.

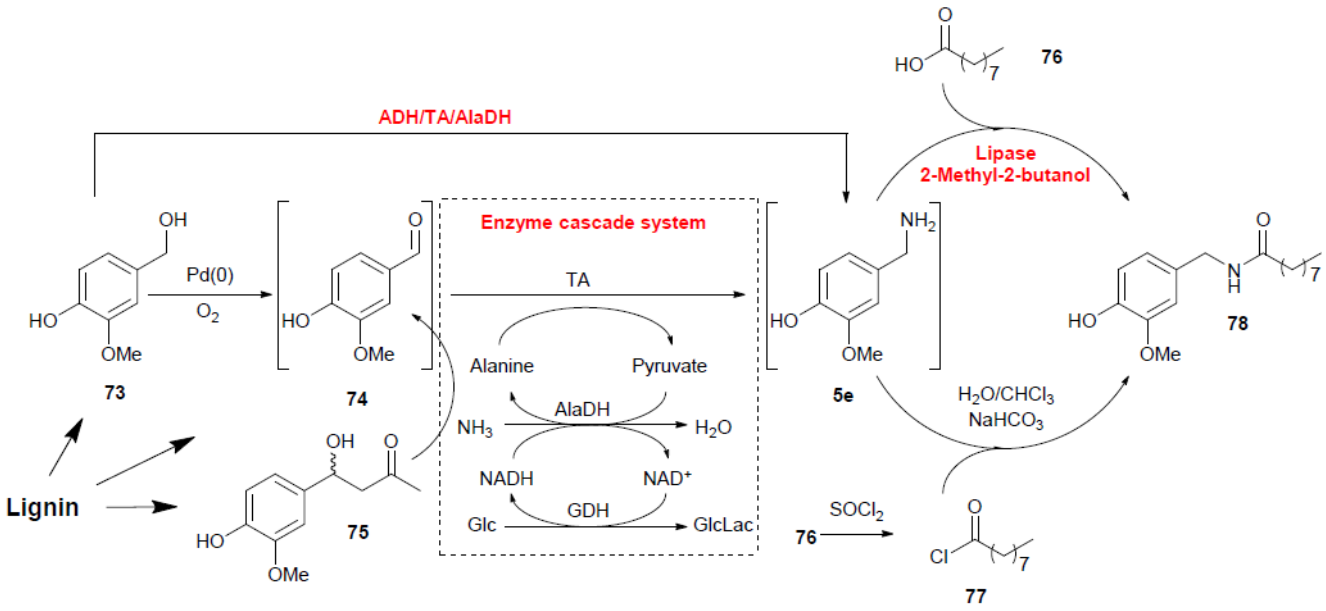


Scheme 39. Diastereoselective synthesis of 2-(3-aminocyclohexyl)acetic acid methyl ester derivatives via a three-step cascade process employing a C-C hydrolase OCH, lipase CALB and an amine transaminase

Apart from the ability to produce optically pure amine compounds, ATAs have been effectively used to obtain highly-valuable non-natural amino acids. For that purpose an ATA from *Paracoccus denitrificans PD1222* has been successfully coupled with threonine deaminase in a one-pot system to obtain L-homoalanine.[153] Furthermore, a one-pot two-step cascade consisting of a kinetic resolution and subsequent stereoselective amination catalysed by two enantiocomplementary ATAs was also described. The resulting amines were obtained with up to quantitative conversion and excellent enantioselectivities giving optically pure pharmacologically relevant amines.[154] The combination of ATAs and deaminases was also described in a study in 2008 as a multienzymatic process for the synthesis of a CGRP receptors antagonist was developed. The synthetic route consisted of an amino acid deaminase followed by an (*R*)-selective transaminase to prepare the key intermediate needed for the synthesis of the target drug.

ATA-catalysed reactions have been successfully integrated with various chemical transformations resulting in a successful combination of chemoenzymatic processes for the synthesis of beneficial compounds. Beside for the already mentioned synthesis of Sitagliptin, ATAs have been successfully applied for the preparation of Rivastigmine, a drug for the treatment of Alzheimer's disease.[155] This highly stereoselective chemoenzymatic process was developed via a four step procedure using the enzyme from *Vibrio fluvialis* for the synthesis of (*S*)-Rivastigmine with an overall isolated yield of 71 %, whereas (*R*)-enantiomer was obtained with complementary ATA-117.

More recently, an excellent example of a one-pot chemoenzymatic cascade was described for the total synthesis of capsaicinoids (**Scheme 40**).[156] The overall environmentally friendly process consisted of a few possible routes starting from lignin-derived compounds, involved different catalytic steps and described the compatibility of biocatalysts with heterogeneous metal catalysts. The process consisted of three steps, namely the production of vanillin (74), the formation of the aminated compound (5e) and the production of amide (78), with the possibility to achieve each of them by either chemo- or bio-catalysis. For this synthesis of Capsaicin analogues, the steps were combined in a one-pot fashion without any protection and purification needed, which resulted in a more efficient, sustainable and economical route.



Scheme 40. Total synthesis of Capsaicin analogues describing the compatibility of chemoenzymatic methods[156]

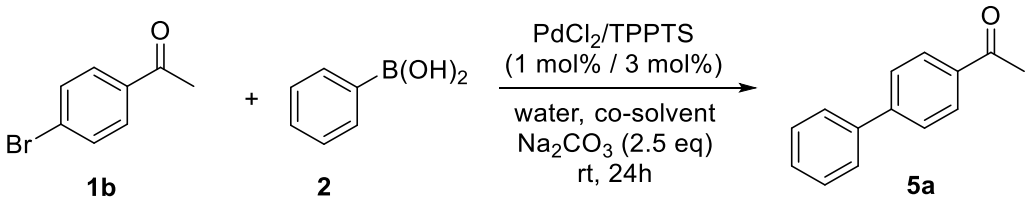
ATAs have been successfully applied in the chemoenzymatic synthetic route towards the preparation of Janus kinase 2 (JAK2) inhibitor AZD1480 to construct the key chiral amine using a two-phase reaction mixture to avoid enzyme inhibition. Similar chemoenzymatic processes have been developed for a number of pharmaceutically relevant compounds, intermediates and APIs. For instance the preparation of the drug to treat insomnia was prepared in a nine-steps three-enzyme system process using ATAs to prepare chiral lactams from prochiral ketones.[157]

These examples highlight the applicability of amine transaminases for the preparation of amines on both laboratory and industrial scale. Moreover, recent technological advancements in the fields of enzyme discovery, protein engineering and process optimisations allowed biocatalysis to prevail as a promising strategy in organic synthesis for an efficient production of valuable pharmaceuticals.

Results and discussion

The previous chapter described the synthesis of biaryl alcohols by means of a Suzuki cross-coupling and subsequent bioreduction catalysed by KREDs of the transiently formed ketones. In regard to the process development of that research a similar pattern takes place for the multi-step synthesis of biaryl amines. Referring to the previous project the use of *DESs* allowed to tackle the solubility hurdles and reach concentrations of 200 mM for the coupling step and 75 mM for the later bioreduction. Having that in mind, it is necessary to evaluate the stability of transaminases in these solvents, and above all to find a reaction mixture suitable for both steps of the cascade. After optimising the first step of the cascade, the focus is switched to the biotransformation process with the goal to select the appropriate transaminases and the efficient amine donor system. Finally, both processes are combined in a one-pot multistep sequence for the synthesis of bulky amines.

3.3.3 Development of the Suzuki cross-coupling reaction in a mixture of *DES* and buffer for the synthesis of biarylamines

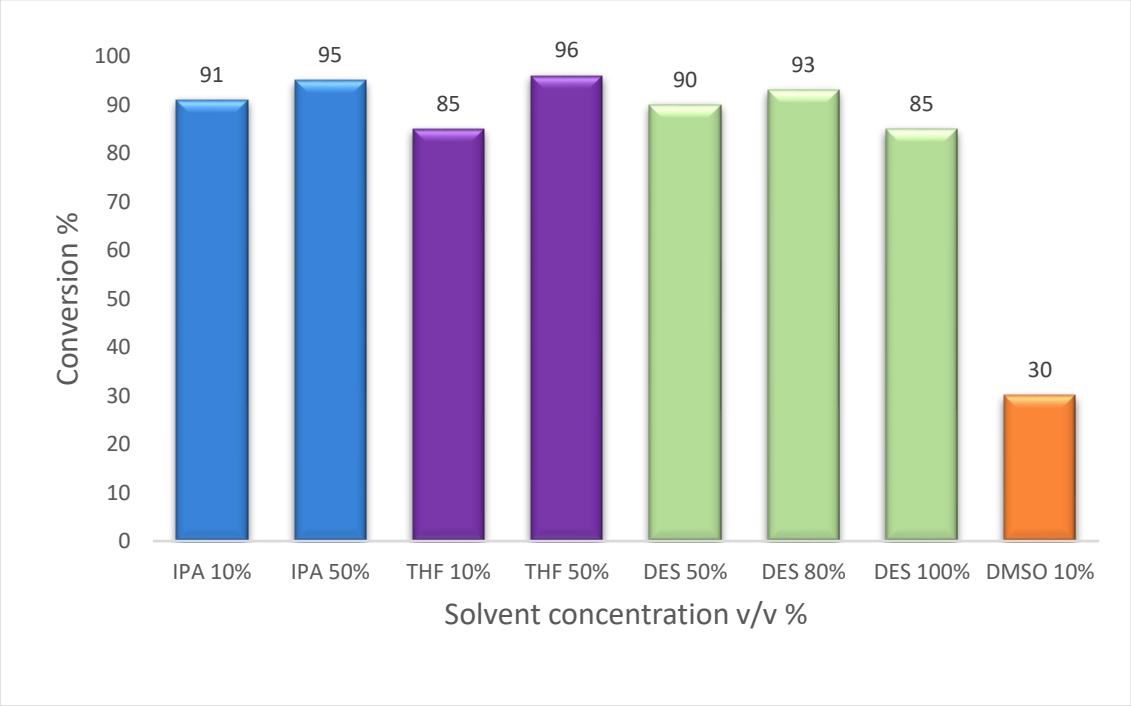


Scheme 41. Suzuki cross-coupling reaction of 4'-bromoacetophenone (**1b**) and phenylboronic acid (**2**)

Taking into account the excellent properties of the 1*ChCl*/2*Gly DES* mixture based on the previous research from **chapter 2**, the same composition was tested along with other water-miscible co-solvents in order to find the reaction media suitable for both steps of the cascade. For this, equimolar amounts of 4'-bromoacetophenone (**1b**) and phenylboronic acid (**2**) were added at 40 mM in a mixture of water and the individual co-solvent at room temperature (**Scheme 41**). As depicted in **Scheme 42**, the measured conversions towards the compound **5a** were very high (≥90%) at 50% of *i*-PrOH, THF and 1*ChCl*/2*Gly* respectively, whereas the reaction did not work at all at 50% of DMSO. When the concentration of these co-solvents was lowered to 10 % v/v, there was no improvement in the formation of the biaryl ketone. On the contrary, 10 % v/v co-solvent concentration seemed to generate lower results, probably caused by inefficient solubility of the rather apolar substrate **1b** at

such a high-water content. The reaction with 10 % DMSO slightly improved compared to higher amounts of this co-solvent. A rather low 30 % conversion in that case was by all means too low to progress with the choice of this co-solvent.

Based on the knowledge gained in the previous chapters 1*ChCl*/2*Gly* turned up to be a suitable candidate for the one-pot process. Furthermore, the first step of the process could be effectively accomplished up to 200 mM substrate concentration in a *DES*-water 4:1 mixture at temperatures ranging from 70 to 100 °C (see **chapter 2**). Nonetheless, it will still be necessary to investigate the feasibility of the transamination step in *Deep Eutectic Solvents* along with the above mentioned *i*-PrOH and THF for their use as co-solvents in the one-pot cascade process.



Scheme 42. Effect of co-solvent on the Suzuki cross-coupling reaction for the synthesis of 4'-acetylbiphenyl **5a**

3.3.4 Transamination reactions employing ω-transaminases

In the joint project with Aline Telzerow from the University of TU Graz, using data mining, six new amine transaminases cloned from different organisms were available and supplied.[158] Five of these available ATAs were demonstrated to be (*R*)-selective and will be studied in the enzymatic transamination reaction of ketones and bulky substrates (**Table 11**).

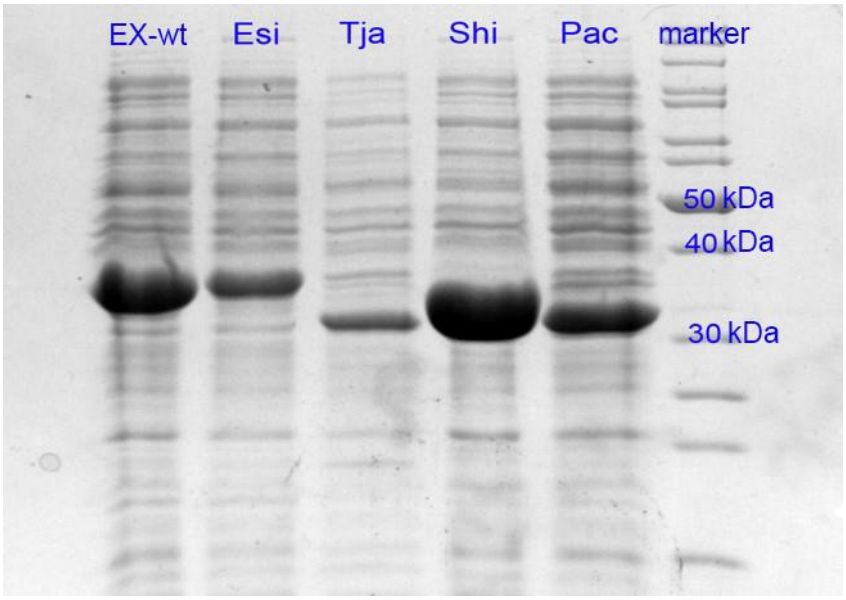


Figure 2. SDS-PAGE of the crude extracts of five new (*R*)-selective ATAs

Table 11. The enzymatic volumetric activity of the new (*R*)-selective ATAs identified by Aline Telzerow[158]

Entry	Enzyme	Organism of origin	Activity (U/ml lysate)
1	EX-wt	*Exophiala xenobiotica*	16.9
2	Esi	*Exophiala sideris*	6.42
3	Pac	*Pseudonocardia acaciae*	4.2
4	Shi	*Shinella*	13.21
5	Tja	*Tetrasphaera japonica*	3.04

The amount of overexpression of the five (*R*)-selective ATAs was studied *via* the SDS-PAGE analysis (**Figure 2**). Furthermore, the acetophenone assay was used to measure the enzymatic performance and confirmed highest activities in EX-wt and Shi amine transaminases (**Table 11**).

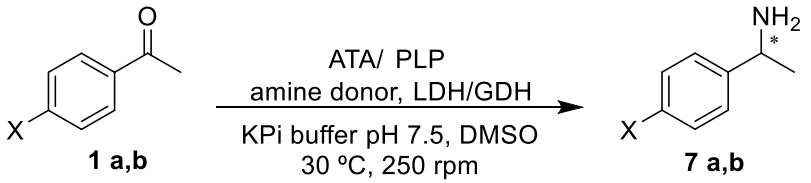
Furthermore, the industrial partner DSM provided four amine transaminases, namely the ATAs from *Vibrio fluvialis* (Vf-ATA)[159], *Chromobacterium violaceum* (Cv-ATA)[160], *Aspergillus terreus* (Ate-ATA)[161] and its mutated variant Ate-ATA_T274S (unpublished) (**Table 12**).

Table 12. The enzymatic volumetric activity of the ATAs from DSM

Entry	Enzyme	Activity (U/ml lysate)
1	Vf-ATA	51.8
2	Cv-ATA	42.3
3	Ate-ATA	28.2
4	Ate-ATA_T274S	44.4

The initial screening strategy involved the selection of the preferred amine donor for the amination of simple monoaryl ketones (**1a** and **1b**). The ATAs provided from DSM showed great conversion ranging from 68 -99 % towards the selected ketones with excellent enantioselectivity (**Table 13**). The course of the reaction could be easily followed by thin-layer chromatography (TLC) analysis (chloroform/methanol/acetic acid, 12:2:1) as the amines had an evidently lower Rf (~0.3) compared to the ketone starting material (~0.9). Simple staining with ninhydrin was sufficient to visualise the obtained aromatic amines as distinct purple spots.

Table 13. Enzymatic asymmetric amination of mono aryl ketones using ATAs from DSM



X = H (**1a**), Br (**1b**)

Entry	Substrate	Amine donor	Enzyme	c (%)	*ee* (%)
1	**1a**	L-alanine	Vf-ATA	>99	>99 (*S*)
2	**1b**	L-alanine	Vf-ATA	90	>99 (*S*)
3	**1a**	L-alanine	Cv-ATA	90	>99 (*S*)
4	**1b**	L-alanine	Cv-ATA	68	>99 (*S*)
5	**1a**	D-alanine	Ate-ATA	98	>99 (*R*)
6	**1b**	D-alanine	Ate-ATA	93	>99 (*R*)
7	**1a**	D-alanine	Ate-ATA_T274S	>99	>99 (*R*)
8	**1b**	D-alanine	Ate-ATA_T274S	95	>99 (*R*)

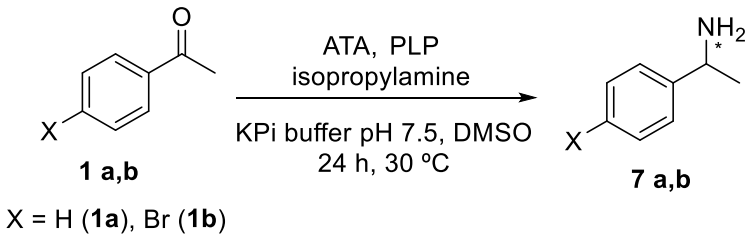
The selection of the appropriate amine donor is a crucial parameter, and although the use of isopropylamine seems a good alternative from the economical perspective, its use is restricted only to certain ATAs. For that reason, the biotransformation is often achieved through the use of other amine donors such as L-alanine. Although being fairly cheap and readily available, the use of L-alanine is limited to (*S*)-selective transaminases, while the (*R*)-selective ATAs demand for the more expensive D-alanine enantiomer. For that reason, the choice of a suitable amine donor poses a challenging and necessary task.

As Ate-TA and ATA_T274S are known to have no trouble accepting isopropylamine as the amine donor, the favoured amine donor for the five recently identified ATAs had yet to be identified. For that purpose, the biotransformation was tested under the simplest reaction conditions comparing the performance using isopropylamine and alanine as the amine donors.

The obtained results displayed very low conversion in most cases with isopropylamine and certain enzymes did not accept this amine donor at all (**Table 14**). Contrarily, D-alanine was revealed

to be the amine donor of choice for the five recently discovered transaminases as a considerably higher conversion was achieved with all five enzymes to obtain the monoaryl amines **7a** and **7b** (**Table 15**).

Table 14. Enzymatic asymmetric amination of monoaromatic ketones using new (*R*)-selective ATAs with isopropylamine amine donor

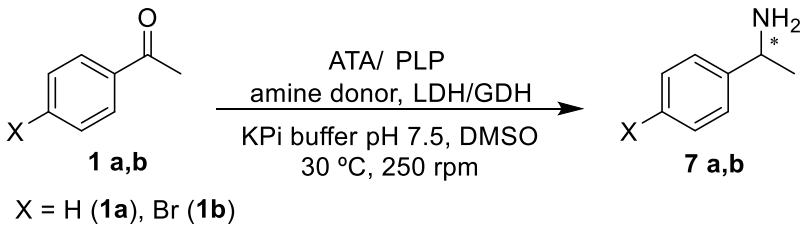


1 a,b **7 a,b**

X = H (**1a**), Br (**1b**)

Entry	Substrate	Amine donor	Enzyme	c (%)
1	**1b**	*i*-PrNH$_2$	Esi	0
2	**1a**	*i*-PrNH$_2$	Esi	0
3	**1b**	*i*-PrNH$_2$	EX-wt	16
4	**1a**	*i*-PrNH$_2$	EX-wt	<5
5	**1b**	*i*-PrNH$_2$	Pac	32
6	**1a**	*i*-PrNH$_2$	Pac	23
7	**1b**	*i*-PrNH$_2$	Shi	20
8	**1a**	*i*-PrNH$_2$	Shi	<5
9	**1b**	*i*-PrNH$_2$	Tja	0
19	**1a**	*i*-PrNH$_2$	Tja	<5

Table 15. Enzymatic asymmetric amination of monoaromatic ketones using new (*R*)-selective ATAs with D-alanine amine donor



X = H (**1a**), Br (**1b**)

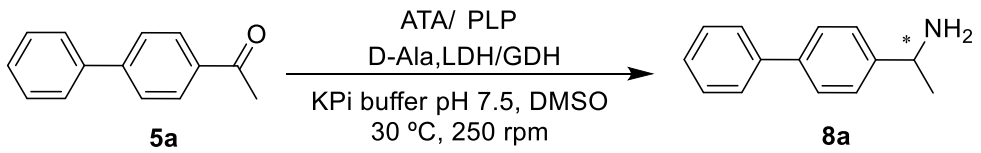
Entry	Substrate	Amine donor	Enzyme	c (%)	*ee* (%)
1	**1a**	D-alanine	Esi	5	>99 (*R*)
2	**1b**	D-alanine	Esi	18	>99 (*R*)
3	**1a**	D-alanine	EX-wt	15	>99 (*R*)
4	**1b**	D-alanine	EX-wt	>99	>99 (*R*)
5	**1a**	D-alanine	Pac	42	>99 (*R*)
6	**1b**	D-alanine	Pac	45	>99 (*R*)
7	**1a**	D-alanine	Shi	51	>99 (*R*)
8	**1b**	D-alanine	Shi	95	>99 (*R*)
9	**1a**	D-alanine	Tja	10	>99 (*R*)
10	**1b**	D-alanine	Tja	13	>99 (*R*)

In order to achieve the goal of the one-pot cascade process towards the bulky biaryl amine the joined ATAs were tested for the conversion of the 4'-acetylbiphenyl (**5a**). After thorough parameterization encompassing not only the involved enzymes and cofactors but also pH, amino donor and co-solvent, the enzymatic system worked efficiently according to the following reaction setup: **5a** (20 mM) in 100 mM phosphate buffer pH 7.5, supplemented with PLP (1 mM), NAD^+ (0.1 mM), glucose (60 mM), D-alanine (6.5 equivalents), DMSO (5% v/v) and 8 U of ATA were used. After 24 h of incubation at 30 °C and 250 rpm, the TLC analysis (chloroform/methanol/acetic acid, 12:2:1) followed by ninhydrin staining revealed the formation of the amine. Further validation was needed and the subsequent HPLC analyses confirmed the production of the corresponding biaryl amine (*R*)-**8a** in enantiomerically pure form (>99% ee, **Table 16**, entry 2) with a conversion of 83% using the EX-wt enzyme. On the other hand, the Shi ATA was able to convert the 4'-acetylbiphenyl to the respective

chiral biaryl amine with a conversion of 17 % (**Table 16**, entry 4). These positive results highlighted the possibility to use of EX-wt in a one-pot system for the synthesis of bulky biaryl amines.

It is also worth noting that although the other enzymes (entry 1, 3, 5-9) effortlessly converted the mono aryl substrates with relatively high conversions (Table 13, 15), they were unable to accept the bulky biaryl ketone and convert it into the corresponding amine under the identical reaction conditions.

Table 16. Enzymatic asymmetric amination of 4'-acetylbiphenyl using the available ATAs



5a **8a**

Entry	Enzyme	c (%)	*ee* (%)
1	Esi	0	n.d.
2	EX-wt	83	≥99 (*R*)
3	Pac	0	n.d.
4	Shi	17	≥99 (*R*)
5	Tja	0	n.d.
6	Vf-ATA	0	n.d.
7	Cv-ATA	0	n.d.
8	Ate-ATA	0	n.d.
9	Ate-ATA_T274S	0	n.d.

3.3.5 (*R*)-selective amine transaminase from *Exophiala xenobiotica* for the synthesis bulky biaryl amines

To identify new (*R*)-selective ATAs that expand the substrate scope within previously inaccessible bulky substrates several sequences from literature were used for sequence-based similarity searches against the NCBI database. *Exophiala xenobiotica*-ATA (EX-ωTA) was chosen based on the habitat of its host organism. The black yeast *E. xenobiotica* was isolated from environments that were associated with toxic, aromatic xenobiotics such as oil sludge, creosote-treated railway tie, soil polluted by gasoline or brown coal rich in phenolic compounds.[162] The hypothesis was that the presence and density of so many different aromatic compounds in the habitat of *E. xenobiotica* might have led to a natural evolution of its transaminase to accept some substrates that other ATAs do not tolerate.

Encouraged by the latest result of the bioamination of the compound **5a** the substrate scope was explored employing a panel of *ortho*-, *meta*-, *para*-biaryl and arylpyridine ketones, some of them exhibiting different patterns of substitution at the phenyl moiety next to the carbonyl group (**Scheme 43**). Additionally, the protein crystal structure of the wild type enzyme (**Figure 3**) was obtained which enabled structure-guided protein engineering. As a result, five variants were prepared with EX-STA and EX-STA5 being the best ones in terms of stability and activity towards various biaryl ketones.

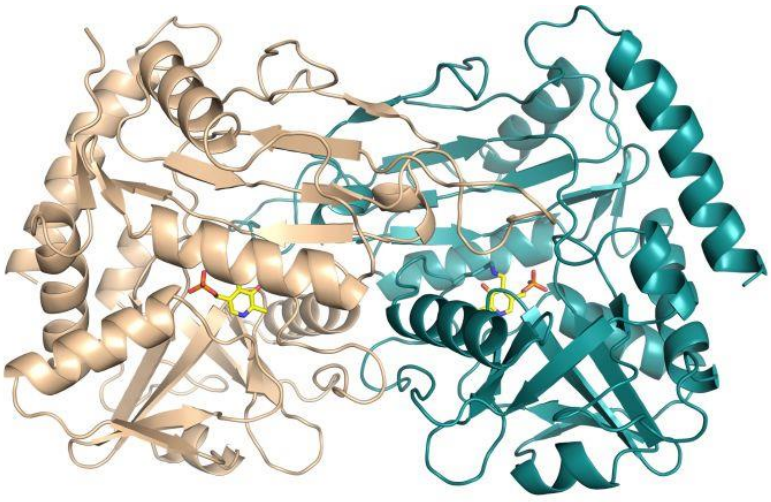
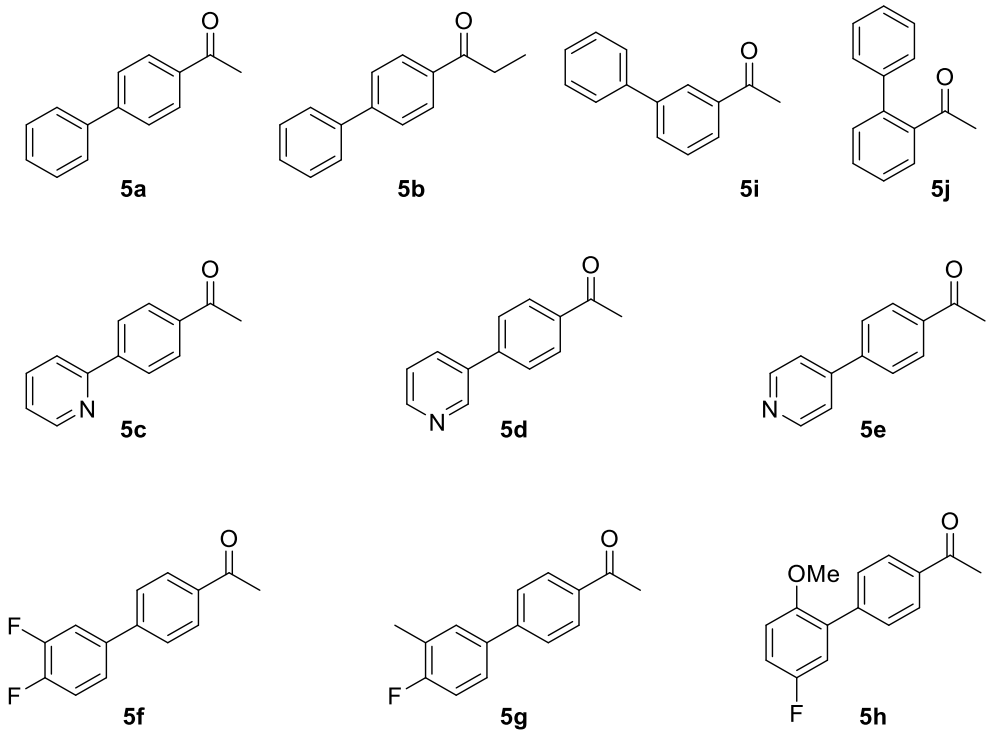


Figure 3. Crystal structure of the EX-ωTA dimer (chain A in orange, chain B in turquoise), PLP is indicated in yellow. The figure was provided by Aline Telzerow and prepared using the program PyMOL

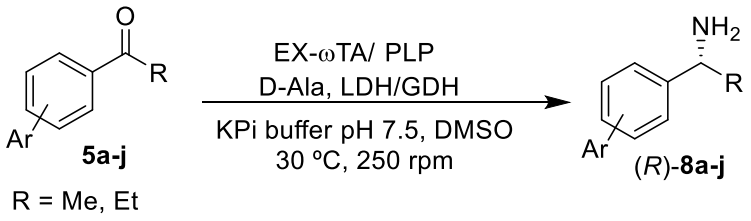


Scheme 43. Substrate scope of biaryl ketones for the enzymatic transamination reaction

While none of the variants showed detectable catalysis toward the *ortho*-biaryl ketone **5j** (**Table 17**, entry 10), a remarkable activity was observed for *meta*- and *para*-biaryl ketones. Formation of the amine was clearly visible via TLC analysis (chloroform/methanol/acetic acid, 12:2:1) followed by staining with ninhydrin, and further confirmed by HPLC measurement. In particular, the unsubstituted arylpyridine ketones **5c-e** rendered conversions higher than 70% (entries 8-10). The inactivity toward 4'-phenylpropiophenone (**5b,** entry 2) unveiled the size restrictions of EX-ωTA in the small binding pocket, which cannot accommodate substituents larger than a methyl group.

Fluorine atoms on different positions on the aromatic group in ketones **5f-h** had a negative impact on the conversion which was attributed to the strong electron-withdrawing effect of fluorine (entries 6-8).

Table 17. Substrate scope of EX-wt and its variants



Entry	Substrate	EX-wt	EX-STA	EX-STA5	*ee* (%)
1	**5a**	83	45	45	>99 *(R)*
2	**5b**	-	-	-	n.d.
3	**5c**	72	>99	80	>99 *(R)*
4	**5d**	82	>99	95	>99 *(R)*
5	**5e**	85	>99	70	>99 *(R)*
6	**5f**	25	72	55	>99 *(R)*
7	**5g**	21	40	30	>99 *(R)*
8	**5h**	25	30	24	>99 *(R)*
9	**5i**	38	85	85	>99 *(R)*
10	**5j**	-	-	-	n.d.

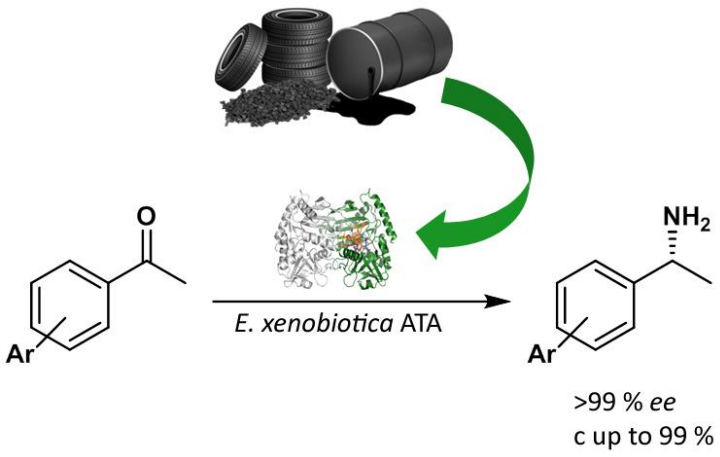


Figure 4. The black yeast *E. xenobiotica*, isolated from harsh and toxic environments, was able to accept bulky substrates that other known ATAs are unable to tolerate

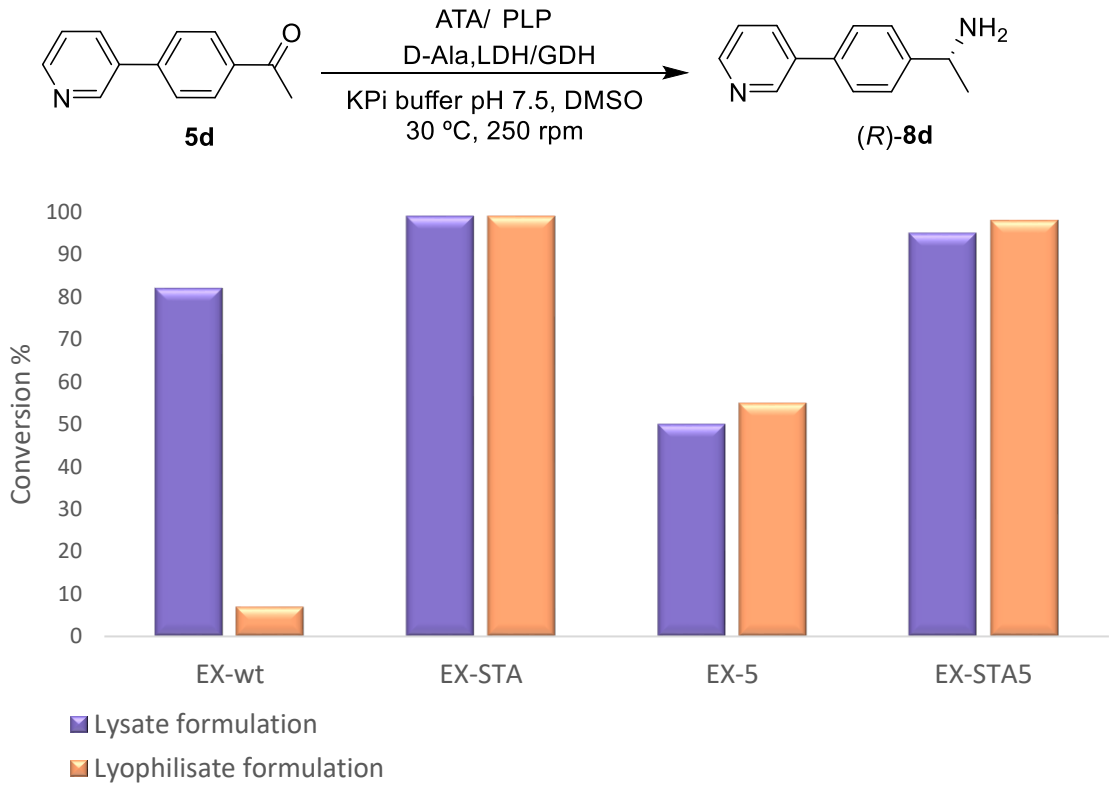
In conclusion, the EX-ωTA displayed perfect asymmetric induction in the reductive amination of the carbonyl group of the biaryl ketones, leading to the (*R*)-enantiomer of each amine with >99% ee. These results already underline the high potential of this enzyme for the synthesis of chiral biaryl amine building blocks of various pharmaceutical intermediates and chemical compounds.

3.3.6 Optimisation of the transamination reaction

Taking the EX-STA-catalysed transamination of **5d** as a model reaction (**Table 17**, entry 4), several process parameters were investigated on an analytical scale. In detail, the formulation of the biocatalyst, the amino donor, the concentration of substrate and the enzyme loading were assessed.

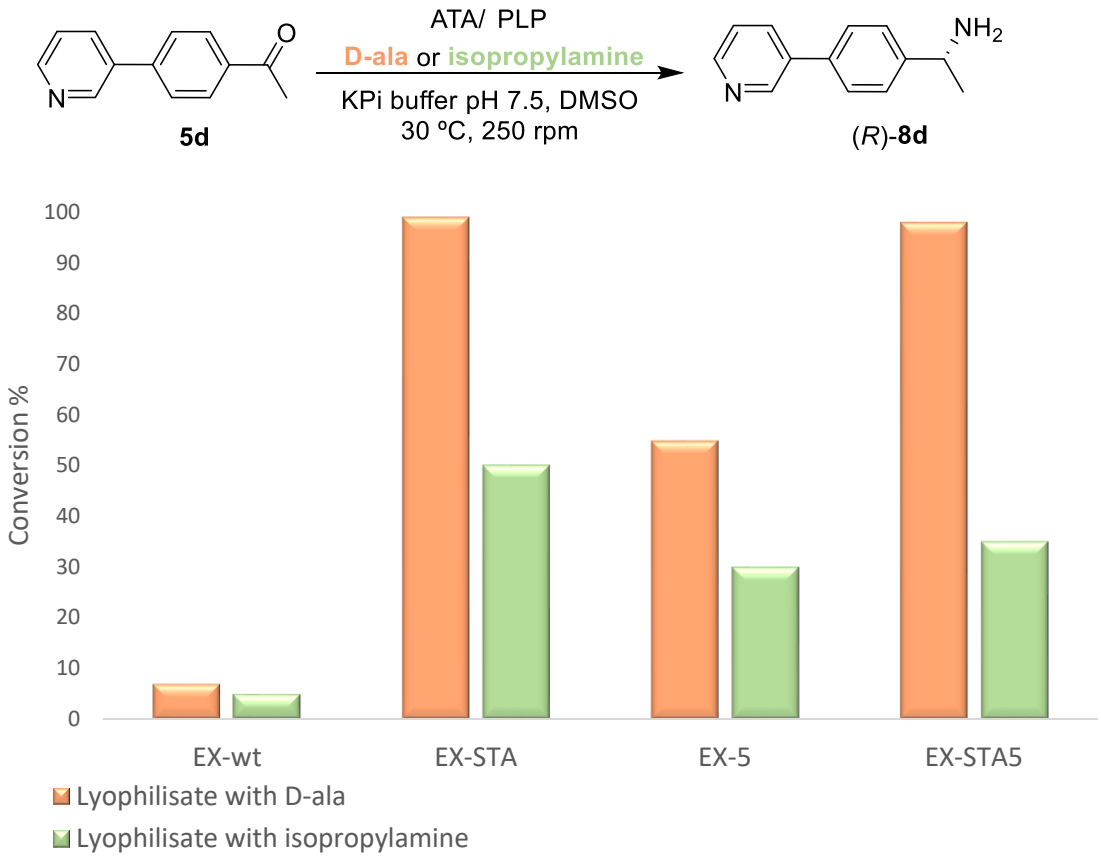
As described in the previous sections, a major limitation in the asymmetric synthesis starting from prostereogenic ketones is the product inhibition and the unfavourable thermodynamic equilibrium. Many studies addressed this issue and a number of methods have been developed to shift the equilibrium towards the desired amination reaction.[106,120,163] One of the suggested methods implies the use of the preferred amine donor in excessive amounts. From the economical aspect it is desirable to lower the amount of the amine donor and find the lowest quantity necessary to efficiently carry out the reaction. For that purpose, the amount of the amine donor was investigated as original protocols suggested the use of 12 equivalents of D-alanine.[116] It was then demonstrated that the reaction ran smoothly with 7 equivalents of D-alanine in regards to the substrate.

Moreover, the analysis of the enzyme formulation displayed that the engineered enzymes EX-5, EX-STA and EX-STA5 retained their activity as a lyophilised powder in contrast to the wild type whose activity dropped dramatically (**Scheme 44**). These findings were beneficial for the preparative-scale and cascade reactions and further confirmed the advantages of protein engineering.



Scheme 44. Study of the performance of EX-ωTA and its variants in different formulations for the transamination of the biaryl ketone **5d**

In addition, the same model reaction was used to investigate the possibility of having isopropylamine as the amine donor with the engineered variants using the lyophilised formulation. Pleasantly these enzymes accepted isopropylamine although with lower conversions than those measured with alanine (**Scheme 45**). This feature again demonstrates the benefits of protein engineering as the possibility to use isopropylamine heavily broadens the spectrum of possible reactions. To complement these studies a kinetic analysis was carried out and it was demonstrated that the transamination of the compound **5d** fully took place after only 3 hours of reaction time (**Scheme 46**). This significantly reduces the duration of the biotransformation and the entire cascade as this step was usually carried out for 24 hours.

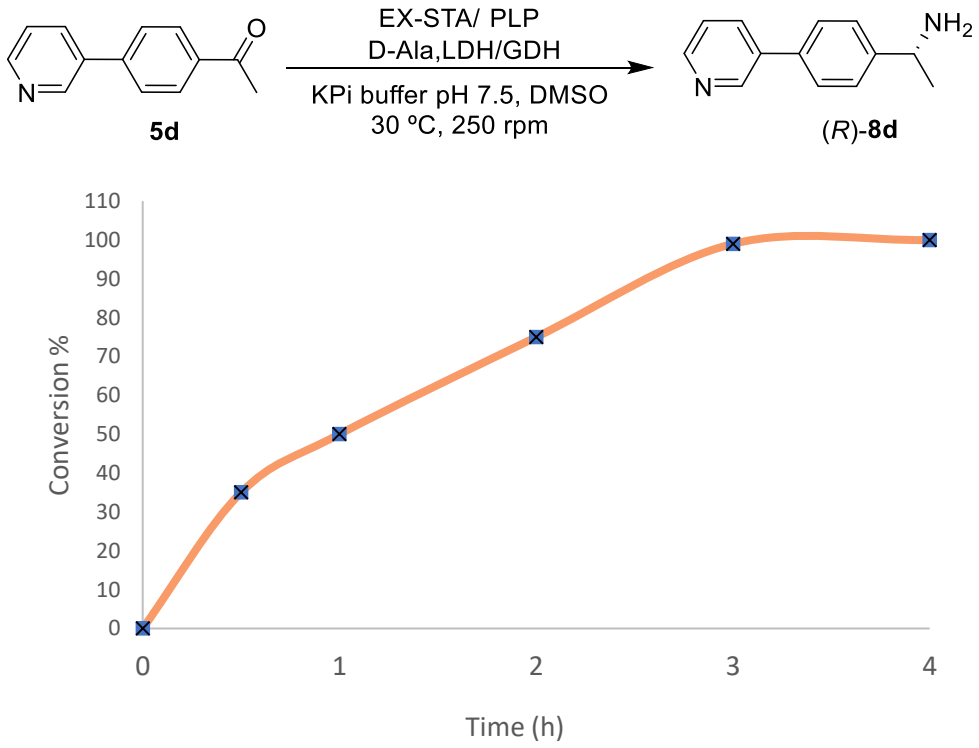


Scheme 45. Enzymatic transamination of the compound **5d** catalysed by lyophilised EX-ωTA and its variants using isopropylamine (1M) as amino donor compared to the use of D-alanine

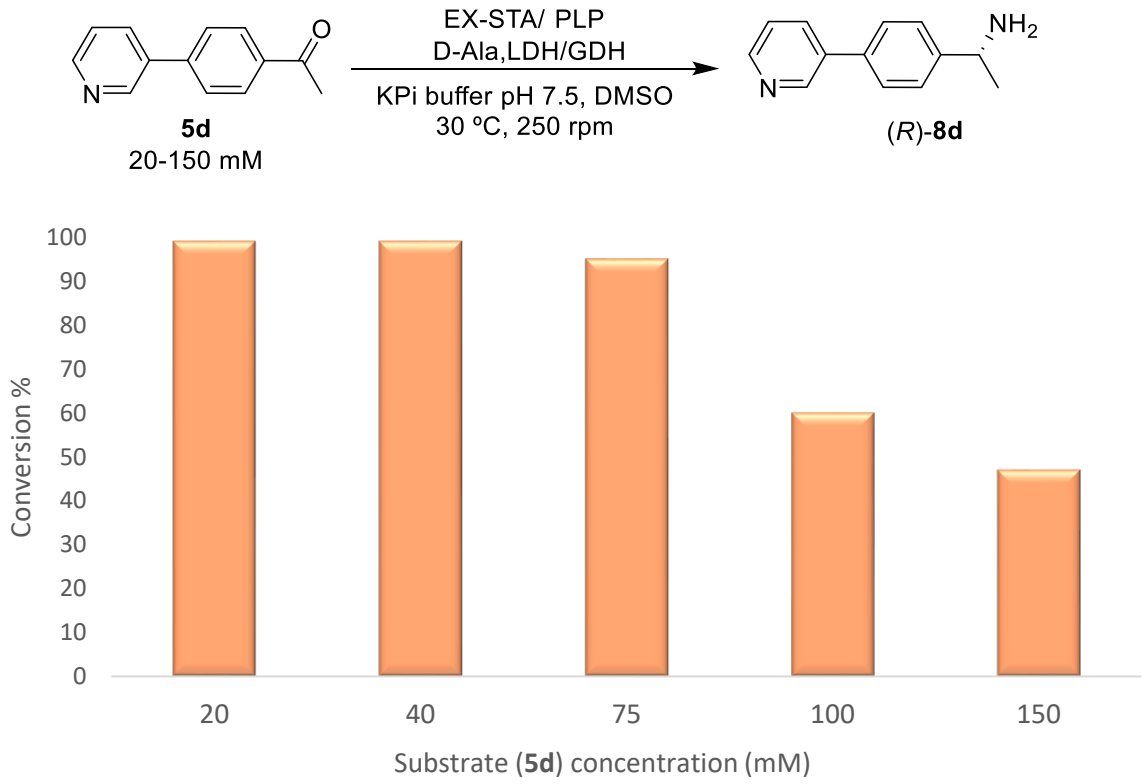
Furthermore, the enzymatic reaction worked efficiently at up to 75 mM substrate concentration (**Scheme 46**) which was a substantial increase from the previously tested reactions at concentrations that barely reached 20 mM.

Another optimisation study was aimed to investigate the lowest amount of enzyme required for the biotransformation which was thus carried out in the range of 1 - 4 U per mg of substrate (**Scheme 48**).

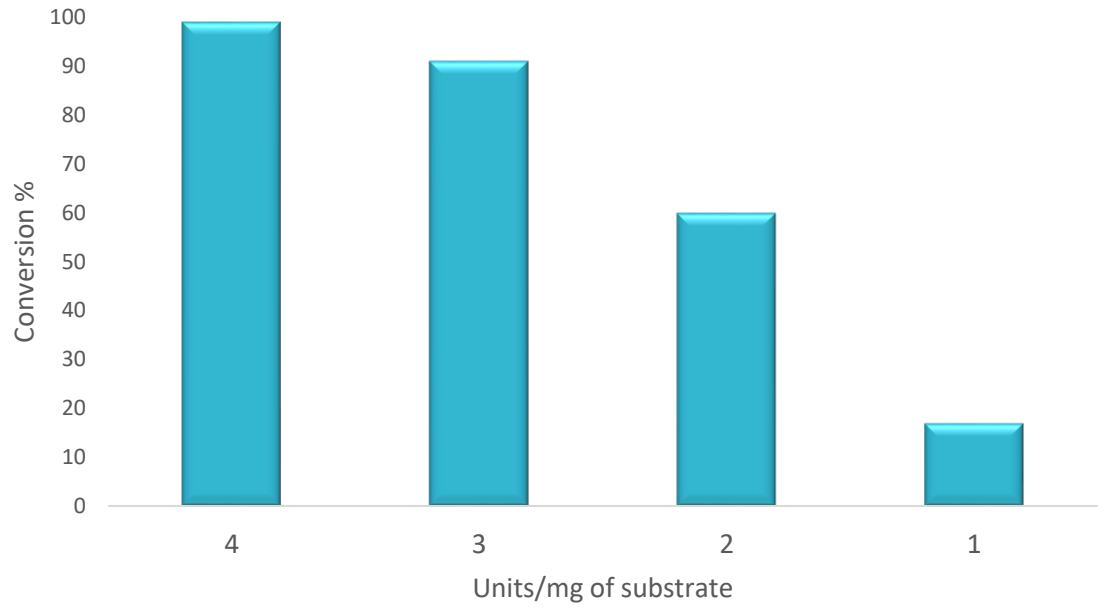
Reducing the amount of enzyme starting from 4 U per mg of substrate gradually showed a decrease of conversion. Thus, 4 U per mg of substrate was defined as optimally the lowest enzyme loading necessary to successfully carry out the biotransformation reaction (**Scheme 47**). This finding is a considerable improvement over the previously used 8 U per mg substrate as it heavily reduces the necessary amount of enzyme and facilitates the upscaling of the process.



Scheme 46. Study of the kinetics for the transamination of **5d** catalysed by lyophilised EX-STA

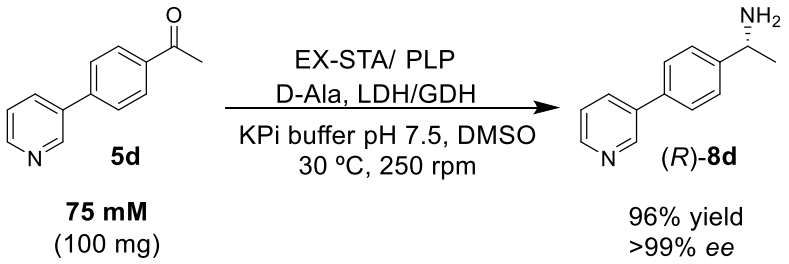


Scheme 47. Enzymatic transamination of **5d** catalysed by lyophilised EX-STA at different substrate loadings



Scheme 48. Enzymatic transamination of **5d** catalysed by lyophilised EX-STA at different enzyme loadings

To illustrate the synthetic applicability of the new ATA, a preparative-scale biotransformation was carried out. The target biotransformation was accomplished at a 100 mg-scale and 75 mM of the compound **5d** to afford the enantiomerically pure (*R*)-**8d** in excellent isolated yield (96%) after extractive workup and without the need for further purification of the crude product (**Scheme 49**).

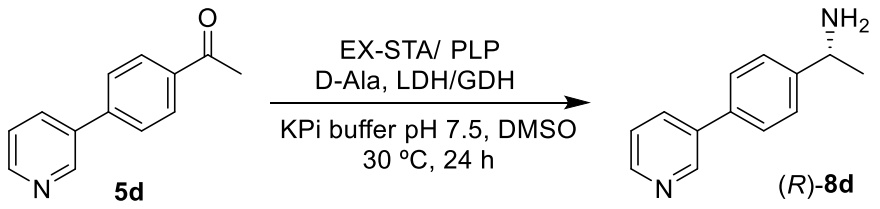


Scheme 49. Preparative-scale transamination of **5d** catalysed by EX-STA

3.3.7 Activity of EX-ωTA in *DES* and other co-solvents

In order for the transaminases to be suitable for the one-pot process a key challenge was to determine if the enzyme is active in the solvents already tested for the Suzuki cross-coupling reaction step. Accordingly, the bioamination of the biaryl ketone **5d** was investigated as a benchmark reaction with the mutant EX-STA, the variant leading to the highest conversions. The biotransformation was conducted under the previously optimised reaction setup, namely based on the use of alanine as amino donor and the LDH/GDH recycling system. As deduced from **Table 18**, the presence of THF and *i*-PrOH was harmful for the enzyme, resulting in a conversion being very low at 15% of co-solvent (entries 2 and 4). Conversely, the ATA remained very active in both DMSO and 1*ChCl*/2*Gly* with conversions almost quantitative at 15% of co-solvent (entries 5-8). An increase to 25% *DES* decreased the enzymatic performance by 40% (entry 9) while the activity of the ATA in *DES*-buffer 1:1 was negligible (entry 10). In conclusion, the variant exhibited perfect enantioselectivity regardless of the co-solvent and its ratio, with the resulting amine (*R*)-**8d** displaying >99% *ee* in all cases.

Table 18. Effect of co-solvent on the conversion of the EX-STA-catalysed bioamination of biaryl ketone **5d**



Entry	Co-solvent (%)	*c* (%)	*ee* (%)
1	THF (5%)	8	>99 (*R*)
2	THF (15%)	-	n.d.
3	*i*-PrOH (5%)	64	>99 (*R*)
4	*i*-PrOH (15%)	<5	n.d.
5	DMSO (5%)	>99	>99 (*R*)
6	DMSO (15%)	>99	>99 (*R*)
7	1*ChCl*/2*Gly* (5%)	>99	>99 (*R*)
8	1*ChCl*/2*Gly* (15%)	95	>99 (*R*)
9	1*ChCl*/2*Gly* (25%)	60	>99 (*R*)
10	1*ChCl*/2*Gly* (50%)	<5	n.d.

3.3.8 ATA-catalysed bioamination of phenylacetone in different *DES*-buffer media

For a better understanding of the unveiled stability of ATAs in *DES*-buffer mixtures the study was extended to enzymes from the Codexis commercial kit, also overexpressed in *E. coli*.[164] Furthermore the enzymes utilized were the (*S)*-selective ATAs from *Chromobacterium violaceum* (Cv),[160] (*S*)-*Arthrobacter* (ArS),[165] the (*R)*-selective ATAs from (*R*)-*Arthrobacter* (ArR)[86] and its evolved variant ArRmut11.[19] For this study, phenylacetone (**1c**) was selected as a substrate, which had been efficiently converted for those ATAs in conventional aqueous medium.[166]

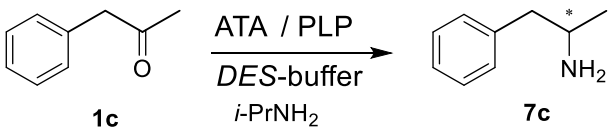
Four choline chloride-based eutectic mixtures, namely 1*ChCl*/2*Gly*, 1*ChCl*/2H_2O, 1*ChCl*/1*Sorb* and 1*ChCl*/2*Urea* were screened at variable water content (**Table 19**). In a typical experiment aimed

at evaluating the enzymatic performance, **1c** (30 mM) was incubated in a mixture (1 mM PLP and 1 M isopropylamine) of *DES* and potassium phosphate buffer 100 mM at pH 7.0, 30 °C and 250 rpm during 24 hours. The first conclusion was that the *DES*-buffer mixtures resulted in being a suitable reaction media for both purified and overexpressed ATAs at 25% or 50% (w/w) *DES*. Conversely, the commercial purified enzymes led to very high conversions in the four media tested (entries 1-4). Indeed, the conversion values were almost identical to those reported in aqueous buffer solution,[166] and in the case of ATA-256 the conversion even increased from 57% to 90-95% in the neoteric mixtures (entry 3).

With regards to the overexpressed enzymes, the transaminase from *C. violaceum* and ArS-TA were less active in 1*ChCl*/2H_2O, 1*ChCl*/1*Sorb* and 1*ChCl*/2*Urea* meanwhile they exhibited comparable activities in 1*ChCl*/2*Gly* to those in aqueous buffer (entries 5-6). On the other hand, the enzymes from ArR and ArRmut11 led to good conversion in all the *DES*-buffer mixtures, especially in the case of the ATA from ArRmut11. For the particular case of 1*ChCl*/2*Gly*, a further increase to 75% (w/w) *DES* proved to be harmless for ATAs with changes in the conversion rate lower than 5%.

It is worth noting the excellent tolerance of *DESs* by ATAs, far greater than organic solvents.[167] The unexpected stability of ATAs in *DESs* could be explained following a recent study in which the *DES* hydration tolerance was investigated and the presence of water in its nanostructured domains was discussed.[83]

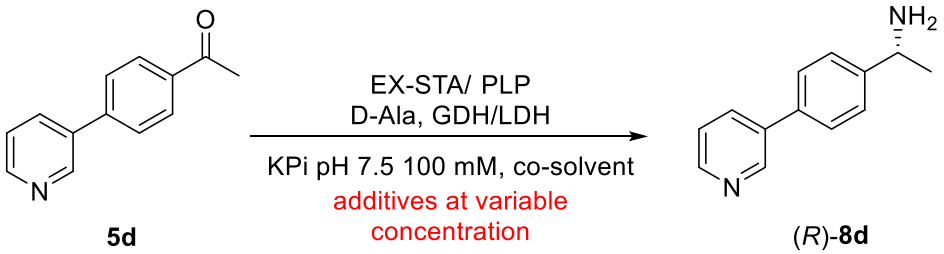
Table 19. Effect of different *DESs*-buffer media on the conversion of the ATA-catalysed bioamination of phenylacetone



Entry	Enzyme	Buffer	1*ChCl*/2*Gly*			1*ChCl*/2H_2O		1*ChCl*/1*Sorb*		1*ChCl*/2*Urea*	
			25% *DES*	**50% *DES***	**75% *DES***	**25% *DES***	**50% *DES***	**25% *DES***	**50% *DES***	**25% *DES***	**50% *DES***
1	ATA-237	95	98	93	93	90	90	93	88	91	88
2	ATA-251	95	97	92	92	93	90	>99	91	95	86
3	ATA-256	57	95	95	95	91	92	85	88	90	85
4	ATA-P1-G06	95	95	95	92	95	90	96	91	95	90
5	Cv	91	94	90	85	5	30	12	15	<5	<5
6	ArS	64	45	42	40	10	25	10	35	<5	<5
7	ArR	91	55	60	72	50	80	85	90	70	65
8	ArRmut11	>99	95	95	95	90	80	73	95	85	90

3.3.9 Inhibition studies

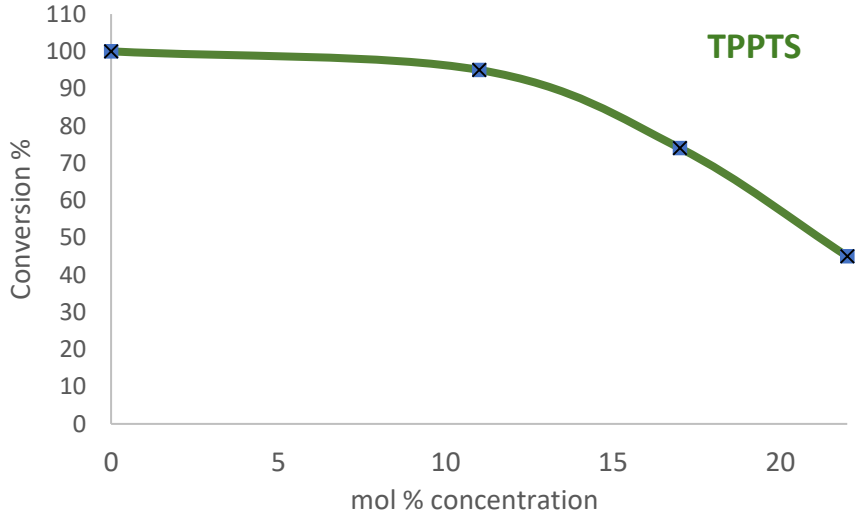
After both reaction steps of the cascade have been studied separately, the focus was to investigate the potential inhibitory effects of the remaining reagents from the Suzuki cross-coupling reaction on the biocatalytic system (**Scheme 50**). Thus, the transamination of the compound **5d** under the optimised setup (**Table 18**, entry 8) was subjected to deferent reaction parameters namely the impact of the formed catalyst [$Pd(TPPTS)_2Cl_2$], the ligand TPPTS and boronic acid on this enzymatic step.



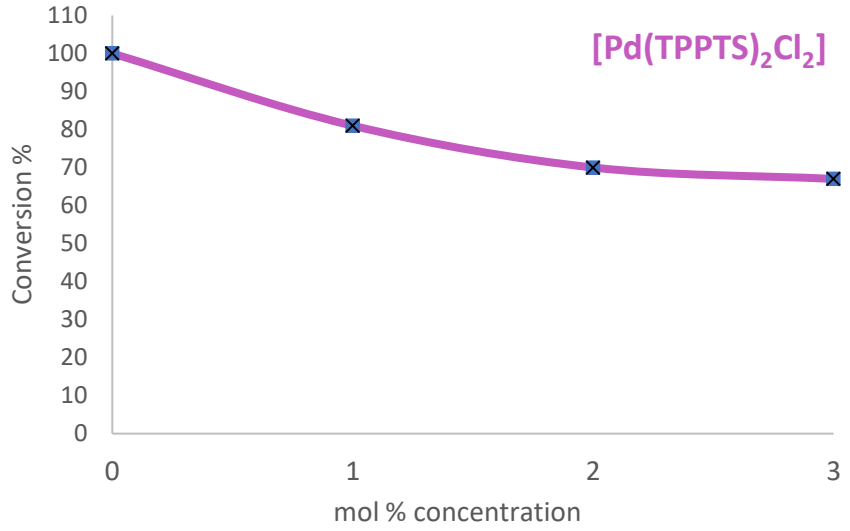
Scheme 50. Study of the potential inhibitory effect of the cross-coupling reagents on the biocatalytic system

The EX-STA enzyme variant tolerated concentrations of TPPTS to up to 10 mol% (**Scheme 51**). The ligand in the solution started to have a negative impact on the enzymatic activity only at concentrations above 10 mol % which in turn generated only 45% of the amine product at 22 mol % concentration. On the other hand the palladium complex did not have such an unfavourable impact, as 1 mol% of $[Pd(TPPTS)_2Cl_2]$ inhibited the enzyme slightly, resulting in a conversion of the biotransformation of 81% at that concentration (**Scheme 52**). Doubling the amount of catalyst gradually decreased the effectiveness of the biocatalytic step, as 3 mol% of the complex resulted in a 67 % final conversion.

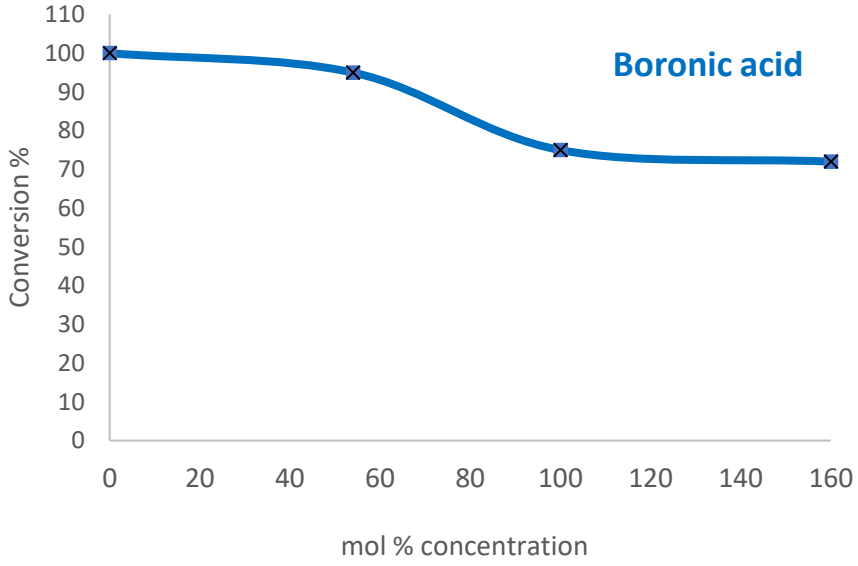
Lastly, the boronic acid component was well-tolerated by the enzyme reaching concentrations of 50 mol% for a nearly complete conversion (**Scheme 53**).



Scheme 51. Enzyme activity at different concentrations of TPPTS



Scheme 52. Enzyme activity at different concentrations of the palladium complex



Scheme 53. Enzyme activity at different concentrations of boronic acid

The presence of the boronic acid and the TPPTS salt in the solution however should not pose a problem to the enzymatic step since the reported Suzuki cross-coupling occurs quantitatively from equimolar amounts of these species, thus leading to their full consumption prior to the addition of the enzyme in the one-pot process.

3.3.10 Synthesis of biaryl amines in a one-pot cascade process

The impact of the Suzuki cross-coupling components and co-solvents on ATAs was essential to setup the chemoenzymatic cascade in a sequential fashion. Attention had to be given to the concentration of the starting components and temperature as the first step was carried out at 100 °C and 200 mM while the enzymatic step on the other hand is limited to the ketone concentration of 75 mM and works efficiently only at temperatures lower than 50 °C. Likewise, although the first step is accomplished at 80% (w/w) *DES*, the EX-STA only tolerates 15% of this solvent (**Table 18**, entry 8).

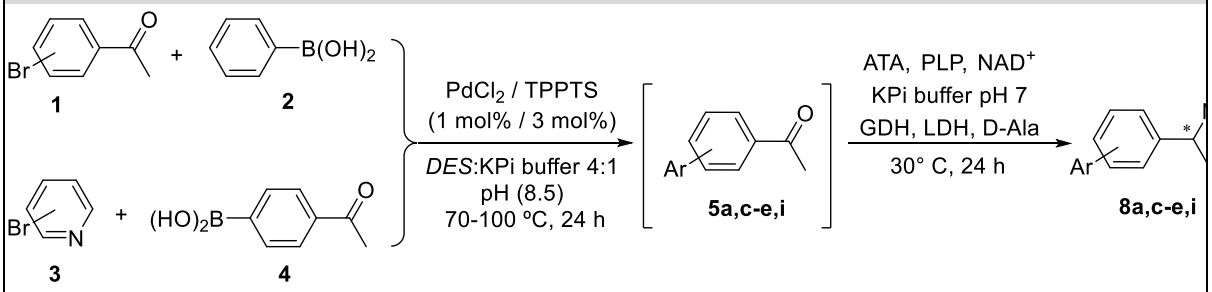
With these premises, the first step was efficiently conducted at 100 °C in *DES*-water 4:1. Once the reaction was completed, the mixture containing 4'-acetylbiphenyl (**5a**) was diluted to 75 mM with the aqueous buffer for the bioamination and supplemented with EX-STA, LDH, GDH, glucose, the cofactors and the amino donor. After 24 h of incubation at 30 °C and 250 rpm, the resulting 1-([1,1'-biphenyl]-4-yl)ethanamine (**8a**) was produced with a conversion of 15%. A plausible explanation for this low conversion was presumably the high content of *DES* during the bioamination step. Actually, the dilution of the *DES*-water 4:1 mixture from 200 mM to 75 mM results in a medium containing approximately 30% *DES*. Considering the previous studies (**Table 18**) over 15% of *DES* v/v had negative effect on the enzyme activity. Accordingly, the dilution of the reaction mixture containing the transiently biaryl ketone was fixed at 50 mM, resulting in a final 20% of *DES* for the biotransformation. As expected, the conversion for **8a** increased up to 30%. Further dilution to 25 mM, which results in 10% of *DES*, led to an optimised conversion of 45% (**Table 20**, entry 1).

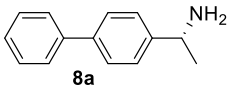
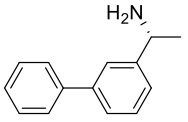
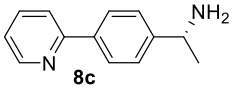
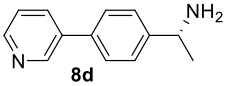
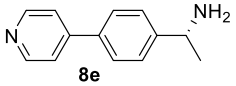
In addition to the EXE-STA variant, the cascade was tested with the wild-type enzyme, which had previously exhibited the highest conversion in the single bioamination of **8a** (c= 83%, **Table 16**, entry 2). However, upon the optimal cascade setup described above (25 mM concentration in the biotransformation) the conversion to the desired amine dropped to only 35% (**Table 20**, entry 2). Nonetheless, encouraged by the potential of this dual catalytic system, the methodology was extended to other substrates with both EX-STA and EX-wt. Thus, the initial metal-catalysed step proceeded quantitatively in all cases, leading to the formation of 3'-acetylbiphenyl **5i** and methyl pyridylphenyl ketones **5c-e**. The subsequent transamination step proceeded according to the optimised conditions and the conversion of ketones to their respective amines was confirmed *via* TLC analysis (chloroform/methanol/acetic acid, 12:2:1). The immediate appearance of purple spots followed by ninhydrin staining indicated the formation of the desired products. According to the HPLC analysis EX-STA converted quantitatively (c >95%) the resulting ketones into the corresponding (*R*)-biaryl amines **8c-e,i** with >99% *ee* (**Table 20**, entries 3, 5, 7, 9). Conversely, as a result of its poor

stability in the *DES*-buffer medium, the EX-wt displayed very low product formation (**Table 20**, entries 6, 8, 10) and even no conversions at all (**Table 20**, entry 4).

Although originally engineered to have a higher enzymatic activity and a larger binding pocket, the EX-STA variant turned out to be more stable than the wild type enzyme in *DES*-buffer mixtures, a feature that made it possible to carry out an efficient one-pot cascade process.

Table 20. One-pot synthesis of enantiomerically pure biaryl amines by palladium-catalysed Suzuki cross-coupling followed by enzymatic transamination in a *DES*-buffer mixture



Entry	Enzyme	Product	*c* (%)	Isolated yield (%)	*ee* (%)
1	EX-STA	 **8a**	45	32	>99 (*R*)
2	EX-wt		35	n.d.	>99 (*R*)
3	EX-STA	 **8i**	95	85	>99 (*R*)
4	EX-wt		-	n.d.	n.d.
5	EX-STA	 **8c**	>99	85	>99 (*R*)
6	EX-wt		20	n.d.	>99 (*R*)
7	EX-STA	 **8d**	>99	88	>99 (*R*)
8	EX-wt		10	n.d.	n.d.
9	EX-STA	 **8e**	>99	90	>99 (*R*)
10	EX-wt		5	n.d.	n.d.

3.4 Conclusion

A chemoenzymatic cascade consisting of a palladium-catalysed Suzuki cross-coupling followed by an enzymatic transamination mediated by ω-transaminases has been efficiently implemented in *ad hoc* mixtures of *DESs* and aqueous media.

At first a suitable (*R*)-selective ATA which naturally converts biaryl ketones to the corresponding enantiomerically pure amines has been identified, followed by accurate protein engineering that improved the biocatalysts activity and stability, allowing for a preparative-scale biaryl amine synthesis.

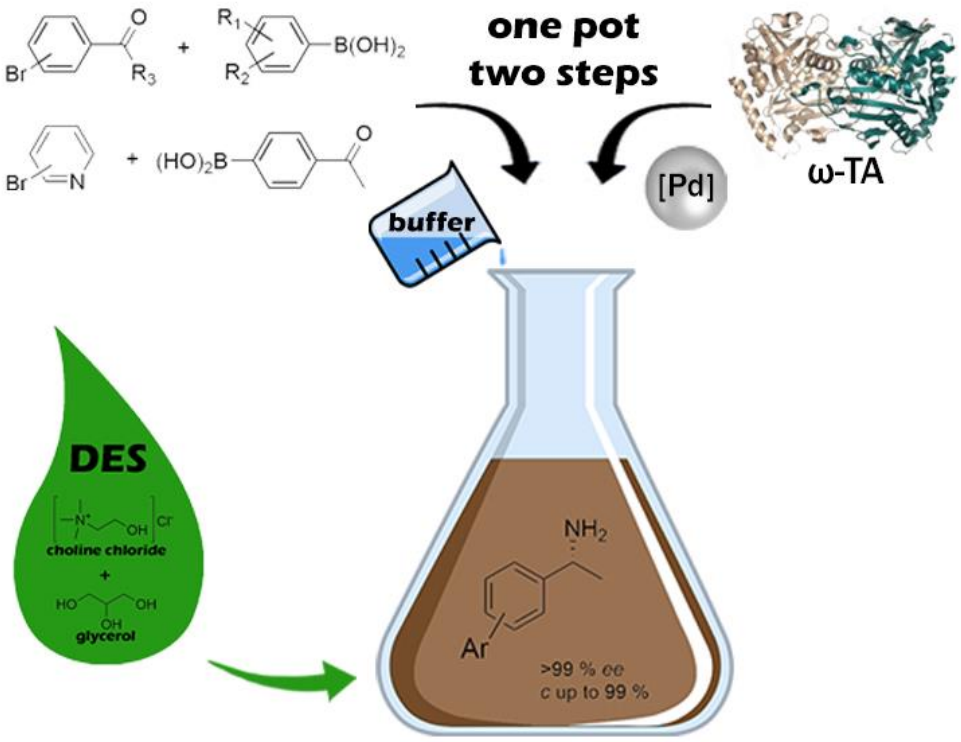


Figure 5. Illustration of a one-pot synthesis of biaryl amines in the mixture of *DES*s and aqueous media

Furthermore, the advantages of the reaction the medium allowed the cross-coupling step to proceed smoothly at high substrate concentration. The ATAs exhibited good stability and catalytic performance at high percentages of *DES* (up to 75%) which allowed the two processes to be effectively combined after thorough reaction optimisation resulting in a successful sequential one-pot process. This unprecedented enzymatic activity of ATAs is an excellent proof of concept of the practical value of biorenewable solvents for synthetic chemists.

4 Summary

Among several achievements realised throughout this research, the highlight is certainly the use of *Deep eutectic solvents* for one-pot chemoenzymatic cascades. Amine transaminases and alcohol dehydrogenases displayed praiseworthy performances in these neoteric solvents for the synthesis of bulky chiral compounds.

Besides the broadening of the substrate scope, a variety of of different enzymes has been tested for both cascade reactions. For the chemoenzymatic ketone reduction a number of commercially available ADHs was first screened in an individual process, followed by its combination with the chemical step resulting in a successful one-pot reaction.

Contrarily to ADHs, for the ATA catalysed reactions there were no reported enzymes able to accept such demanding biaryl substrates. It was thus inevitable to screen for ATAs that would work with these challenging compounds, leading to the discovery of two enzymes that could naturally convert the bulky ketones. The (*R*)-selective transaminase from *Exophiala xenobiotica* showed exceptional results for this purpose and the acquisition of its crystal structure enabled to explain the substrate acceptance, thus making it possible to further improve its performance by means of protein engineering.

The substrate scope for both enzymatic processes was in principle closely related and consisted of *ortho-, meta-* and *para*-biaryl and arylpyridine ketones, some of them exhibiting different patterns of substitution. A first process development by conductive small-scale experiments for the Suzuki cross-coupling was successful with the best performing model substrate. Consequently, intensive process optimisation led to lower utilization of the metal catalyst and allowed a higher substrate loading with the biotransformations being carried out in the range of 25-75 mM substrate concentration.

Besides the possibility to reach high substrate concentration the unique propeties of *DESs* allowed to obtain chiral amines and alcohols in good yields (70 – 90 %) and a very high enantiomeric excess (>99 %).

These results highlight the value of nature's biodiversity as an ever-increasing source of new genomes and metagenomes to identify unprecedented catalytic activities which can lead to novel robust biocatalysts. Furthermore medium engineering was demonstrated to be a valuable solution for the optimisation of chemical transformations and biocatalytic reactions with the possibility to overcome a set of hurdles and and reach improvement of the overall process.

5 Experimental section

5.1 Materials and general methods

Enzymes

Codex® KRED Screening Kit was purchased from Codexis.

Codex® Transaminase Screening Kit (ATASK-000250) was purchased from Codexis.

The (*S*)-alcohol dehydrogenase from *Rhodococcus ruber* DSM 44541[81] was provided as a cell-free extract (CFE) by InnoSyn. The total protein concentration is about 46 mg/ml with a good expression level of the ADH in the soluble fraction. The activity determined spectrophotometrically at pH 6.0 with acetone and NADH as substrates was 225 U/ml or 4.85 U/mg total protein, respectively.

The (*R*)-alcohol dehydrogenase from *Lactobacillus kefir* DSM 20587[80] was provided as a cell-free extract (CFE) by the Industrial Organic Chemistry and Biotechnology group in Bielefeld. The determination of the protein amount revealed ca. 22 mg/mL. The activity of the enzyme is about 1468 U/mL. The activities for both enzymes were measured by the above-mentioned groups referring to the activity for acetophenone following the appropriate assay known in the literature.[168]

LDH (Lactate dehydrogenase) from rabbit muscle (Sigma-Aldrich, Type II, ammonium sulfate suspension, 800-1,200 U mg^{-1} protein).

GDH (Glucose dehydrogenase) from *Bacillus megaterium* was expressed in *Escherichia coli*. The activity was determined as 1884 U mL^{-1} according to the assay described in the literature.[169]

E. xenobiotica ATA gene codon-optimised for expression in *Escherichia coli* was ordered from Geneart/LifeTech (Vienna, Austria).

The (*S*)-selective ω-TA from *Chromobacterium violaceum* (Cv-TA) and *Vibrio fluvialis* (Vf-ATA), and the (*R*)-selective ω-TA from *Aspergillus terreus* (Ate-ATA) and its mutated variant (Ate-ATA_T274S) were provided as a cell-free extracts (CFE) by DSM. Other ω-TAs were provided by InnoSyn B.V. (Geleen,

The Netherlands) or, when stated, were expressed in *Escherichia coli* and the activity was measured spectrophotometrically.

Transaminases from *Arthrobacter* sp. [ArR (pEG23), ArS (pEG29) and ArRmut11 (pEG90)] were overexpressed in *E.coli* and used and lyophilised cells. The protein content of these four transaminases was established by the Pierce's method (mg protein/mg catalyst) according to the manufacturer's instructions: ArS (0.37), Cv (0.31), ArRmut11 (0.38), ArR (0.19).

Reagents

The ligand TPPTS (Triphenylphosphine-3,3',3''-trisulfonic acid trisodium salt hydrate, tech. 85%) was purchased from Alfa Aesar. 1-(Biphenyl-4-yl)ethanone (**5a**) was purchased from Merck Schuchardt. Palladium(II) chloride was purchased from TCI. Arylbromides (**1a,b,f-j** and **3c-e**) and phenylboronic acids (**2a,b,f-j** and **4c-e**) were purchased from Sigma Aldrich. D-(+)-glucose was purchased from VWR. D-alanine, PLP (Pyridoxal 5'-phosphate hydrate) and NAD^+ were purchased from Sigma Aldrich.

Protein expression and purification:

After transformation of *E. coli* BL21-Gold(DE3) (Stratagene, La Jolla, CA, USA), the cells were grown at 37 °C in terrific broth medium supplemented with ampicillin (Amp: 100 µg mL-1). Expression was induced with 0.1 mM Isopropyl β-D-1-thiogalactopyranoside (IPTG) at an OD600 between 0.6-0.8 and carried out at 25 °C overnight. The cells were harvested at 4000 x g for 15 min and resuspended in buffer (50 mM KPi buffer, pH 7.5, 0.1 mM PLP). The cells were disrupted by sonication (Branson Sonifier S-250, 6 min, 80% duty cycle, 70% output) and centrifuged at 50000 x g for 1 h. The cleared lysates were filtered through 0.45 µm syringe filters. The protein concentration of the lysate was determined by Bradford protein assay. The expression and solubility of the proteins was analyzed by SDS-PAGE (NuPAGE Bis-Tris PreCast Gels/Life Technologies). The protein was frozen and stored at -20 °C until further use.

Spectrophotometric activity assay

The activity of the ATA variants was determined with an adjusted acetophenone photometric assay. The substrate phenylethylamine is converted to acetophenone, which can be detected

spectrophotometrically at 300 nm. The absorbance increases with rising product concentration. To measure the activities of the ATA variants, reactions were set up in 1 mL volume with 100 mM KPi buffer pH 7.5 containing 0.1 mM PLP, 5 mM phenylethylamine and 5 mM pyruvic acid. For the reaction, 50 µg to 250 µg of total protein were used. The production of acetophenone was measured for several minutes at 300 nm at 30 °C. The volumetric activity in U mL^{-1} and the specific activity in U mg^{-1} total protein of the enzyme samples was calculated using the Beer-Lambert law with the molar extinction coefficient of acetophenone ε300 = 0.28 cm2 μmol^{-1}.

NMR

^{1}H-NMR and proton-decoupled ^{13}C-NMR spectra ($CDCl_3$) were obtained using a Bruker DPX-300 (^{1}H, 300.13 MHz and ^{13}C, 75.5 MHz) spectrometer using the δ scale (ppm) for chemical shifts. Calibration was made on the signal of the solvent (^{13}C: $CDCl_3$, 77.16; ^{1}H: $CDCl_3$, 7.26).[170]

Optical activity

Optical rotations were measured using a Perkin-Elmer 241 polarimeter and are quoted in units of 10^{-1}deg cm^2 g^{-1}.

HPLC for conversion (c) and enantiomeric excess (*ee*) determination

The determination of conversion (c) of ketones, amines and alcohols was measured at the following settings:

Conversion was determined monitoring and comparing the integrated areas of the product with the starting material in the HPLC chromatogram.

HPLC Method for **5a-b**, **5f-j, 6a-b**, **6f-j, 7a-c**, **8a-b**, **8f-j** (Method A): HPLC analyses were carried out in an Agilent chromatographic system, using a reversed phase column (Zorbax Eclipse XDB-C18, RR, 1.8 µm, 4.6 x 50 mm, Agilent) and acetonitrile (MeCN) and 0.1% trifluoroacetic acid (TFA) in water as solvents. Samples were eluted with three linear gradients from 10% to 60% MeCN during 5.70 min, followed by another from 60% to 100% MeCN during 0.5 min and a third gradient from 100% to 10% MeCN during 1.90 min, at flow rate of 2 ml/min. Detection and spectral characterization of peaks were performed at 220 nm with a diode array detector and ChemStation Rev.B.03.01 software (Agilent).
HPLC Method for **5c-e, 6c-e, 8c-e** (Method B): HPLC analyses (for 5c-e) were carried out in an Agilent chromatographic system, using a reversed phase column (Zorbax Eclipse XDB-C18, RR, 1.8 µm, 4.6 x 50 mm, Agilent) and acetonitrile (MeCN) and 0.1% triethylamine (Et_3N) in water as solvents. Samples

were eluted with three linear gradients from 10% to 60% MeCN during 5.70 min, followed by another from 60% to 100% MeCN during 0.5 min and a third gradient from 100% to 10% MeCN during 1.90 min, at flow rate of 1.50 ml/min. Detection and spectral characterization of peaks were performed at 278 nm with a diode array detector and ChemStation Rev.B.03.01 software (Agilent).

The determination of enantiomeric excess was measured at the following settings:
HPLC analyses to determine the enantiomeric excess were performed on a Hewlett Packard 1100 LC liquid chromatograph using hexane/isopropanol mixtures and 0.8 mL/min flow. Detection of peaks (UV absorption) was performed at 210 and 278 nm on normal-phase AD-H or OD-H columns.

Column chromatography

The column-chromatographic purification was carried out with Merck® Silica Gel 60 (0.04-0.063 nm) as the stationary phase and different proportions of ethyl acetate and Hexane were used as mobile phases.

Thin Layer Chromatography (TLC)

Glass TLC plates coated with silica were used. To detect the substances, fluorescence quenching was used under UV light at the wavelength of 254 nm. The detection of alcohols was determined with using the permanganate staining procedure. To follow the formation of the amine products the TLC plates were stained with ninhydrin resulting in the formation of purple or orange spots after heating.

APCI$^+$-MS

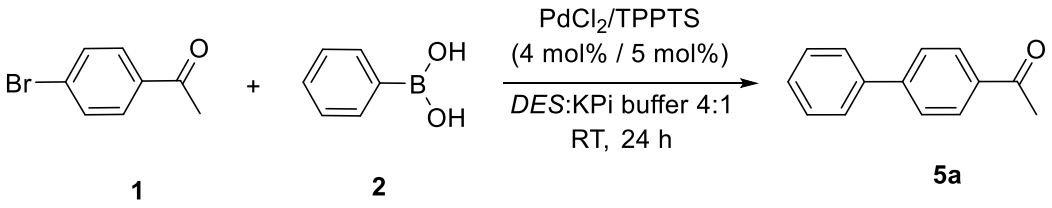
High resolution mass spectra were recorded on a Bruker Impact II instrument.

Preparation of *DESs*

Deep Eutectic Solvents *ChCl-Gly* (1:2 w/w), *ChCl*-H_2O (1:2 w/w), *ChCl-Sorb* (1:1 w/w) and *ChCl-Urea* (1:2 w/w) were prepared by adding choline chloride to a round-bottom flask followed by the addition of the second component. The mixture was then gently heated under stirring at 60-80 °C for 1 hour. The corresponding individual components were homogeneously dissolved and a clear solution was obtained signalling the completion of the process. For simplicity purposes the *DES* mixtures in the text were expressed as follows: 1*ChCl/*2*Gly,* 1*ChCl*/2H_2O, 1*ChCl*/1*Sorb* and 1*ChCl*/2Urea.

5.2 Pd-catalysed Suzuki cross-coupling reaction

5.2.1 Study of different Deep Eutectic Solvents



1 **2** **5a**

At first, a suspension of $PdCl_2$ (2.8 mg; 0.016 mmol; 4 mol %) and TPPTS (11.3 mg; 0.020 mmol; 5 mol %) in 1.0 mL of phosphate buffer 150 mM pH 8.5 was prepared. After 30 min the resulting catalyst solution was added to a mixture consisting of 4'-bromoacetophenone (**1b,** 0.389 mmol), boronic acid (**2**, 0.389 mmol), *DES* (8.0 mL) and phosphate buffer 150 mM pH 8.5 (1.0 mL). The pH was adjusted to 8.5 by adding aqueous 3 N NaOH and the reaction mixture stirred at room temperature for 24 h. After this time, the reaction was quenched with saturated aqueous NH_4Cl (10 mL) and extracted with ethyl acetate (2 × 20 mL). The combined organic layers were combined, dried with $NaSO_4$, filtered and concentrated under vacuum to provide the crude product **5a**. The degree of conversion (*c*) was determined via HPLC and the results obtained in these reactions are shown in **Table 21**.

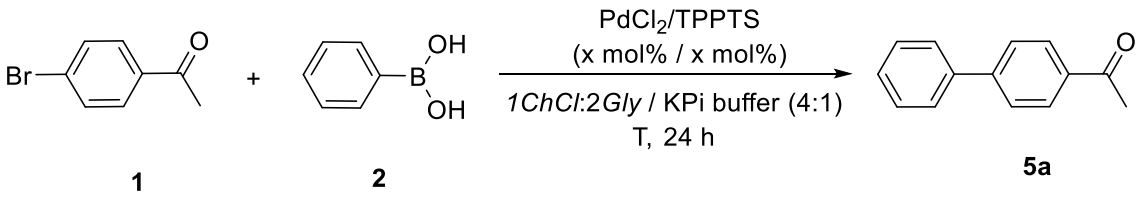
Table 21. Screening of different *DESs* to obtain the ketone **5a**

Entry	*DES*	c (%)
1	1*ChCl*/2*Gly*	92
2	1*ChCl*/1*Sorb*	82
3	1*ChCl*/2Urea	0
4	1*ChCl*/2H_2O	80

5.2.2 Study of amount of catalyst and temperature

It was performed following the procedure detailed above. The degree of conversion (*c*) of each reaction was determined from HPLC analysis of the crude material. The results obtained in these reactions are shown in **Table 22**.

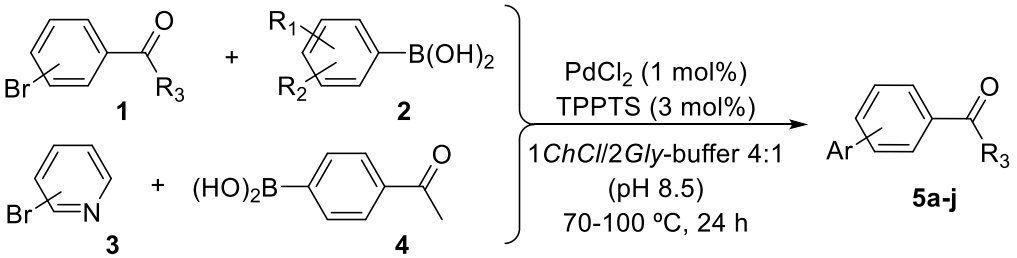
Table 22. Screening of amount of catalyst and temperature to obtain the ketone **5a**



1 **2** **5a**

Entry	[Pd]/ligand [mol%]	T (°C)	*c* (%)
1	4/5	rt	92
2	1/3	rt	78
3	1/3	70	>99

5.2.3 Synthesis of biarylketones in *DES*-Buffer medium after process optimisation



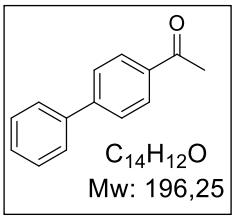
R_1, R_2 = H, Me, OMe, F

R_3 = Me,Et

General procedure for the preparation of biaryl ketones:

At first, a suspension of $PdCl_2$ (3.5 mg; 0.02 mmol; 1 mol%) and TPPTS (34 mg; 0.06 mmol; 3 mol%) in 1 mL of phosphate buffer 150 mM pH 8.5 was prepared. After 30 min the resulting catalyst solution was added to a mixture, consisting of arylbromide (**1** or **3**, 1.945 mmol; 1 eq, 200 mM), boronic acid (**2** or **4**, 1.945 mmol; 1 eq, 200 mM), *DES* (8 mL) and phosphate buffer 150 mM pH 8.5 (1 mL). The pH was adjusted to 8.5 by dropwise addition of aq 3 N NaOH and the reaction mixture was heated according to the substrate of choice for 24 h. Then, 20 mL of aq saturated NH_4Cl was added and extracted with ethyl acetate (2x20 mL). The combined organic layers were combined, dried with Na_2SO_4, filtered and concentrated under vacuum providing the crude product (**5 a-j**).

5.2.3.1 *Synthesis of 1-([1,1'-biphenyl]-4-yl)ethanone (5a)*

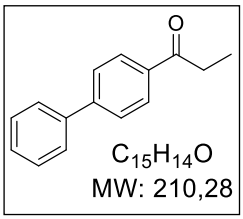


At first, a suspension of $PdCl_2$ (3.5 mg; 0.02 mmol; 1 mol%) and TPPTS (34 mg; 0.06 mmol; 3 mol%) in 1 mL of phosphate buffer 150 mM pH 8.5 was prepared. After 30 min the resulting catalyst solution was added to a mixture, consisting of 4'-bromoacetophenone (386 mg, 1.945 mmol; 1 eq, 200 mM), phenylboronic acid (**2**, 237,76 mg, 1.945 mmol; 1 eq, 200 mM), *DES* (8 mL) and phosphate buffer 150 mM pH 8.5 (1 mL). The pH was adjusted to 8.5 by dropwise addition of aq 3 N NaOH and the reaction mixture was heated to 70 °C for 24 h. Then, 20 mL of aq saturated NH_4Cl was added and extracted with ethyl acetate (2x20 mL). The combined organic layers were combined, dried with Na_2SO_4, filtered and concentrated under vacuum providing the crude product. With further purification by flash chromatography (silica gel 60 Å, hexane-ethyl acetate 14: 1) a white solid was obtained.

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 2.64 (s, 3H), 7.43 – 7.38 (m, 1H), 7.51 – 7.45 (m, 2H), 7.65 – 7.61 (m, 2H), 7.71 – 7.67 (m, 2H), 8.07 – 8.01 (m, 2H). These spectroscopic data are in good agreement with the characterized compound in the literature.[23]

RP-HPLC (Method A): t_R = 5.8 min

5.2.3.2 *Synthesis of 1-([1,1'-biphenyl]-4-yl)propan-1-one (5b)*

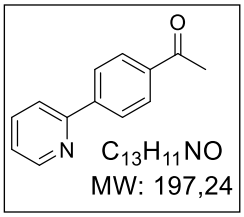


At first, a suspension of $PdCl_2$ (3.5 mg; 0.02 mmol; 1 mol%) and TPPTS (34 mg; 0.06 mmol; 3 mol%) in 1 mL of phosphate buffer 150 mM pH 8.5 was prepared. After 30 min the resulting catalyst solution was added to a mixture, consisting of 4-bromopropiophenone (414,42 mg, 1.945 mmol; 1 eq, 200 mM), phenylboronic acid (**2**, 237,8 mg, 1.945 mmol; 1 eq, 200 mM), *DES* (8 mL) and phosphate buffer 150 mM pH 8.5 (1 mL). The pH was adjusted to 8.5 by dropwise addition of aq 3 N NaOH and the reaction mixture was heated to 70 °C for 24 h. Then, 20 mL of aq saturated NH_4Cl was added and extracted with ethyl acetate (2x20 mL). The combined organic layers were combined, dried with Na_2SO_4, filtered and concentrated under vacuum providing the crude product. With further purification by flash chromatography (silica gel 60 Å, hexane-ethyl acetate 14: 1) a white solid was obtained.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.26 (t, *J* 7.5 Hz, 3H), 3.04 (q, *J* 7.5 Hz, 2H), 7.37-7.51 (m, 3H), 7.60-7.72 (m, 4H), 8.04 (d, *J* 8.4 Hz, 2H). These spectroscopic data are in good agreement with the characterized compound in the literature.[171]

RP-HPLC (Method A): t_R = 6.5 min

5.2.3.3 *Synthesis of 1-(4-(pyridin-2-yl)phenyl)ethanone (5c)*

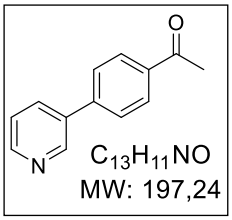


At first, a suspension of $PdCl_2$ (3.5 mg; 0.02 mmol; 1 mol%) and TPPTS (34 mg; 0.06 mmol; 3 mol%) in 1 mL of phosphate buffer 150 mM pH 8.5 was prepared. After 30 min the resulting catalyst solution was added to a mixture, consisting of 2-bromopyridine (307,3 mg, 1.945 mmol; 1 eq, 200 mM), 4-acetylphenylboronic acid (**2**, 318,9 mg, 1.945 mmol; 1 eq, 200 mM), *DES* (8 mL) and phosphate buffer 150 mM pH 8.5 (1 mL). The pH was adjusted to 8.5 by dropwise addition of aq 3 N NaOH and the reaction mixture was heated to 100 °C for 24 h. Then, 20 mL of aq saturated NH_4Cl was added and extracted with ethyl acetate (2x20 mL). The combined organic layers were combined, dried with Na_2SO_4, filtered and concentrated under vacuum providing the crude product. With further purification by flash chromatography (silica gel 60 Å, hexane-ethyl acetate 3: 1) a yellow solid was obtained.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.64 (s, 3H), 7.28 (q, *J* 3.9 Hz, 1H), 7.71 (d, *J* 8.4 Hz, 1H), 7.79 (d, *J* 4.8 Hz, 1H), 8.04-8.11 (m, 4H), 8.72 (d, *J* 4.8 Hz, 1H). These spectroscopic data are in good agreement with the characterized compound in the literature. [172]

RP-HPLC (Method B): t_R = 5.6 min

5.2.3.4 *Synthesis of 1-(4-(pyridin-3-yl)phenyl)ethanone (5d)*

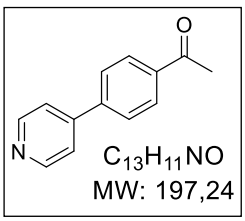


At first, a suspension of $PdCl_2$ (3.5 mg; 0.02 mmol; 1 mol%) and TPPTS (34 mg; 0.06 mmol; 3 mol%) in 1 mL of phosphate buffer 150 mM pH 8.5 was prepared. After 30 min the resulting catalyst solution was added to a mixture, consisting of 3-bromopyridine (307,3 mg, 1.945 mmol; 1 eq, 200 mM), 4-acetylphenylboronic acid (**2**, 318,9 mg, 1.945 mmol; 1 eq, 200 mM), *DES* (8 mL) and phosphate buffer 150 mM pH 8.5 (1 mL). The pH was adjusted to 8.5 by dropwise addition of aq 3 N NaOH and the reaction mixture was heated to 100 °C for 24 h. Then, 20 mL of aq saturated NH_4Cl was added and extracted with ethyl acetate (2x20 mL). The combined organic layers were combined, dried with Na_2SO_4, filtered and concentrated under vacuum providing the crude product. With further purification by flash chromatography (silica gel 60 Å, hexane-ethyl acetate 3: 1) a white solid was obtained.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.65 (s, 3H), 7.40 (dd, *J* 8.1 and 4.8 Hz, 1H), 7.68 (d, *J* 8.4 Hz, 1H), 7.91 (dt, *J* 7.8 and 2.1 Hz, 1H), 8.07 (d, *J* 8.4 Hz, 2H), 8.65 (dd, *J* 4.8 and 1.5 Hz, 1H), 8.88 (d, *J* 2.1 Hz, 1H). These spectroscopic data are in good agreement with the characterized compound in the literature. [173]

RP-HPLC (Method B): t_R = 3.8 min

5.2.3.5 *Synthesis of 1-(4-(pyridin-4-yl)phenyl)ethanone (5e)*



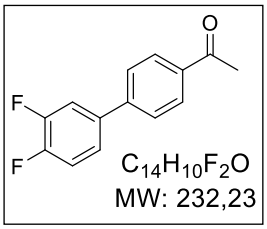
At first, a suspension of $PdCl_2$ (3.5 mg; 0.02 mmol; 1 mol%) and TPPTS (34 mg; 0.06 mmol; 3 mol%) in 1 mL of phosphate buffer 150 mM pH 8.5 was prepared. After 30 min the resulting catalyst solution was added to a mixture, consisting of 4-bromopyridine hydrochloride (378,2 mg, 1.945 mmol; 1 eq, 200 mM), 4-acetylphenylboronic acid (318,9 mg, 1.945 mmol; 1 eq, 200 mM), *DES* (8 mL) and phosphate buffer 150 mM pH 8.5 (1 mL). The pH was adjusted to 8.5 by dropwise addition of aq 3 N NaOH and the reaction mixture was heated to 100 °C for 24 h. Then, 20 mL of aq saturated NH_4Cl was added and extracted with ethyl acetate (2x20 mL). The combined organic layers were combined,

dried with Na_2SO_4, filtered and concentrated under vacuum providing the crude product. With further purification by flash chromatography (silica gel 60 Å, hexane-ethyl acetate 1: 2) a white solid was obtained.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.66 (s, 3H), 7.53 (d, *J* 6.0 Hz, 2H), 7.73 (d, *J* 8.4 Hz, 2H), 8.07 (d, *J* 8.4 Hz, 2H), 8.70 (d, *J* 6.0 Hz, 2H), 8.65 (dd, *J* 4.8 and 1.5 Hz, 1H), 8.88 (d, *J* 2.1 Hz, 1H). These spectroscopic data are in good agreement with the characterized compound in the literature. [174]

RP-HPLC (Method B): t_R = 3.8 min

5.2.3.6 *Synthesis of 1-(3',4'-difluoro-[1,1'-biphenyl]-4-yl)ethanone (5f)*

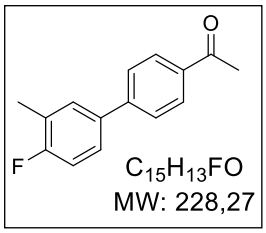


At first, a suspension of $PdCl_2$ (3.5 mg; 0.02 mmol; 1 mol%) and TPPTS (34 mg; 0.06 mmol; 3 mol%) in 1 mL of phosphate buffer 150 mM pH 8.5 was prepared. After 30 min the resulting catalyst solution was added to a mixture, consisting of 4'-bromoacetophenone (386 mg, 1.945 mmol; 1 eq, 200 mM), 3,4-difluorophenylboronic acid (306,4 mg, 1.945 mmol; 1 eq, 200 mM), *DES* (8 mL) and phosphate buffer 150 mM pH 8.5 (1 mL). The pH was adjusted to 8.5 by dropwise addition of aq 3 N NaOH and the reaction mixture was heated to 70 °C for 24 h. Then, 20 mL of aq saturated NH_4Cl was added and extracted with ethyl acetate (2x20 mL). The combined organic layers were combined, dried with Na_2SO_4, filtered and concentrated under vacuum providing the crude product. With further purification by flash chromatography (silica gel 60 Å, hexane-ethyl acetate 8: 1) a white solid was obtained.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.63 (s, 3H), 7.18-7.46 (m, 3H), 7.60 (d, *J* 8.1 Hz, 2H), 8.01 (d, *J* 8.1 Hz, 2H), 8.70 (d, *J* 6.0 Hz, 2H), 8.65 (dd, *J* 4.8 and 1.5 Hz, 1H), 8.88 (d, *J* 2.1 Hz, 1H). These spectroscopic data are in good agreement with the characterized compound in the literature.[175]

RP-HPLC (Method A): t_R = 6.0 min

5.2.3.7 *Synthesis of 1-(4'-fluoro-3'-methyl-[1,1'-biphenyl]-4-yl)ethanone (5g)*

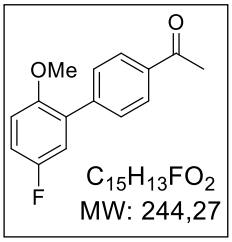


At first, a suspension of $PdCl_2$ (3.5 mg; 0.02 mmol; 1 mol%) and TPPTS (34 mg; 0.06 mmol; 3 mol%) in 1 mL of phosphate buffer 150 mM pH 8.5 was prepared. After 30 min the resulting catalyst solution was added to a mixture, consisting of 4'-bromoacetophenone (386 mg, 1.945 mmol; 1 eq, 200 mM), 4-fluoro-3-methylphenylboronic acid (299,4 mg, 1.945 mmol; 1 eq, 200 mM), *DES* (8 mL) and phosphate buffer 150 mM pH 8.5 (1 mL). The pH was adjusted to 8.5 by dropwise addition of aq 3 N NaOH and the reaction mixture was heated to 70 °C for 24 h. Then, 20 mL of aq saturated NH_4Cl was added and extracted with ethyl acetate (2x20 mL). The combined organic layers were combined, dried with Na_2SO_4, filtered and concentrated under vacuum providing the crude product. With further purification by flash chromatography (silica gel 60 Å, hexane-ethyl acetate 8: 1) a white solid was obtained.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.35 (d, *J* 1.8 Hz, 3H), 2.64 (s, 3H), 7.09 (t, *J* 9.0 Hz, 1H), 7.36-7.47 (m, 2H), 7.62 (d, *J* 8.4 Hz, 2H), 8.01 (d, *J* 8.4 Hz, 2H). These spectroscopic data are in good agreement with the characterized compound in the literature. [176]

RP-HPLC (Method A): t_R = 6.4 min

5.2.3.8 *Synthesis of 1-(5'-fluoro-2'-methoxy-[1,1'-biphenyl]-4-yl)ethanone (5h)*



At first, a suspension of $PdCl_2$ (3.5 mg; 0.02 mmol; 1 mol%) and TPPTS (34 mg; 0.06 mmol; 3 mol%) in 1 mL of phosphate buffer 150 mM pH 8.5 was prepared. After 30 min the resulting catalyst solution was added to a mixture, consisting of 4'-bromoacetophenone (386 mg, 1.945 mmol; 1 eq, 200 mM), 5-Fluoro-2-methoxyphenylboronic acid (330,6 mg, 1.945 mmol; 1 eq, 200 mM), *DES* (8 mL) and phosphate buffer 150 mM pH 8.5 (1 mL). The pH was adjusted to 8.5 by dropwise addition of aq 3 N NaOH and the reaction mixture was heated to 70 °C for 24 h. Then, 20 mL of aq saturated NH_4Cl was added and extracted with ethyl acetate (2x20 mL). The combined organic layers were combined, dried with Na_2SO_4, filtered and concentrated under vacuum providing the crude product. With further purification by flash chromatography (silica gel 60 Å, hexane-ethyl acetate 8: 1) a white solid was obtained (Mp: 113-115 °C).

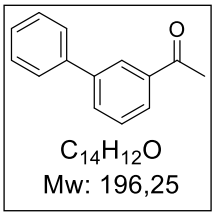
^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.63 (s, 3H), 3.79 (s, 3H), 6.87-6.92 (m, 1H), 7.00-7.08 (m, 2H), 7.61 (d, J 8.4 Hz, 2H), 8.01 (d, J 8.4 Hz, 2H).

^{13}C NMR (CDCl3, 75.5 MHz): δ 26.68 (CH_3), 56.19 (CH_3), 112.40 (CH, d, J_{CF}= 8 Hz), 115.22 (CH, d, J_{CF}= 23 Hz), 117.27 (CH, d, J_{CF}= 23 Hz), 128.17 (2CH), 129.61 (2CH), 130.63 (C, d, J_{CF}= 7 Hz), 135.86 (C), 142.35 (C), 152.26 (C), 157.08 (C, d, J_{CF}= 239 Hz), 197.80 (C)

MS ($APCI^+$, m/z): 245 [$(M+H)^+$, 100%].

RP-HPLC (Method A): t_R = 5.9 min

5.2.3.9 *Synthesis of 1-([1,1'-biphenyl]-3-yl)ethanone (5i)*

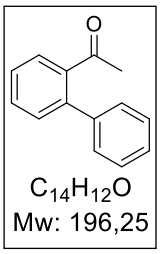


At first, a suspension of $PdCl_2$ (3.5 mg; 0.02 mmol; 1 mol%) and TPPTS (34 mg; 0.06 mmol; 3 mol%) in 1 mL of phosphate buffer 150 mM pH 8.5 was prepared. After 30 min the resulting catalyst solution was added to a mixture, consisting of 3'-bromoacetophenone (386 mg, 1.945 mmol; 1 eq, 200 mM), phenylboronic acid (**2**, 237,76 mg, 1.945 mmol; 1 eq, 200 mM), *DES* (8 mL) and phosphate buffer 150 mM pH 8.5 (1 mL). The pH was adjusted to 8.5 by dropwise addition of aq 3 N NaOH and the reaction mixture was heated to 70 °C for 24 h. Then, 20 mL of aq saturated NH_4Cl was added and extracted with ethyl acetate (2x20 mL). The combined organic layers were combined, dried with Na_2SO_4, filtered and concentrated under vacuum providing the crude product. With further purification by flash chromatography (silica gel 60 Å, hexane-ethyl acetate 14: 1) a yellowish oil was obtained.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.66 (s, 3H), 7.35-7.57 (4H, m), 7.60-7.65 (2H, m), 7.79 (dt, *J* 7.8 and 1.5 Hz, 1H), 7.94 (dt, *J* 7.8 and 1.5 Hz, 1H), 8.21 (t, *J* 4.8 Hz, 1H). These spectroscopic data are in good agreement with the characterized compound in the literature.[172]

HPLC (Method A): t_R = 5.9 min

5.2.3.10 *Synthesis of 1-([1,1'-biphenyl]-2-yl)ethanone (5j)*

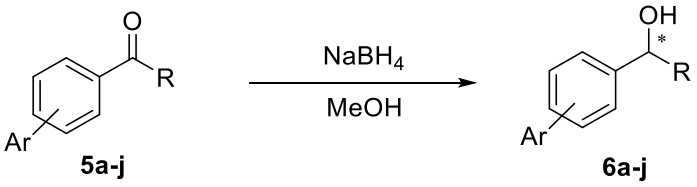


At first, a suspension of $PdCl_2$ (3.5 mg; 0.02 mmol; 1 mol%) and TPPTS (34 mg; 0.06 mmol; 3 mol%) in 1 mL of phosphate buffer 150 mM pH 8.5 was prepared. After 30 min the resulting catalyst solution was added to a mixture, consisting of 2'-bromoacetophenone (386 mg, 1.945 mmol; 1 eq, 200 mM), phenylboronic acid (**2**, 237,76 mg, 1.945 mmol; 1 eq, 200 mM), *DES* (8 mL) and phosphate buffer 150 mM pH 8.5 (1 mL). The pH was adjusted to 8.5 by dropwise addition of aq 3 N NaOH and the reaction mixture was heated to 70 °C for 24 h. Then, 20 mL of aq saturated NH_4Cl was added and extracted with ethyl acetate (2x20 mL). The combined organic layers were combined, dried with Na_2SO_4, filtered and concentrated under vacuum providing the crude product. With further purification by flash chromatography (silica gel 60 Å, hexane-ethyl acetate 14: 1) a yellowish oil was obtained.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.01 (s, 3H), 7.32-7.36 (2H, m), 7.37-7.46 (5H, m), 7.47-7.61 (m, 2H). These spectroscopic data are in good agreement with the characterized compound in the literature.[177]

HPLC (Method A): t_R = 5.7 min

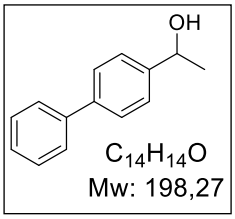
5.3 Reduction of the products from the Suzuki cross-coupling reaction using $NaBH_4$



General procedure for the reduction of biaryl ketones using sodium borohydride

After dissolving the biaryl ketone (**5 a-j**, 0.1 mmol) in methanol (2 mL), sodium borohydride (0.3 mmol) is added and the reaction is stirred for 24 hours at room temperature. The product (**6 a-j**) is then washed with water (10 mL) and the reaction mixture extracted with ethyl acetate (3x 10 mL). The organic layers are combined, dried with Na_2SO_4, filtered and concentrated under vacuum providing the final racemic alcohol.

5.3.1 Synthesis of racemic -1-([1,1'-biphenyl]-4-yl)ethanol (6a)

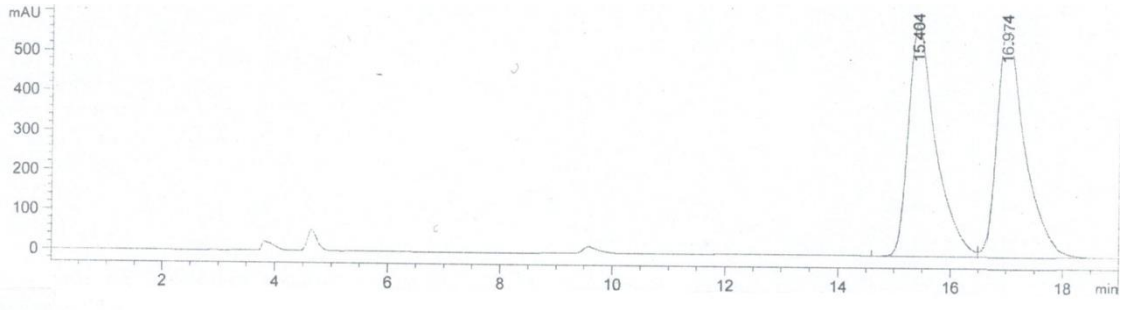


The title compound was obtained according to the general procedure (Yield >95%).

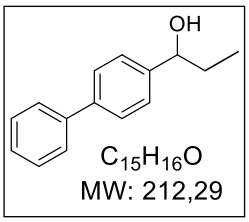
^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.52 (d, *J* 6.6 Hz, 3H), 1.70 (brs, OH), 4.88 (q, *J* 6.6 Hz, 1H), 7.32-7.38 (m, 1H), 7.40-7.48 (m, 4H), 7.58 (d, *J* 8.1 Hz, 4H). These spectroscopic data are in good agreement with those previously published.[172]

NP-HPLC: (OD, hexane/isopropanol 95:5 v/v, 0.8 mL/min, 258 nm) t_R = 15.4 min (*S*), 16.9 min (*R*)

HPLC separation for both enantiomers of compound (±)-**6a**



5.3.2 Synthesis of racemic - 1-([1,1'-biphenyl]-4-yl)propan-1-ol (6b)

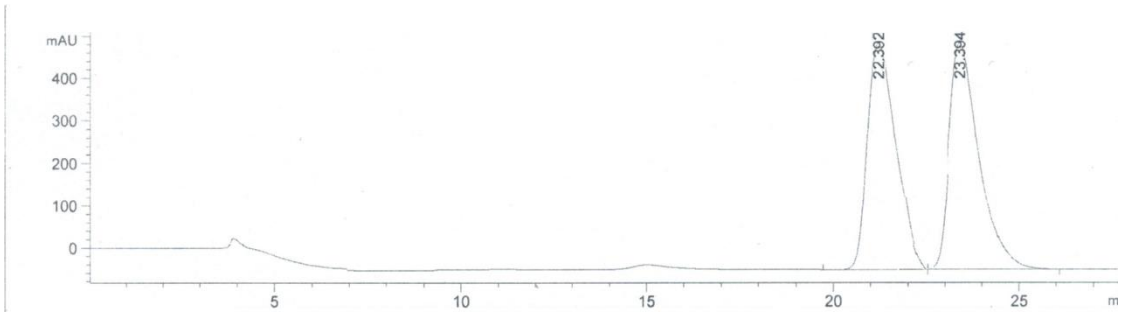


The title compound was obtained according to the general procedure (Yield >95%).

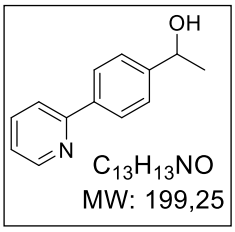
^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 0.97 (d, *J* 6.3 Hz, 3H), 1.74-1.97 (m, 2H+OH), 4.68 (t, *J* 6.3 Hz, 1H), 7.33-7.51 (m, 5H), 7.57-7.65 (m, 4H). These spectroscopic data are in good agreement with those previously published.[172]

NP-HPLC: (OD, hexane/isopropanol 97:3 v/v, 0.8 mL/min, 258 nm) t_R = 22.3 min (*S*), 23.3 min (*R*)

HPLC separation for both enantiomers of compound (±)-**6b**



5.3.3 Synthesis of racemic - 1-(4-(pyridin-2-yl)phenyl)ethanol (6c)

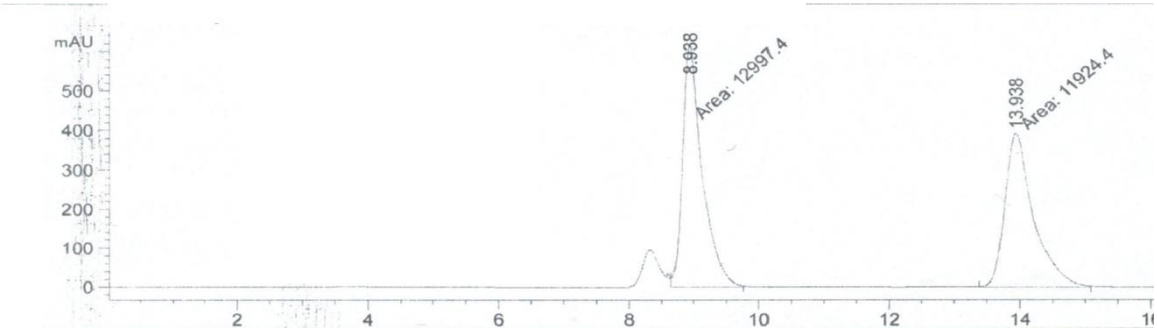


The title compound was obtained according to the general procedure (Yield >95%).

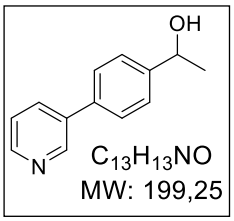
^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.53 (d, *J* 6.6 Hz, 3H), 2.11 (brs, OH), 4.96 (q, *J* 6.6 Hz, 1H), 7.20-7.26 (m, 1H), 7.43-7.58 (m, 3H), 7.70-7.79 (m, 2H), 7.96 (d, *J* 8.1 Hz, 2H), 8.68 (d, *J* 4.8 Hz, 1H). These spectroscopic data are in good agreement with those previously published.[172]

NP-HPLC: (OD, hexane/isopropanol 75:25 v/v, 0.8 mL/min, 258 nm) t_R = 8.9 min (*S*), 13.9 min (*R*)

HPLC separation for both enantiomers of compound (±)-**6c**



5.3.4 Synthesis of racemic - 1-(4-(pyridin-3-yl)phenyl)ethanol (6d)



The title compound was obtained according to the general procedure (Yield >95%).

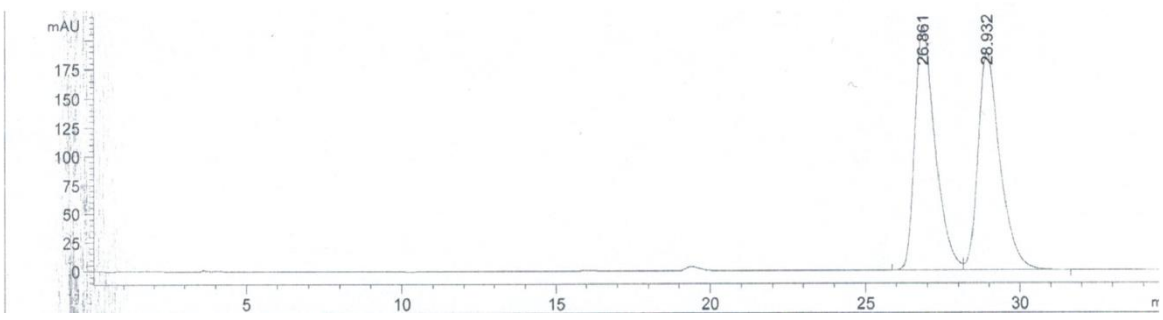
^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.52 (d, *J* 6.6 Hz, 3H), 3.39 (brs, OH), 4.93 (q, *J* 6.6 Hz, 1H), 7.34 (dd, *J* 8.1 and 4.8 Hz, 1H), 7.43-7.51 (m, 4H), 7.83 (dt, *J* 4.8 and 1.8 Hz, 1H), 8.50-8.54 (m, 2H)

^{13}C NMR (75.5 MHz, CDCl3): δ 25.37 (CH_3), 69.90 (CH),123.75 (CH), 126.45 (2CH), 127.31 (2CH), 134.51 (CH), 136.59 (2C), 146.52 (C), 148.15 (CH), 148.22 (CH)

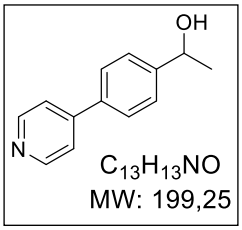
MS (APCI$^+$, m/z): 200 [$(M+H)^+$, 100%].

NP-HPLC: (AD-H, hexane/isopropanol 90:19 v/v, 0.8 mL/min, 258 nm) t_R = 26.8 min (*S*), 28.9 min (*R*)

HPLC separation for both enantiomers of compound (±)-**6d**



5.3.5 Synthesis of racemic- 1-(4-(pyridin-4-yl)phenyl)ethanol (6e)

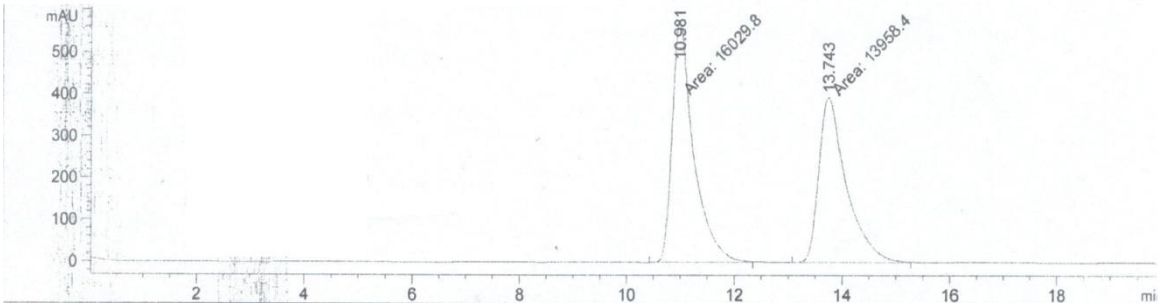


The title compound was obtained according to the general procedure (Yield >95%).

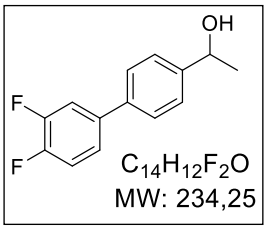
^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.54 (d, *J* 6.6 Hz, 3H), 2.48 (brs, OH), 4.98 (q, *J* 6.6 Hz, 1H), 7.47-7.5 (m, 4H), 7.63 (d, *J* 8.4 Hz, 2H), 8.53 (d, *J* 6.3 Hz, 1H). These spectroscopic data are in good agreement with those previously published.[178]

NP-HPLC: (AD-H, hexane/isopropanol 75:25 v/v, 0.8 mL/min, 258 nm) t_R = 10.9 min (*S*), 13.7 min (*R*)

HPLC separation for both enantiomers of compound (±)-**6e**



5.3.6 Synthesis of racemic - 1-(3',4'-difluoro-[1,1'-biphenyl]-4-yl)ethanol (6f)



The title compound was obtained according to the general procedure (Yield >95%).

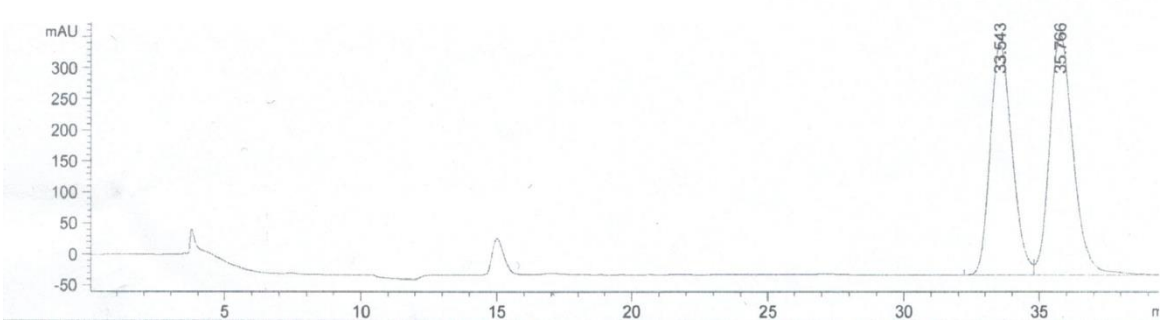
^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.60 (d, *J* 6.6 Hz, 3H), 2.16 (brs, OH), 5.02 (q, *J* 6.6 Hz, 1H), 7.23-77 (m, 3H), 7.51 (d, *J* 8.4 Hz, 2H), 7.58 (d, *J* 8.4 Hz, 2H).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ 25.21 (CH_3), 70.04 (CH), 115.84 (CH, d, J_{CF}= 17 Hz), 117.51 (CH, d, J_{CF}= 17 Hz), 122.93 (CH, dd, J_{CF}= 6 and 2 Hz), 126.02 (2CH), 127.04 (2CH), 137.90 (C, d, J_{CF}= 5 Hz), 138.29 (C), 145.44 (C), 148.52 (C, dd, J_{CF}= 45 and 12 Hz), 151.81 (C, dd, J_{CF}= 44 and 12 Hz).

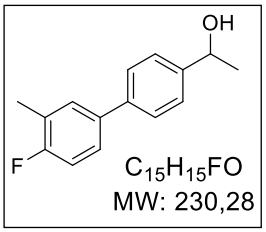
MS (APCI$^+$, m/z): 235 [$(M+H)^+$, 100%]

NP-HPLC: (AD-H, hexane/isopropanol 98:2 v/v, 0.8 mL/min, 258 nm) t_R = 33.5 min (*S*), 35.7 min (*R*)

HPLC separation for both enantiomers of compound (±)-**6f**



5.3.7 Synthesis of racemic- 1-(4'-fluoro-3'-methyl-[1,1'-biphenyl]-4-yl)ethanol (6g)



The title compound was obtained according to the general procedure (Yield >95%).

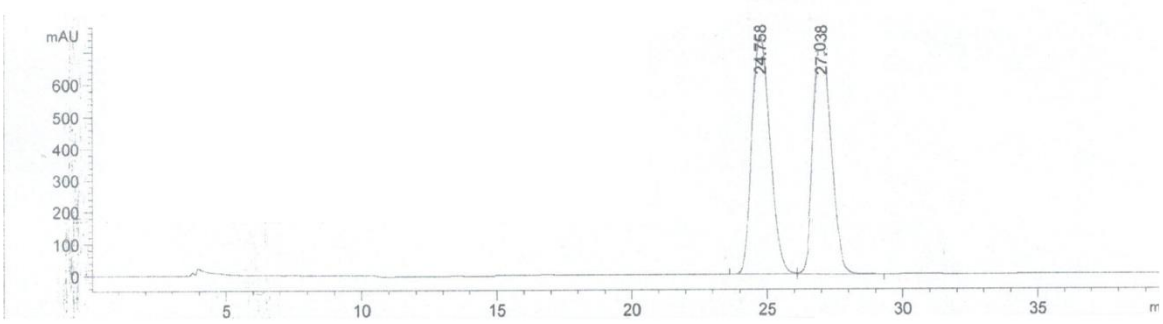
^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.53 (d, *J* 6.3 Hz, 3H), 1.91 (brs, OH), 2.34 (s ,3H), 4.94 (q, *J* 6.3 Hz, 1H), 7.06 (t, *J* 9.0 Hz, 1H), 7.31-7.47 (m, 4H), 7.51 (d, *J* 8.1 Hz, 2H).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ 14.68 (CH_3), 25.15 (CH_3), 70.14 (CH), 115.25 (CH, d, J_{CF}= 22 Hz), 125.02 (C, d, J_{CF}= 17 Hz), 125.85 (2CH), 127.09 (2CH), 130.13 (CH, d, J_{CF}= 5 Hz), 136.37 (C), 139.66 (C), 144.66 (C), 161.03 (C, d, J_{CF}= 245 Hz).

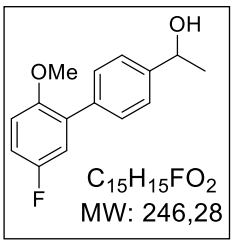
MS (APCI$^+$, m/z): 231 [$(M+H)^+$, 100%].

NP-HPLC: (AD-H, hexane/isopropanol 98:2 v/v, 0.8 mL/min, 258 nm) t_R = 24.7 min (*S*), 27.0 min (*R*)

HPLC separation for both enantiomers of compound (±)-**6g**



5.3.8 Synthesis of racemic - 1-(5'-fluoro-2'-methoxy-[1,1'-biphenyl]-4-yl)ethanol (6h)



The title compound was obtained according to the general procedure (Yield >95%).

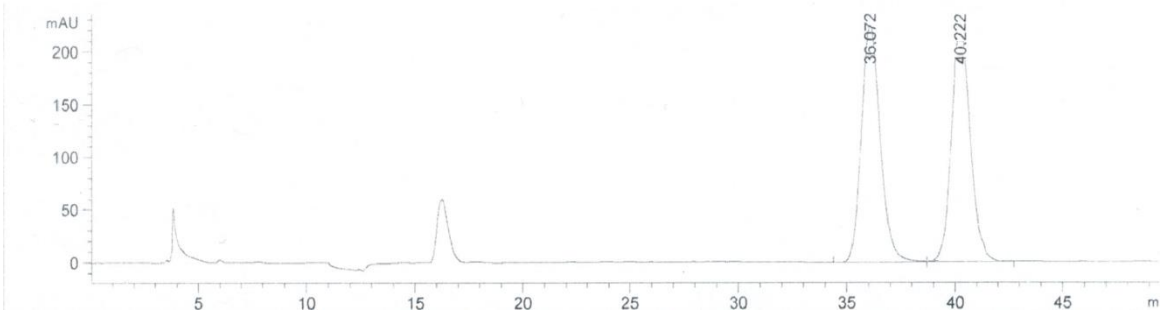
^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.54 (d, *J* 6.6 Hz, 3H), 1.97 (brs, OH), 3.78 (s ,3H), 4.93 (q, *J* 6.6 Hz, 1H), 6.86-6.94 (m, 1H), 6.95-7.08 (m, 2H), 7.42 (d, *J* 8.4 Hz, 2H), 7.51 (d, *J* 8.4 Hz, 2H);

^{13}C NMR (75.5 MHz, $CDCl_3$): δ 25.18 (CH_3), 56.30 (CH_3), 70.33 (CH), 112.39 (CH, d, J_{CF}= 7 Hz), 114.39 (CH, d, J_{CF}= 23 Hz), 117.45 (CH, d, J_{CF}= 23 Hz), 125.35 (2CH), 129.62 (2CH), 131.77 (C, d, J_{CF}= 7 Hz), 136.74 (C), 145.02 (C), 152.77 (C), 157.24 (C, d, J_{CF}= 239 Hz);

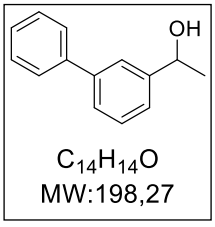
MS (APCI$^+$, m/z): 247 [$(M+H)^+$, 100%].

NP-HPLC: (AD-H, hexane/isopropanol 98:2 v/v, 0.8 mL/min, 258 nm) t_R = 35.0 min (*R*), 40.2 min (*S*)

HPLC separation for both enantiomers of compound (±)-**6h**



5.3.9 Synthesis of racemic - 1-([1,1'-biphenyl]-3-yl)ethanol (6i)

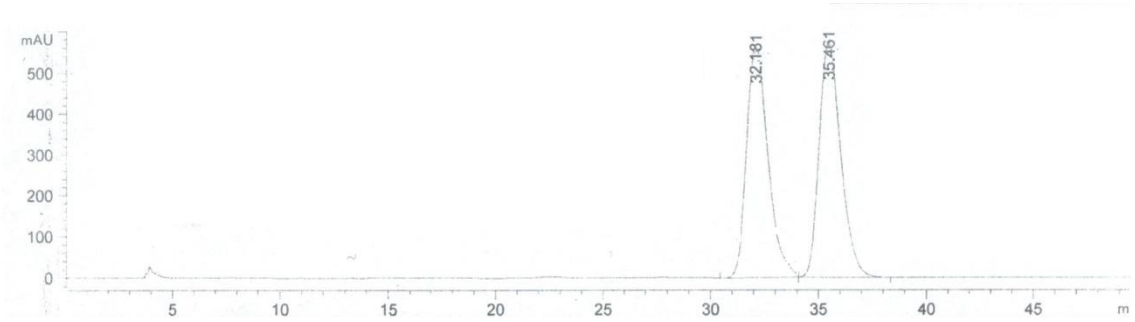


The title compound was obtained according to the general procedure (Yield >95%).

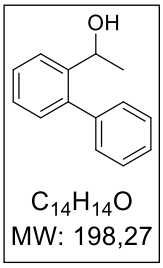
^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.55 (d, *J* 6.3 Hz, 3H), 1.89 (brs, OH), 4.99 (q, *J* 6.3 Hz, 1H), 7.32-7.55 (m, 6H), 7.57-7.64 (m, 3H). These spectroscopic data are in good agreement with those previously reported.[172]

NP-HPLC: (AD-H, hexane/isopropanol 98:2 v/v, 0.8 mL/min, 258 nm) t_R = 32.1 min (*R*), 35.4 min (*S*)

HPLC separation for both enantiomers of compound (±)-**6i**



5.3.10 Synthesis of racemic - 1-([1,1'-biphenyl]-2-yl)ethanol (6j)

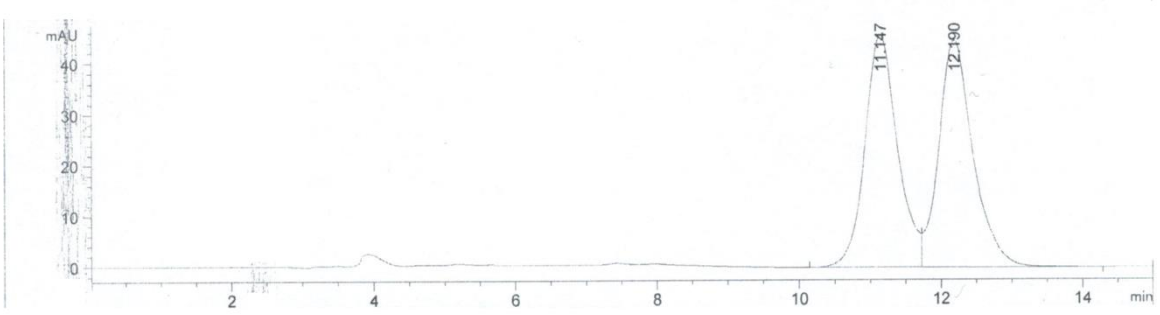


The title compound was obtained according to the general procedure (Yield >95%).

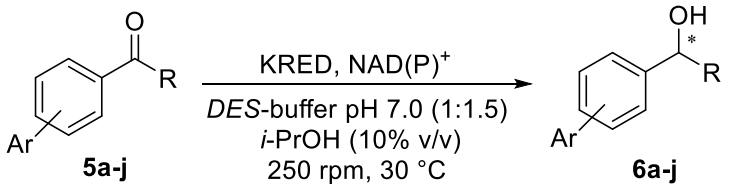
^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.42 (d, *J* 6.3 Hz, 3H), 1.69 (brs, OH), 4.98 (q, *J* 6.3 Hz, 1H), 7.21 (dd, *J* 7.5 and 1.5 Hz, 1H), 7.28-7.35 (m, 3H), 7.37-7.45 (m, 4H), 7.68 (d, *J* 7.8 Hz, 1H). These spectroscopic data are in good agreement with those previously reported.[172]

NP-HPLC: (OD, hexane/isopropanol 99:1 v/v, 0.8 mL/min, 258 nm) t_R = 11.1 min (*R*), 12.1 min (*S*).

HPLC separation for both enantiomers of compound (±)-**6j**



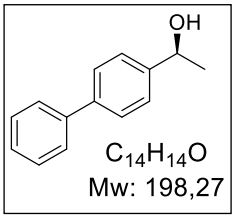
5.4 Enzymatic reduction of biaryl ketones in a *DES*-buffer mixture



General procedure for the bioreduction of biaryl ketones in a *DES*-buffer mixture:

In a 2.0 mL Eppendorf tube, ketone (**5 a-j**,5 mM) and purified KRED (1.0 mg) were added to a 1*ChCl:*2*Gly* (215 mL)/125 mM KH_2PO_4 buffer pH 7.0 (325 mL) mixture (containing 1.25 mM $MgSO_4$, 1mM $NADP^+$), and *i*-PrOH (60 mL, 10% v/v). For *L. kefir* reactions, 15 U of ADH and KPi buffer 50 mM pH 7.0 were used, the mixture containing 1 mM $MgCl_2$ and 1 mM $NADP^+$; For *R. ruber* reactions, 5 U of ADH and KPi buffer 50 mM pH 7.0 were used, the mixture containing 1 mM NAD^+. In all the cases, the reaction mixture was shaken at 30 °C and 250 rpm for 24 h. To determine the conversion, 10 mL of the mixture were diluted with 90 mL of Milli-Q water and analysed by achiral reverse phase. The mixture was then extracted with AcOEt (2x500 mL) and aq saturated NH_4Cl (110 mL), the organic layers separated by centrifugation (120 sec, 1300 rpm), combined and dried over Na_2SO_4. The enantiomeric excess of alcohols (**6 a-j**) was measured by chiral HPLC.

5.4.1 Synthesis of *(S)*-1-([1,1'-biphenyl]-4-yl)ethanol (6a)



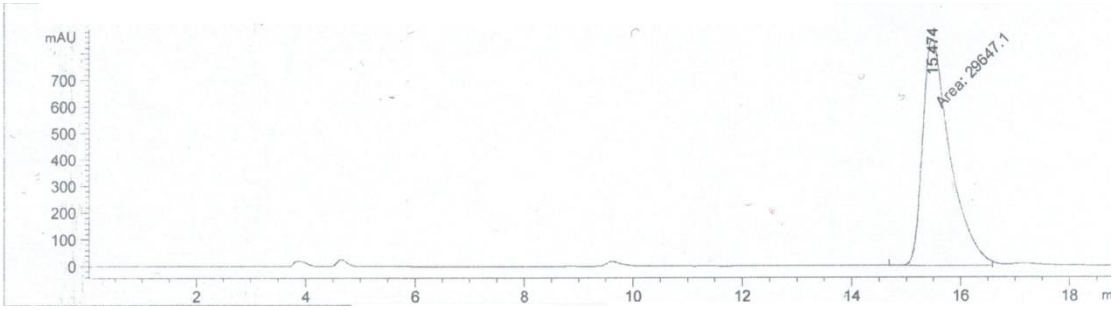
In a 15 mL Falcon tube, **5a** (5 mM) and ADH from *Rhodococcus ruber* (10 U) were added to a 1*ChCl*:2*Gly* (2.15 mL)/50 mM KH_2PO_4 buffer pH 7.0 (3.25 mL) mixture (containing 1 mM NAD^+), and *i*-PrOH (600 µL). The reaction was shaken at 30 °C and 250 rpm for 24 h. To determine the conversion, 10 µL of the mixture were diluted with 90 µL of Milli-Q water and analysed by achiral reverse phase with previous centrifuging and filtering of the sample. Then, the mixture was treated with aq saturated NH_4Cl (1 × 10 mL) and extracted with ethyl acetate (2 × 5 mL), the organic layers combined, filtered and concentrated under vacuum to provide the crude product (*S*)-**6a** (yield >95%) as a colourless oil.

$[\alpha]_D^{20}$ −58.5 (*c* 0.2, $CHCl_3$), *ee* >99%. Lit. [172] for (*S*)-**6a**: $[\alpha]_D^{20}$ −44.1 (*c* 0.97, $CHCl_3$), *ee* >99%.

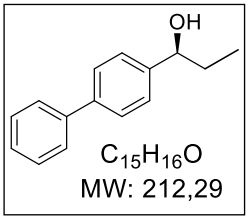
^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.52 (d, *J* 6.6 Hz, 3H), 1.70 (brs, OH), 4.88 (q, *J* 6.6 Hz, 1H), 7.32-7.38 (m, 1H), 7.40-7.48 (m, 4H), 7.58 (d, *J* 8.1 Hz, 4H).

NP-HPLC: (OD, hexane/isopropanol 95:5 v/v, 0.8 mL/min, 258 nm) t_R = 15.4 min (*S*).

Compound (*S*)-**6a** in >99% *ee* after bioreduction with *R.ruber*



5.4.2 Synthesis of *(S)*-1-([1,1'-biphenyl]-4-yl)propan-1-ol (6b)



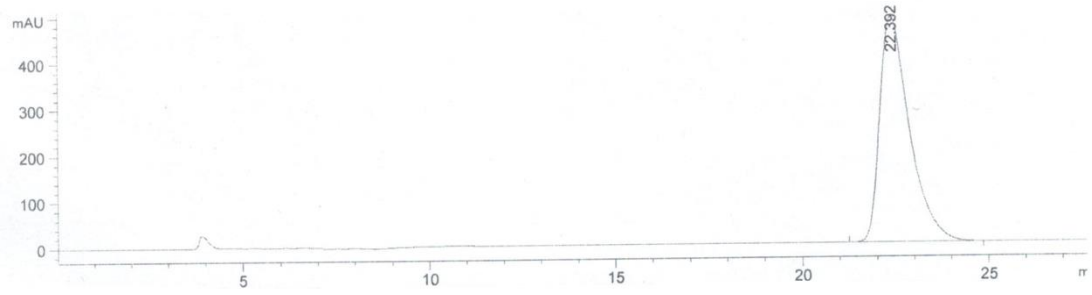
In a 15 mL Falcon tube, **5b** (5 mM) and ADH from *Rhodococcus ruber* (10 U) were added to a 1*ChCl*:2*Gly* (2.15 mL)/50 mM KH_2PO_4 buffer pH 7.0 (3.25 mL) mixture (containing 1 mM NAD^+), and *i*-PrOH (600 µL). The reaction was shaken at 30 °C and 250 rpm for 24 h. To determine the conversion, 10 µL of the mixture were diluted with 90 µL of Milli-Q water and analysed by achiral reverse phase with previous centrifuging and filtering of the sample. Then, the mixture was treated with aq saturated NH_4Cl (1 × 10 mL) and extracted with ethyl acetate (2 × 5 mL), the organic layers combined, filtered and concentrated under vacuum to provide the crude product (*S*)-**6b** (yield >95%) as a white solid.

$[\alpha]_D^{20}$ −22.1 (*c* 0.2, $CHCl_3$), *ee* >99%. Lit.[172] for (*S*)-**6b**: $[\alpha]_D^{20}$ −13.0 (*c* 0.98, $CHCl_3$), *ee* = 36%.

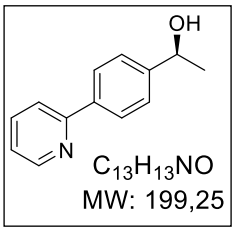
^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 0.97 (d, *J* 6.3 Hz, 3H), 1.74-1.97 (m, 2H+OH), 4.68 (t, *J* 6.3 Hz, 1H), 7.33-7.51 (m, 5H), 7.57-7.65 (m, 4H).

NP-HPLC: (OD, hexane/isopropanol 97:3 v/v, 0.8 mL/min, 258 nm) t_R = 22.3 min (*S*).

Compound (*S*)-**6b** in >99% *ee* after bioreduction with *R.ruber*



5.4.3 Synthesis of *(S)*-1-(4-(pyridin-2-yl)phenyl)ethanol (6c)



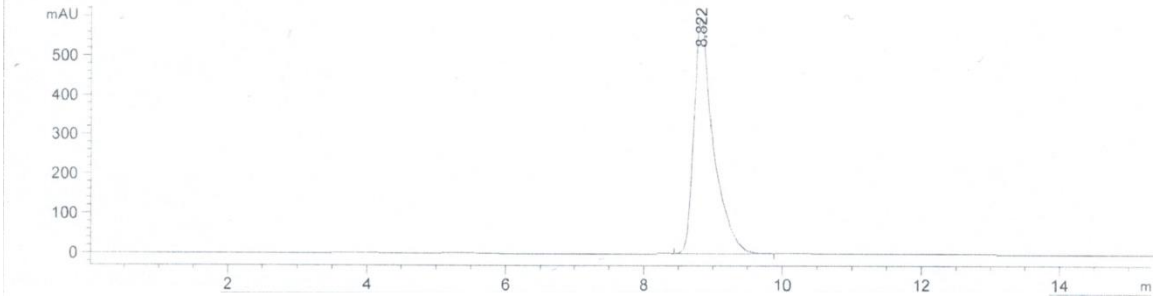
In a 15 mL Falcon tube, **5c** (5 mM) and ADH from *Rhodococcus ruber* (10 U) were added to a 1*ChCl*:2*Gly* (2.15 mL mL)/50 mM KH_2PO_4 buffer pH 7.0 (3.25 mL) mixture (containing 1 mM NAD^+), and *i*-PrOH (600 µL). The reaction was shaken at 30 °C and 250 rpm for 24 h. To determine the conversion, 10 µL of the mixture were diluted with 90 µL of Milli-Q water and analysed by achiral reverse phase with previous centrifuging and filtering of the sample. Then, the mixture was treated with aq saturated NH_4Cl (1 × 10 mL) and extracted with ethyl acetate (2 × 5 mL), the organic layers combined, filtered and concentrated under vacuum to provide the crude product (*S*)-**6c** (yield >95%) as a colourless oil.

$[\alpha]_D^{20}$ −70.5 (*c* 0.2, $CHCl_3$), *ee* >99%. Lit.[172] for (*S*)-**6c**: $[\alpha]_D^{20}$ −42.2 (*c* 0.82, $CHCl_3$), *ee* = 93%.

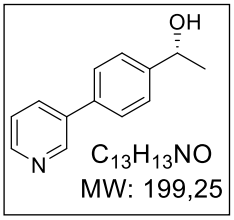
^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.53 (d, *J* 6.6 Hz, 3H), 2.11 (brs, OH), 4.96 (q, *J* 6.6 Hz, 1H), 7.20-7.26 (m, 1H), 7.43-7.58 (m, 3H), 7.70-7.79 (m, 2H), 7.96 (d, *J* 8.1 Hz, 2H), 8.68 (d, *J* 4.8 Hz, 1H).

NP-HPLC: (OD, hexane/isopropanol 75:25 v/v, 0.8 mL/min, 258 nm) t_R = 8.9 min (*S*).

Compound (*S*)-**6c** in >99% *ee* after bioreduction with *R. ruber*



5.4.4 Synthesis of *(R)*-1-(4-(pyridin-3-yl)phenyl)ethanol (6d)



In a 15 mL Falcon tube, **5d** (5 mM) and ADH from *L. kefir* (30 U) were added to a 1*ChCl*:2*Gly* (2.15 mL mL)/50 mM KH_2PO_4 buffer pH 7.0 (3.25 mL) mixture (containing 1 mM $MgCl_2$ and 1 mM $NADP^+$), and *i*-PrOH (600 µL). The reaction was shaken at 30 °C and 250 rpm for 24 h. To determine the conversion, 10 µL of the mixture were diluted with 90 µL of Milli-Q water and analysed by achiral reverse phase with previous centrifuging and filtering of the sample. Then, the mixture was treated with aq saturated NH_4Cl (1 × 10 mL) and extracted with ethyl acetate (2 × 5 mL), the organic layers combined, filtered and concentrated under vacuum to provide the crude product (*R*)-**6d** (yield >95%) as a white solid.

Mp: 108-110 °C

$[\alpha]_D^{20}$ +21.5 (*c* 0.2, $CHCl_3$), *ee* >99%.

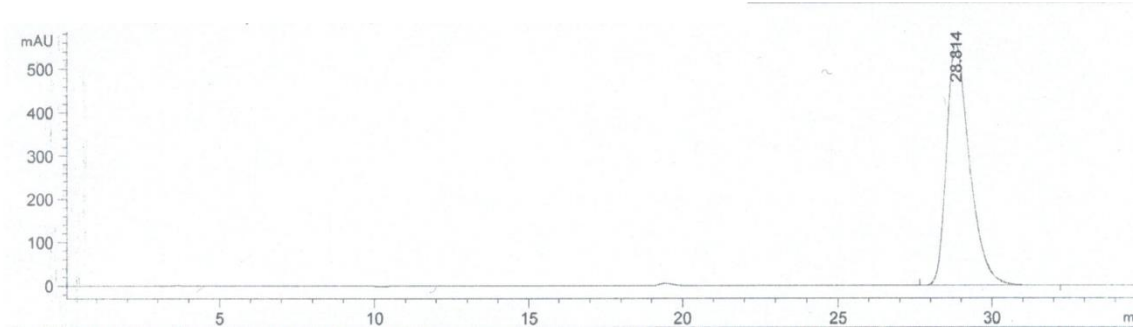
^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.52 (d, *J* 6.6 Hz, 3H), 3.39 (brs, OH), 4.93 (q, *J* 6.6 Hz, 1H), 7.34 (dd, *J* 8.1 and 4.8 Hz, 1H), 7.43-7.51 (m, 4H), 7.83 (dt, *J* 4.8 and 1.8 Hz, 1H), 8.50-8.54 (m, 2H).

^{13}C NMR (75.5 MHz, CDCl3): δ 25.37 (CH_3), 69.90 (CH),123.75 (CH), 126.45 (2CH), 127.31 (2CH), 134.51 (CH), 136.59 (2C), 146.52 (C), 148.15 (CH), 148.22 (CH).

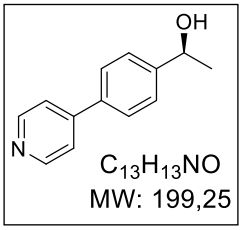
MS (APCI$^+$, m/z): 200 [$(M+H)^+$, 100%].

NP-HPLC: (AD-H, hexane/isopropanol 90:19 v/v, 0.8 mL/min, 258 nm) t_R = 28.9 min (*R*).

Compound (*R*)-**6d** in >99% *ee* after bioreduction with *L. kefir*



5.4.5 Synthesis of *(S)*-1-(4-(pyridin-4-yl)phenyl)ethanol (6e)



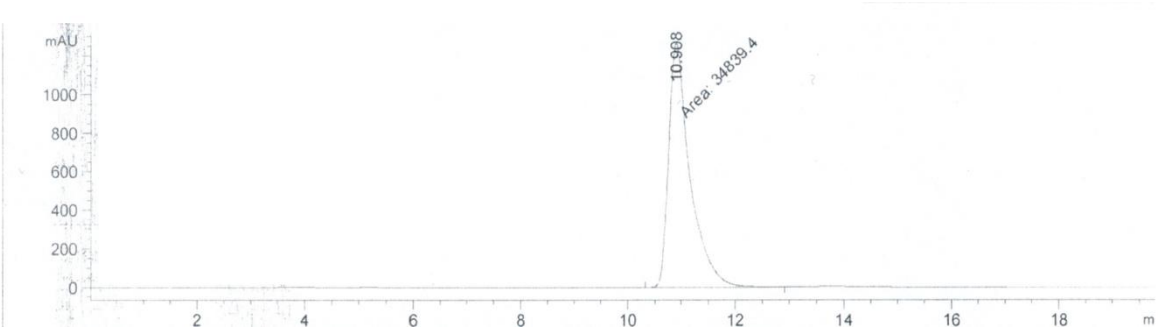
In a 15.0 mL Falcon tube, **5e** (5 mM) and ADH from *Rhodococcus ruber* (10 U) were added to a 1*ChCl*:2*Gly* (2.15 mL)/50 mM KH_2PO_4 buffer pH 7.0 (3.25 mL) mixture (containing 1 mM NAD^+), and *i*-PrOH (600 µL). The reaction was shaken at 30 °C and 250 rpm for 24 h. To determine the conversion, 10 µL of the mixture were diluted with 90 µL of Milli-Q water and analysed by achiral reverse phase with previous centrifuging and filtering of the sample. Then, the mixture was treated with aq saturated NH_4Cl (1 × 10 mL) and extracted with ethyl acetate (2 × 5 mL), the organic layers combined, filtered and concentrated under vacuum to provide the crude product (*S*)-**6e** (yield >95%) as a white oil.

$[\alpha]_D^{20}$ –41.0 (*c* 0.2, $CHCl_3$), *ee* >99%;

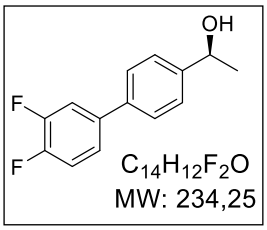
^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.54 (d, *J* 6.6 Hz, 3H), 2.48 (brs, OH), 4.98 (q, *J* 6.6 Hz, 1H), 7.47-7.5 (m, 4H), 7.63 (d, *J* 8.4 Hz, 2H), 8.53 (d, *J* 6.3 Hz, 1H).

NP-HPLC: (AD-H, hexane/isopropanol 75:25 v/v, 0.8 mL/min, 258 nm) t_R = 10.9 min (*S*)

Compound (*S*)-**6e** in >99% *ee* after bioreduction with *R. ruber*



5.4.6 Synthesis of *(S)*-1-(3',4'-difluoro-[1,1'-biphenyl]-4-yl)ethanol (6f)



In a 15.0 mL Falcon tube, **5f** (5 mM) and ADH from *Rhodococcus ruber* (10 U) were added to a 1*ChCl*:2*Gly* (2.15 mL)/50 mM KH_2PO_4 buffer pH 7.0 (3.25 mL) mixture (containing 1 mM NAD^+), and *i*-PrOH (600 µL). The reaction was shaken at 30 °C and 250 rpm for 24 h. To determine the conversion, 10 µL of the mixture were diluted with 90 µL of Milli-Q water and analysed by achiral reverse phase with previous centrifuging and filtering of the sample. Then, the mixture was treated with aq saturated NH_4Cl (1 × 10 mL) and extracted with ethyl acetate (2 × 5 mL), the organic layers combined, filtered and concentrated under vacuum to provide the crude product (*S*)-**6f** (yield >95%) as a colourless oil.

$[\alpha]_D^{20}$ −24.5 (*c* 0.2, $CHCl_3$), *ee* >99%.

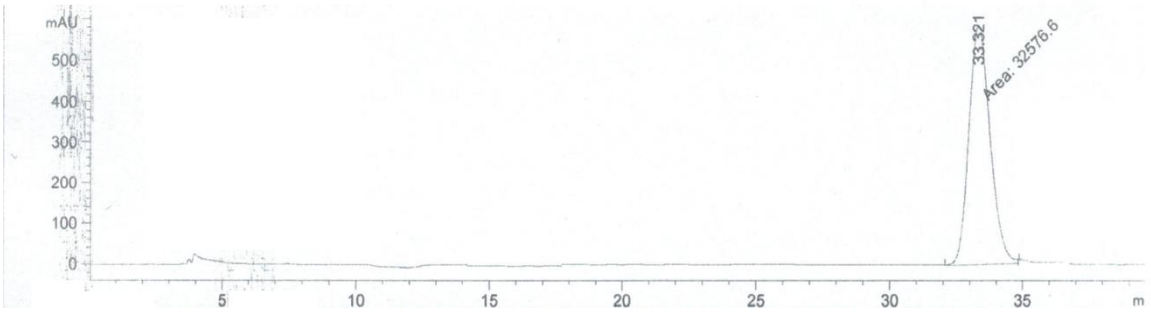
^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.60 (d, *J* 6.6 Hz, 3H), 2.16 (brs, OH), 5.02 (q, *J* 6.6 Hz, 1H), 7.23-77 (m, 3H), 7.51 (d, *J* 8.4 Hz, 2H), 7.58 (d, *J* 8.4 Hz, 2H).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ 25.21 (CH_3), 70.04 (CH), 115.84 (CH, d, J_{CF}= 17 Hz), 117.51 (CH, d, J_{CF}= 17 Hz), 122.93 (CH, dd, J_{CF}= 6 and 2 Hz), 126.02 (2CH), 127.04 (2CH), 137.90 (C, d, J_{CF}= 5 Hz), 138.29 (C), 145.44 (C), 148.52 (C, dd, J_{CF}= 45 and 12 Hz), 151.81 (C, dd, J_{CF}= 44 and 12 Hz).

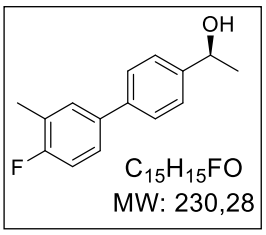
MS (APCI$^+$, m/z): 235 [$(M+H)^+$, 100%].

NP-HPLC: (AD-H, hexane/isopropanol 98:2 v/v, 0.8 mL/min, 258 nm) t_R = 33.5 min (*S*).

Compound (*S*)-**6f** in >99% *ee* after bioreduction with *R. ruber*



5.4.7 Synthesis of *(S)*-1-(4'-fluoro-3'-methyl-[1,1'-biphenyl]-4-yl)ethanol (6g)



In a 15.0 mL Falcon tube, **5g** (5 mM) and ADH from *Rhodococcus ruber* (10 U) were added to a 1*ChCl*:2*Gly* (2.15 mL)/50 mM KH_2PO_4 buffer pH 7.0 (3.25 mL) mixture (containing 1 mM NAD^+), and *i*-PrOH (600 µL). The reaction was shaken at 30 °C and 250 rpm for 24 h. To determine the conversion, 10 µL of the mixture were diluted with 90 µL of Milli-Q water and analysed by achiral reverse phase with previous centrifuging and filtering of the sample. Then, the mixture was treated with aq saturated NH_4Cl (1 × 10 mL) and extracted with ethyl acetate (2 × 5 mL), the organic layers combined, filtered and concentrated under vacuum to provide the crude product (*S*)-**6g** (yield >95%) as a white solid.

Mp: 59-61 °C.

$[\alpha]_D^{20}$ −17.0 (*c* 0.2, $CHCl_3$), *ee* >99% (*S*).

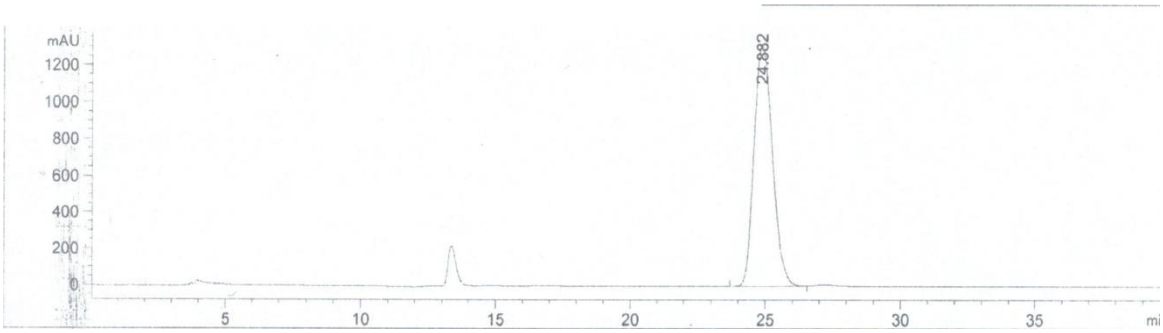
^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.53 (d, *J* 6.3 Hz, 3H), 1.91 (brs, OH), 2.34 (s ,3H), 4.94 (q, *J* 6.3 Hz, 1H), 7.06 (t, *J* 9.0 Hz, 1H), 7.31-7.47 (m, 4H), 7.51 (d, *J* 8.1 Hz, 2H).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ 14.68 (CH_3), 25.15 (CH_3), 70.14 (CH), 115.25 (CH, d, J_{CF}= 22 Hz), 125.02 (C, d, J_{CF}= 17 Hz), 125.85 (2CH), 127.09 (2CH), 130.13 (CH, d, J_{CF}= 5 Hz), 136.37 (C), 139.66 (C), 144.66 (C), 161.03 (C, d, J_{CF}= 245 Hz).

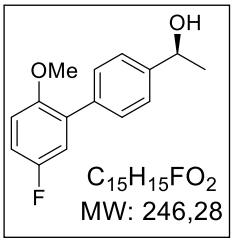
MS (APCI$^+$, m/z): 231 [$(M+H)^+$, 100%].

NP-HPLC: (AD-H, hexane/isopropanol 98:2 v/v, 0.8 mL/min, 258 nm) t_R = 24.7 min (*S*).

Compound (*S*)-**6g** in >99% *ee* after bioreduction with *R. ruber*



5.4.8 Synthesis of *(S)*-1-(5'-fluoro-2'-methoxy-[1,1'-biphenyl]-4-yl)ethanol (6h)



In a 15.0 mL Falcon tube, **5h** (5 mM) and ADH from *Rhodococcus ruber* (10 U) were added to a 1*ChCl*:2*Gly* (2.15 mL)/50 mM KH_2PO_4 buffer pH 7.0 (3.25 mL) mixture (containing 1 mM NAD^+), and *i*-PrOH (600 µL). The reaction was shaken at 30 °C and 250 rpm for 24 h. To determine the conversion, 10 µL of the mixture were diluted with 90 µL of Milli-Q water and analysed by achiral reverse phase with previous centrifuging and filtering of the sample. Then, the mixture was treated with aq saturated NH_4Cl (1 × 10 mL) and extracted with ethyl acetate (2 × 5 mL), the organic layers combined, filtered and concentrated under vacuum to provide the crude product (*S*)-**6h** (yield >95%).

Brownish oil; $[\alpha]_D^{20}$ −23.5 (*c* 0.2, $CHCl_3$), *ee* >99%.

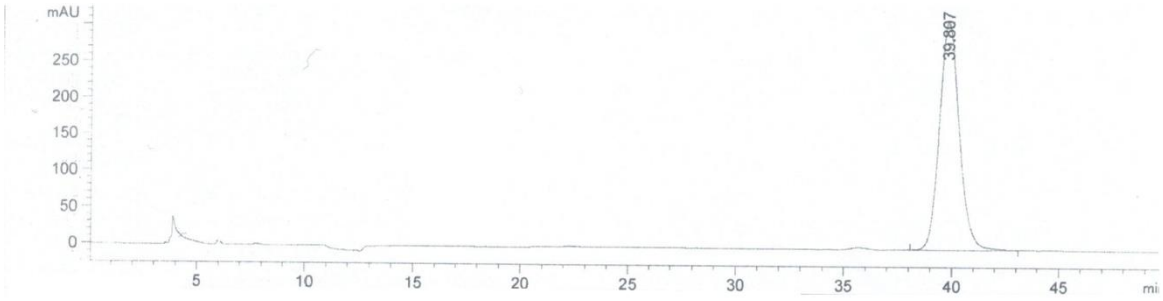
^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.54 (d, *J* 6.6 Hz, 3H), 1.97 (brs, OH), 3.78 (s ,3H), 4.93 (q, *J* 6.6 Hz, 1H), 6.86-6.94 (m, 1H), 6.95-7.08 (m, 2H), 7.42 (d, *J* 8.4 Hz, 2H), 7.51 (d, *J* 8.4 Hz, 2H).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ 25.18 (CH_3), 56.30 (CH_3), 70.33 (CH), 112.39 (CH, d, J_{CF}= 7 Hz), 114.39 (CH, d, J_{CF}= 23 Hz), 117.45 (CH, d, J_{CF}= 23 Hz), 125.35 (2CH), 129.62 (2CH), 131.77 (C, d, J_{CF}= 7 Hz), 136.74 (C), 145.02 (C), 152.77 (C), 157.24 (C, d, J_{CF}= 239 Hz).

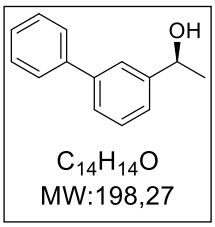
MS (APCI$^+$, m/z): 247 [$(M+H)^+$, 100%].

NP-HPLC: (AD-H, hexane/isopropanol 98:2 v/v, 0.8 mL/min, 258 nm) t_R = 40.2 min (*S*).

Compound (*S*)-**6h** in >99% *ee* after bioreduction with *R. ruber*



5.4.9 Synthesis of *(S)*-1-([1,1'-biphenyl]-3-yl)ethanol (6i)

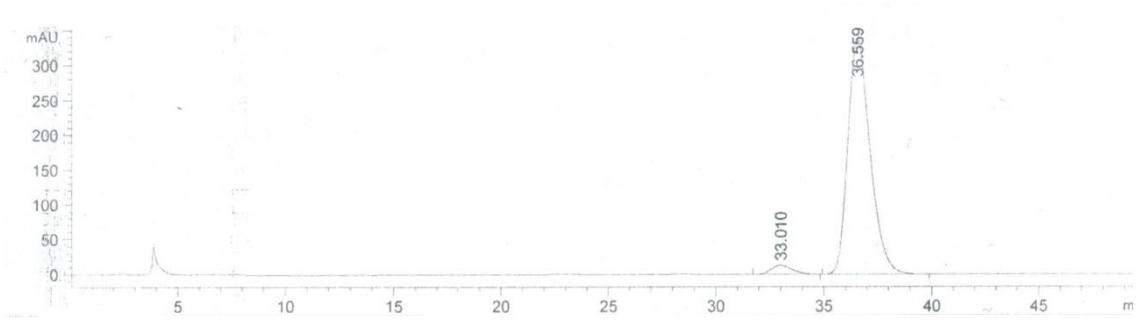


In a 15.0 mL Falcon tube, **5i** (5 mM) and ADH from *Rhodococcus ruber* (10 U) were added to a 1*ChCl*:2*Gly* (2.15 mL)/50 mM KH_2PO_4 buffer pH 7.0 (3.25 mL) mixture (containing 1 mM NAD^+), and *i*-PrOH (600 µL). The reaction was shaken at 30 °C and 250 rpm for 24 h. To determine the conversion, 10 µL of the mixture were diluted with 90 µL of Milli-Q water and analysed by achiral reverse phase with previous centrifuging and filtering of the sample. Then, the mixture was treated with aq saturated NH_4Cl (1 × 10 mL) and extracted with ethyl acetate (2 × 5 mL), the organic layers combined, filtered and concentrated under vacuum to provide the crude product (*S*)-**6i** (yield >95%) as a colourless oil.
$[\alpha]_D^{20}$ -26.5 (*c* 0.2, $CHCl_3$), *ee* >99%. Lit.[172] for (*S*)-**6i**: $[\alpha]_D^{20}$ –38.0 (*c* 1.09, $CHCl_3$), *ee* = 98%.

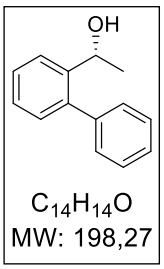
^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.55 (d, *J* 6.3 Hz, 3H), 1.89 (brs, OH), 4.99 (q, *J* 6.3 Hz, 1H), 7.32-7.55 (m, 6H), 7.57-7.64 (m, 3H).

NP-HPLC: (AD-H, hexane/isopropanol 98:2 v/v, 0.8 mL/min, 258 nm) t_R = 35.4 min (*S*).

Compound (*S*)-**6i** in >99% *ee* after bioreduction with *R. ruber*



5.4.10 Synthesis of *(R)*-1-([1,1'-biphenyl]-2-yl)ethanol (6j)



$C_{14}H_{14}O$
MW: 198,27

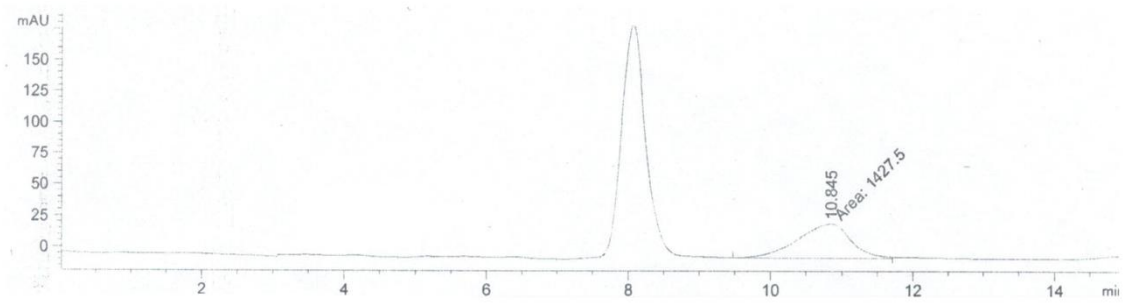
In a 15 mL Falcon tube, **5j** (5 mM) and KRED-P2-H07 (10 mg) were added to a 1*ChCl*:2*Gly* (2.15 mL)/125 mM KH_2PO_4 buffer pH 7.0 (3.25 mL) mixture (containing 1.25 mM $MgSO_4$ and 1 mM NAD^+), and *i*-PrOH (600 µL). The reaction was shaken at 30 °C and 250 rpm for 24 h. To determine the conversion, 10 µL of the mixture were diluted with 90 µL of Milli-Q water and analysed by achiral reverse phase with previous centrifuging and filtering of the sample. Then, the mixture was treated with aq saturated NH_4Cl (1 × 10 mL) and extracted with ethyl acetate (2 × 5 mL), the organic layers combined, filtered and concentrated under vacuum to provide the crude product (*S*)-**6j** (yield >95%) as a colourless oil.

$[\alpha]_D^{22}$ -29.5 (*c* 0.20, $CHCl_3$), *ee* >99%. Lit.[172] for (*S*)-**6j**: $[\alpha]_D^{20}$ -26.5 (*c* 1.00, $CHCl_3$), *ee* 45%.

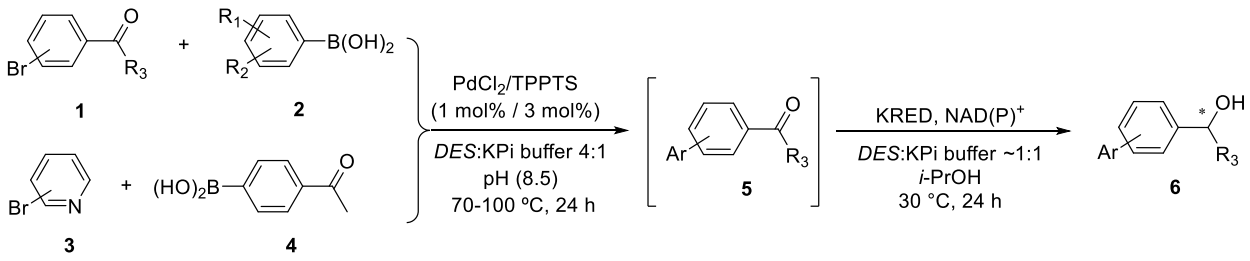
^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.42 (d, *J* 6.3 Hz, 3H), 1.69 (brs, OH), 4.98 (q, *J* 6.3 Hz, 1H), 7.21 (dd, *J* 7.5 and 1.5 Hz, 1H), 7.28-7.35 (m, 3H), 7.37-7.45 (m, 4H), 7.68 (d, *J* 7.8 Hz, 1H).

NP-HPLC: (OD, hexane/isopropanol 99:1 v/v, 0.8 mL/min, 258 nm) t_R = 10.8 min (*R*)

Compound (*R*)-**6j** in >99% *ee* after bioreduction with KRED-P2-H07



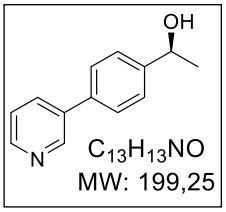
5.5 Preparative-scale synthesis of biaryl alcohols in a one-pot sequential process



General procedure for the synthesis of biaryl alcohols in a one-pot sequential process:

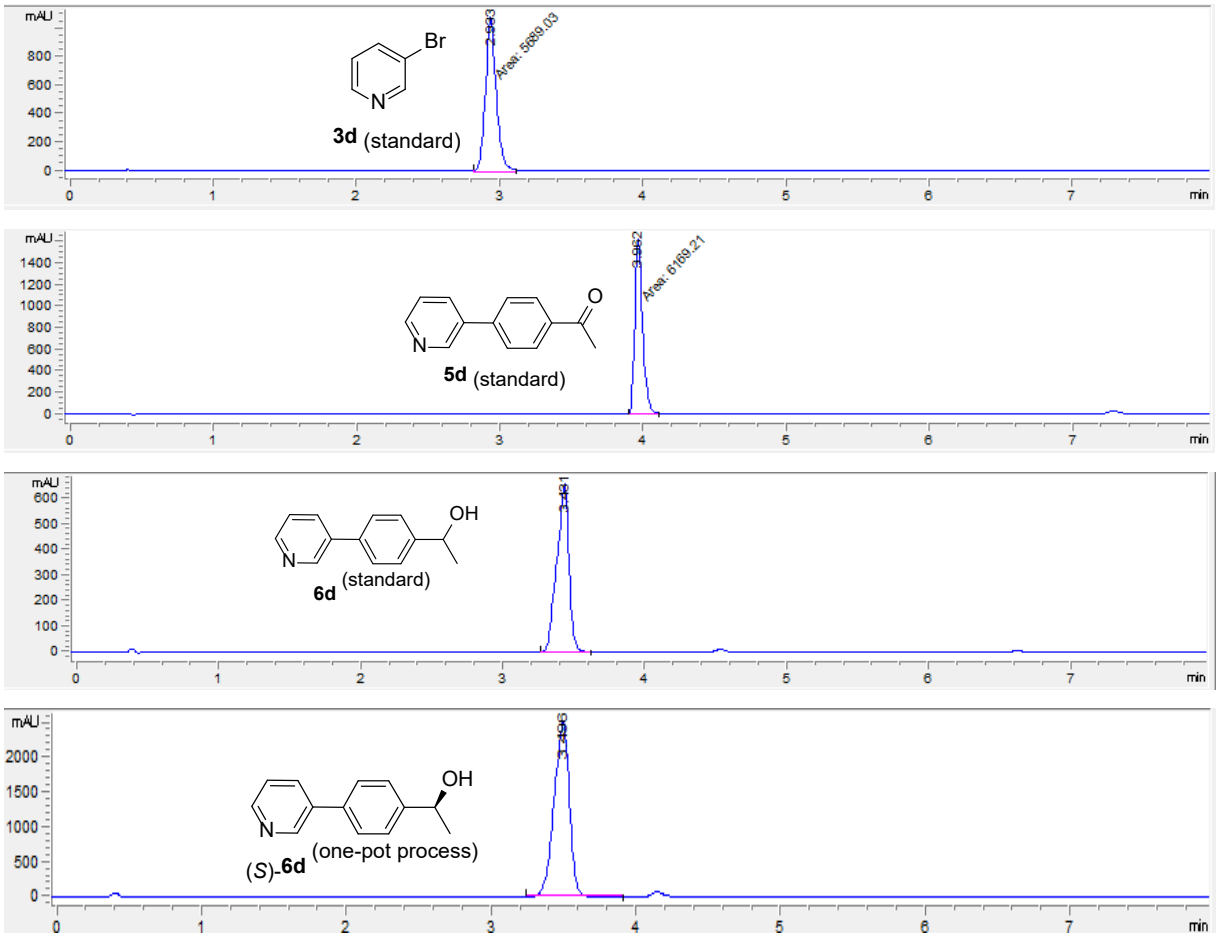
A suspension of $PdCl_2$ (3.5 mg; 0.02 mmol; 1 mol%) and TPPTS (34 mg; 0.06 mmol; 3 mol%) in 1 mL of phosphate buffer 150 mM pH 8.5 was prepared. After 30 min the resulting catalyst solution was added to a mixture, consisting of arylbromide (**1** or **3**, 1.945 mmol), phenylboronic acid (**2** or **4**, 1.945 mmol), 1*ChCl*:2*Gly* (8 mL) and phosphate buffer 150 mM pH 8.5 (1 mL). Then, the pH was adjusted to 8.5 by dropwise addition of aq 3 N NaOH and the reaction mixture was stirred at 70 °C or 100 °C for 24 h. To prepare the *(S)*-alcohol after cooling at rt, KPi buffer pH 8.5 (6.05 mL), *i*-PrOH (1.95 mL), NAD^+ (1 mM) and ADH from *R. ruber* DSM 44541 (360 U) were added and the mixture stirred for 24 h at 30 °C. To prepare the *(R)*-alcohol after cooling at rt, KPi buffer 150 mM pH 8.5 (6.05 mL), *i*-PrOH (1.95 mL), $NADP^+$ (1 mM), $MgCl_2$ (1 mM) and ADH from *L. kefir* DSM 20587 (690 U) were added and the mixture stirred for 24 h at 30 °C. Finally, saturated aq NH_4Cl (25 mL) was added and the reaction mixture was extracted with ethyl acetate (3x40 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under vacuum to provide the crude product (**6**). Further purification by flash chromatography (silica gel 60 Å, hexane-ethyl acetate 1: 1) yielded the final alcohol in high yields (70-86 %).

5.5.1 Synthesis of *(S)*- 1-(4-(pyridin-3-yl)phenyl)ethanol (6d)



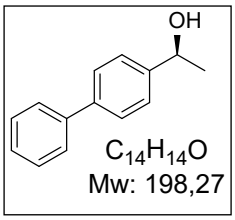
A suspension of $PdCl_2$ (3.5 mg; 0.02 mmol; 1 mol%) and TPPTS (34 mg; 0.06 mmol; 3 mol%) in 1 mL of phosphate buffer 150 mM pH 8.5 was prepared. After 30 min the resulting catalyst solution was added to a mixture, consisting of 3-bromopyridine (307,3 mg, 1.945 mmol), 4-acetylphenylboronic acid (318,9 mg,

1.945 mmol), 1*ChCl*:2*Gly* (8 mL) and phosphate buffer 150 mM pH 8.5 (1 mL). Then, the pH was adjusted to 8.5 by dropwise addition of aq 3 N NaOH and the reaction mixture was stirred at 100 °C for 24 h. After cooling to rt, phosphate buffer 150 mM pH 8.5 (6.05 mL), *i*-PrOH (1.95 mL, 11% v/v), NAD^+ (1 mM) and ADH from *Rhodococcus ruber* DSM 44541 (360 U) were added. After stirring for another 24 h at 30°C, saturated aq NH_4Cl (25 mL) was added and extracted with ethyl acetate (3x40 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under vacuum to provide the crude product. Further purification by flash chromatography (silica gel 60 Å, hexane-ethyl acetate 1: 1) yielded 364 mg of (*S*)-**6d** as a yellowish oil (85 %).

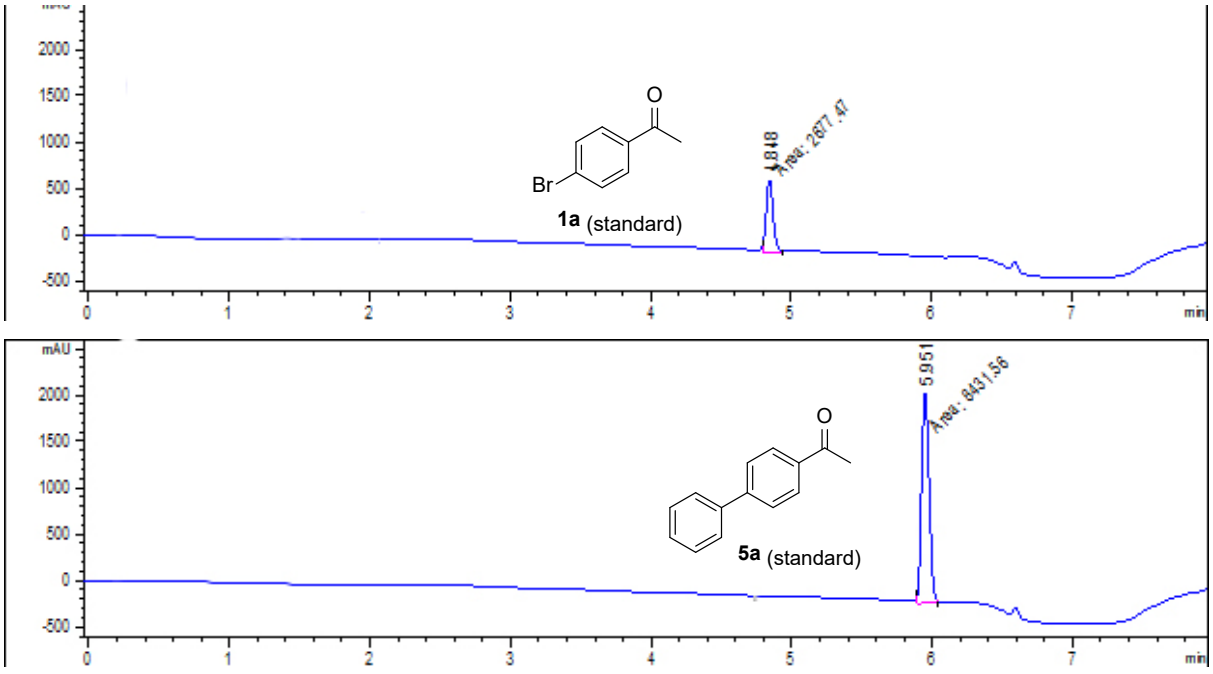


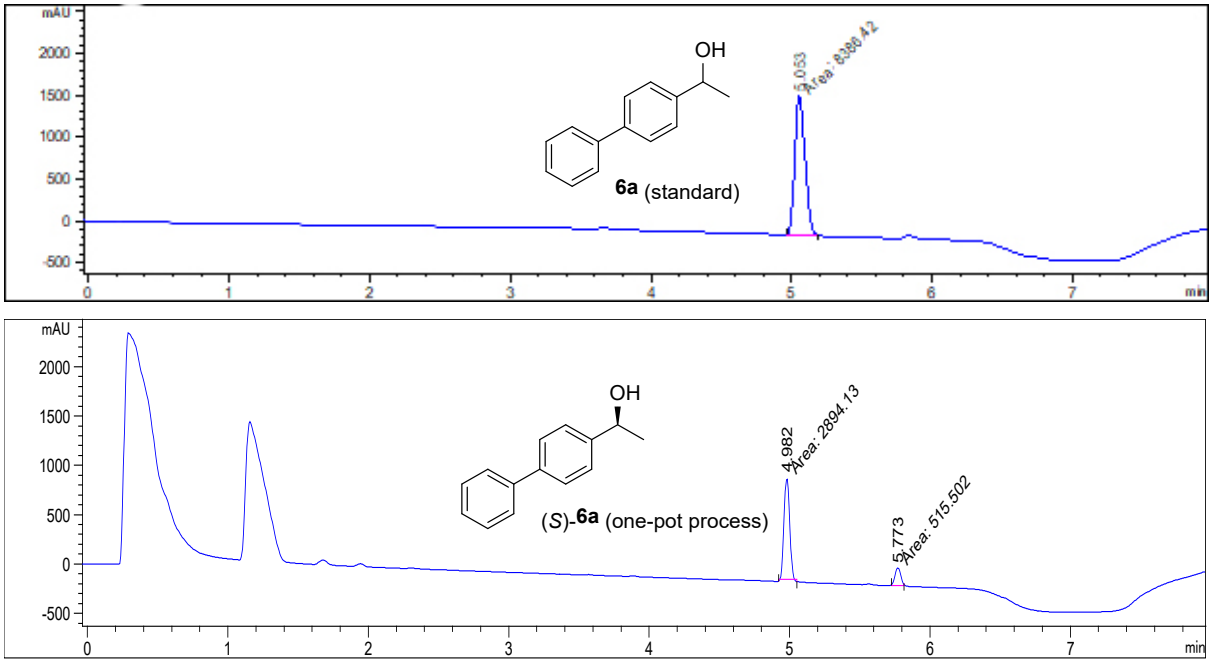
Scheme 54. In process HPLC monitoring for the preparation scale experiment of **6d**

5.5.2 Synthesis of *(S)*-1-([1,1'-biphenyl]-4-yl)ethanol (6a)



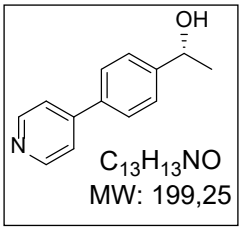
A suspension of $PdCl_2$ (3.5 mg; 0.02 mmol; 1 mol%) and TPPTS (34 mg; 0.06 mmol; 3 mol%) in 1 mL of phosphate buffer 150 mM pH 8.5 was prepared. After 30 min the resulting catalyst solution was added to a mixture, consisting of 4'-bromoacetophenone (387,1 mg, 1.945 mmol), 4-acetylphenylboronic acid (318,9 mg, 1.945 mmol), 1*ChCl*:2*Gly* (8 mL) and phosphate buffer 150 mM pH 8.5 (1 mL). Then, the pH was adjusted to 8.5 by dropwise addition of aq 3 N NaOH and the reaction mixture was stirred at 70 °C for 24 h. After cooling to rt, phosphate buffer 150 mM pH 8.5 (6.05 mL), *i*-PrOH (1.95 mL, 11% v/v), NAD^+ (1 mM) and ADH from *R. ruber* DSM 44541 (360 U) were added. After stirring for another 24 h at 30°C, aq saturated NH_4Cl (25 mL) was added and extracted with ethyl acetate (3x40 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under vacuum to provide the crude product. Further purification by flash chromatography (silica gel 60 Å, hexane-ethyl acetate 1: 1) yielded 270 mg of (*S*)-**6a** as a yellowish oil (70 %).



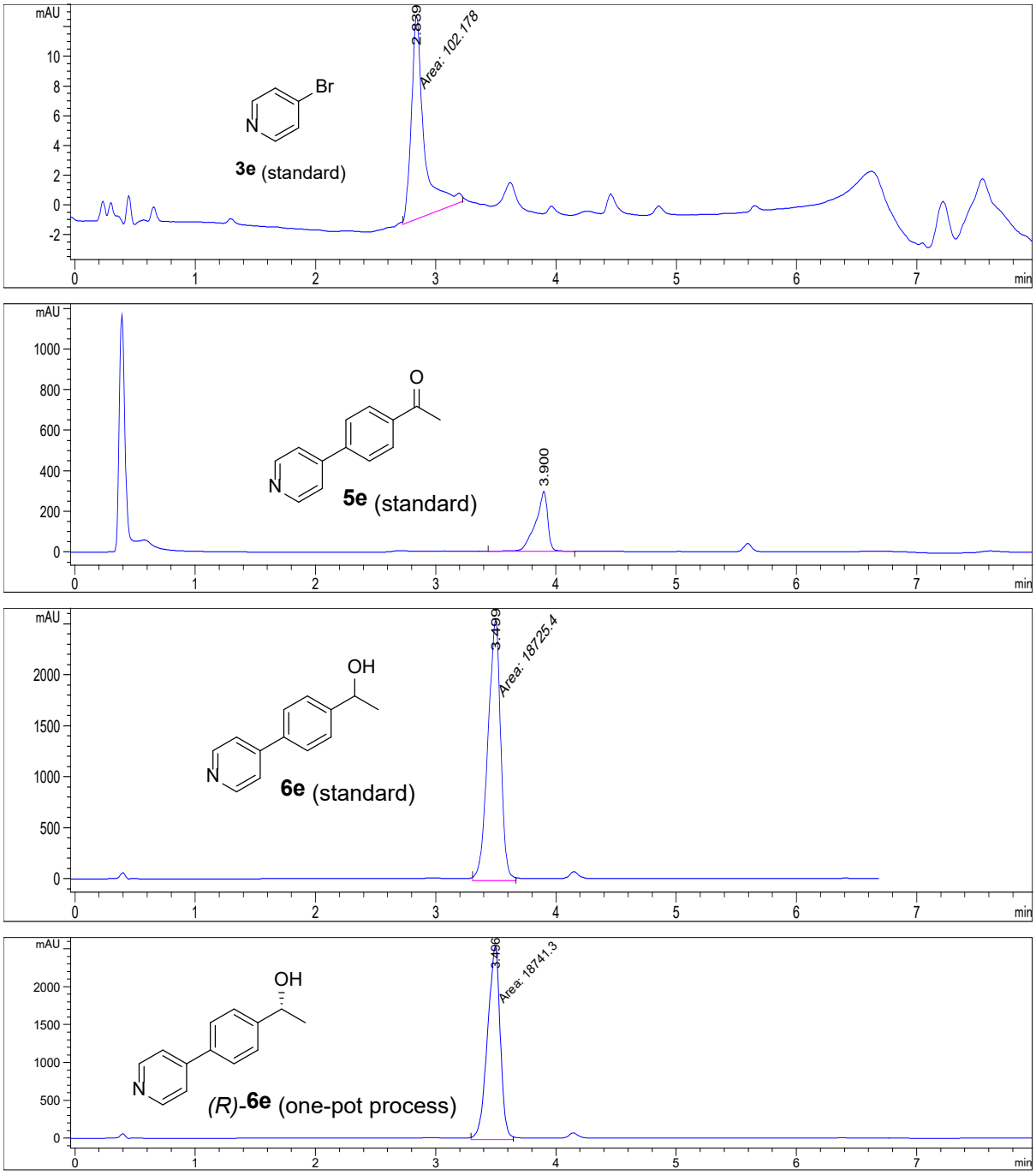


Scheme 55. In process HPLC monitoring for the preparation scale experiment of **6a**

5.5.3 Synthesis of *(R)*- 1-(4-(pyridin-4-yl)phenyl)ethanol (6e)

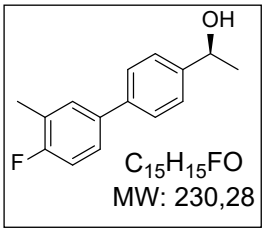


A suspension of $PdCl_2$ (3.5 mg; 0.02 mmol; 1 mol%) and TPPTS (34 mg; 0.06 mmol; 3 mol%) in 1 mL of phosphate buffer 150 mM pH 8.5 was prepared. After 30 min the resulting catalyst solution was added to a mixture, consisting of 4-bromopyridine (377,1 mg, 1.945 mmol), 4-acetylphenylboronic acid (318,9 mg, 1.945 mmol), 1*ChCl*:2*Gly* (8 mL) and phosphate buffer 150 mM pH 8.5 (1 mL). Then, the pH was adjusted to 8.5 by dropwise addition of aq 3 N NaOH and the reaction mixture was stirred at 100 °C for 24 h. After cooling to rt, phosphate buffer 150 mM pH 8.5 (6.05 mL), *i*-PrOH (1.95 mL, 11% v/v), NAD^+ (1 mM) and ADH from *L. kefir* DSM 20587 (690 U) were added. After stirring for another 24 h at 30°C, aq saturated NH_4Cl (25 mL) was added and extracted with ethyl acetate (3x40 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under vacuum to provide the crude product. Further purification by flash chromatography (silica gel 60 Å, hexane-ethyl acetate 1: 1) yielded 332 mg of (*R*)-**6e** as a white oil (86 %).

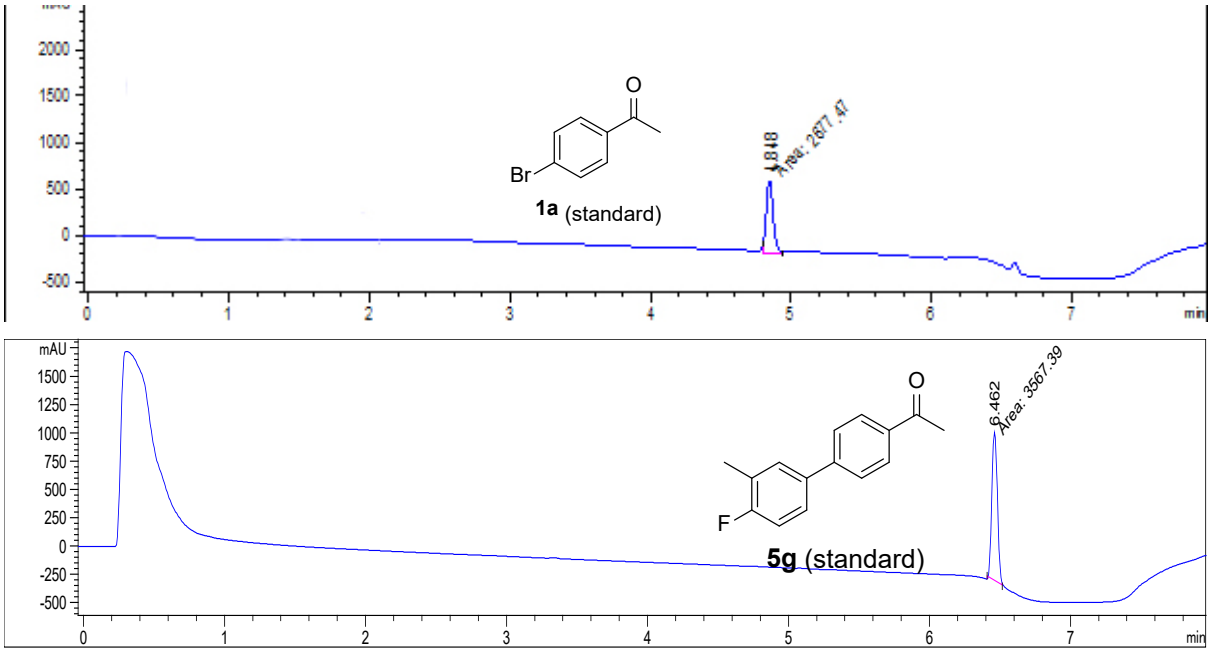


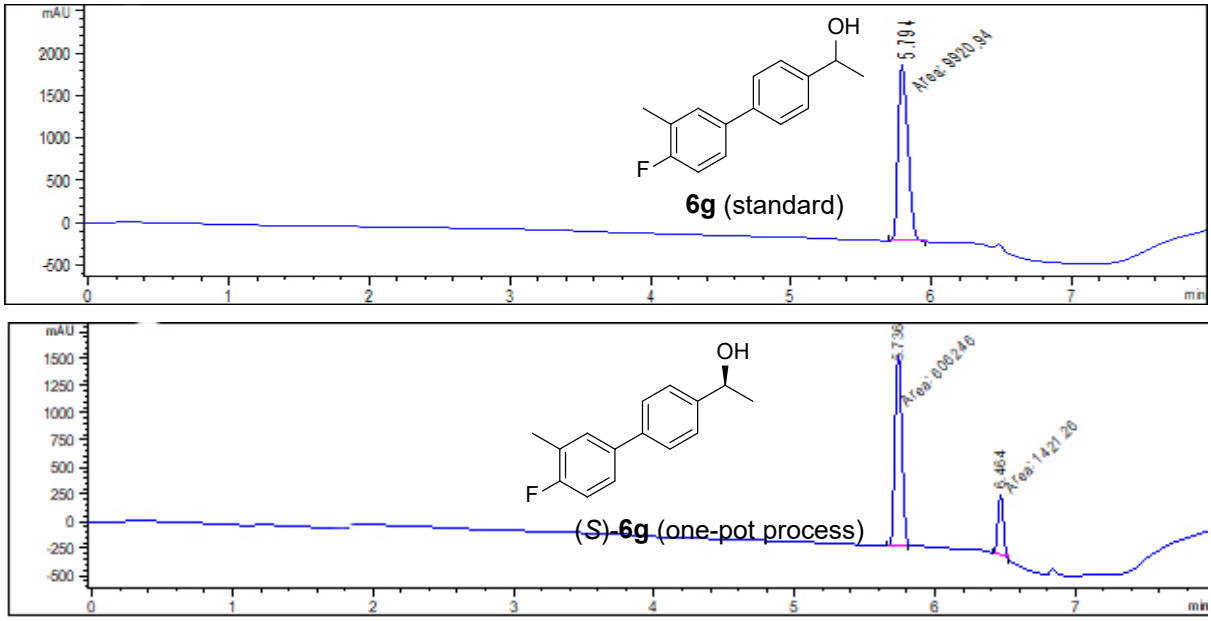
Scheme 56. In process HPLC monitoring for the preparation scale experiment of **6e**

5.5.4 Synthesis of *(S)*- 1-(4'-fluoro-3'-methyl-[1,1'-biphenyl]-4-yl)ethanol (6g)



A suspension of $PdCl_2$ (3.5 mg; 0.02 mmol; 1 mol%) and TPPTS (34 mg; 0.06 mmol; 3 mol%) in 1 mL of phosphate buffer 150 mM pH 8.5 was prepared. After 30 min the resulting catalyst solution was added to a mixture, consisting of 4-fluoro-3-methylphenylboronic acid (300 mg, 1.945 mmol), 4'-bromoacetophenone (387,1 mg, 1.945 mmol), 1*ChCl*:2*Gly* (8 mL) and phosphate buffer 150 mM pH 8.5 (1 mL). Then, the pH was adjusted to 8.5 by dropwise addition of aq 3 N NaOH and the reaction mixture was stirred at 70 °C for 24 h. After cooling to rt, phosphate buffer 150 mM pH 8.5 (6.05 mL), *i*-PrOH (1.95 mL, 11% v/v), NAD^+ (1 mM) and ADH from *R. ruber* DSM 44541 (360 U) were added. After stirring for another 24 h at 30°C, aq saturated NH_4Cl (25 mL) was added and extracted with ethyl acetate (3x40 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under vacuum to provide the crude product. Further purification by flash chromatography (silica gel 60 Å, hexane-ethyl acetate 1: 1) yielded 371 mg of (*S*)-**6g** as a white oil (83 %).





Scheme 57. In process HPLC monitoring for the preparation scale experiment of **6g**

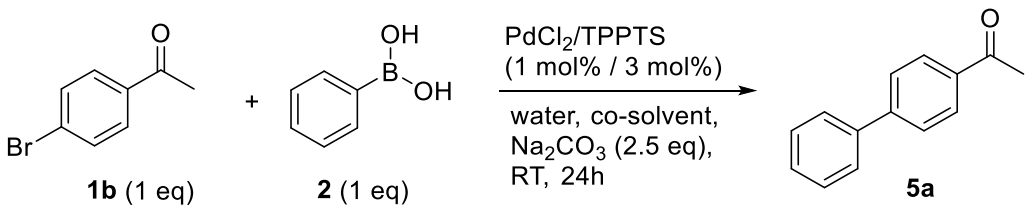
5.6 Optimisation of the Pd-catalysed Suzuki cross-coupling reaction for the one-pot synthesis of biaryl amines

5.6.1 Study of different solvents

General procedure:

At first, a suspension of $PdCl_2$ (3.5 mg; 0.02 mmol; 1 mol%) and TPPTS (34 mg; 0.06 mmol; 3 mol%) in 1 mL of phosphate buffer 150 mM pH 8.5 was prepared. After 30 min the resulting catalyst solution was added to a mixture, consisting of 4'-bromoacetophenone (**1b**, 0.389 mmol; 1 eq, 40 mM), phenylboronic acid (**2**, 0.389 mmol; 1 eq, 40 mM), sodium carbonate (0.97 mmol, 2.5 eq), co-solvent and phosphate buffer 150 mM pH 8.5. The reaction mixture was stirred at room temperature for 24 h. Then, 20 mL of aq saturated NH_4Cl was added and extracted with ethyl acetate (2x20 mL). The combined organic layers were combined, dried with Na_2SO_4, filtered and concentrated under vacuum providing the crude product ketone **5a**.

Table 23. Effect of co-solvent on the Suzuki cross-coupling reaction of 4'-bromoacetophenone (**1b**) and phenylboronic acid (**2**)



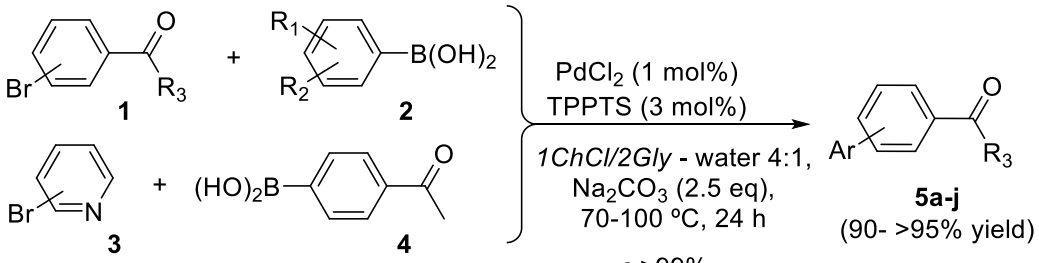
Entry	co-solvent (%)	*c* (%)
1	*i*-PrOH (10%)	91
2	*i*-PrOH (50%)	95
3	THF (10%)	85
4	THF (50%)	96
5	1*ChCl*/2*Gly* (50%)	90
6	1*ChCl*/2*Gly* (80%)	93
7	1*ChCl*/2*Gly* (100%)	85
8	DMSO (10%)	30

5.6.2 Cross-coupling reaction in a *DES*-buffer mixture

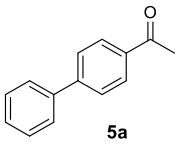
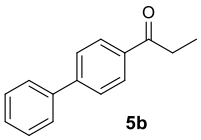
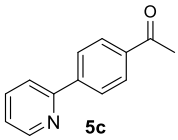
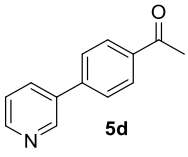
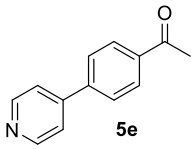
General procedure under the optimised reaction conditions:

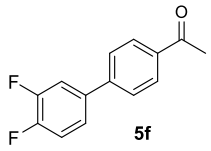
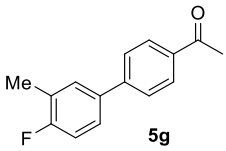
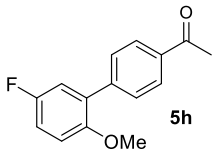
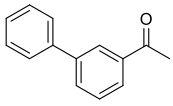
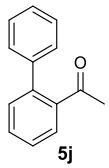
At first, a suspension of $PdCl_2$ (3.5 mg; 0.02 mmol; 1 mol%) and TPPTS (34 mg; 0.06 mmol; 3 mol%) in 1 mL of phosphate buffer 150 mM pH 8.5 was prepared. After 30 min the resulting catalyst solution was added to a mixture, consisting of arylbromide (**1** or **3**, 1.945 mmol; 1 eq, 200 mM), boronic acid (**2** or **4**, 1.945 mmol; 1 eq, 200 mM), sodium carbonate (2.5 eq, 4.9 mmol), *DES* (8 mL) and phosphate buffer 150 mM pH 8.5 (1 mL). The reaction mixture was heated according to the substrate of choice for 24 h. Then, 20 mL of aq saturated NH_4Cl was added and extracted with ethyl acetate (2x20 mL). The combined organic layers were combined, dried with Na_2SO_4, filtered and concentrated under vacuum providing the crude product (**5 a-j**).

Table 24. Scope of the Suzuki cross-coupling reaction in *DES*-buffer medium under the optimised reaction conditions

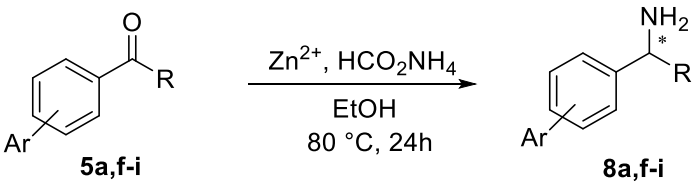


R_1, R_2 = H, Me, OMe, F
R_3 = Me,Et

Entry	Product	[Pd]/ligand [mol%]	T (℃)	Substrate concentration (mM)	*c* (%)
1	 **5a**	1/3	70	200	>99
2	 **5b**	1/3	70	200	>99
3	 **5c**	1/3	100	200	>99
4	 **5d**	1/3	100	200	>99
5	 **5e**	1/3	100	200	>99

6	 **5f**	1/3	70	200	>99
7	 **5g**	1/3	70	200	>99
8	 **5h**	1/3	70	200	>99
9	 **5i**	1/3	70	200	>99
10	 **5j**	1/3	70	200	>99

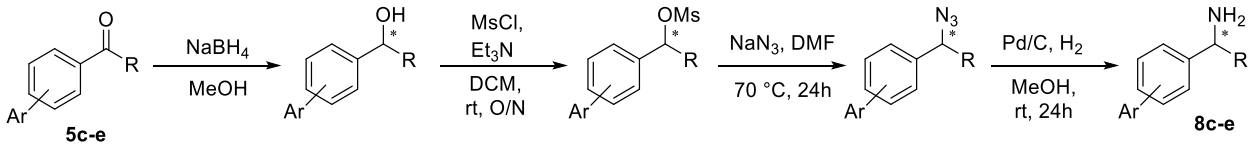
5.7 Synthesis of racemic amine standards



General procedure for the synthesis of racemic amine standards 8a,f-i:

Ammonium formate (300 mg) and zinc (150 mg) were added to a solution of 80 mg ketone (**5a,f-i**) in 1.5 mL of ethanol and the mixture was stirred overnight at 80 °C under nitrogen. Then, the reaction mixture was cooled at room temperature, filtered through Celite and washed with ethanol. The solvent was removed under vacuum to obtain an oily crude, followed by the addition of 10 mL of aqueous 1 N HCl and washing with diethyl ether (2 × 20 mL). The aqueous layer was basified to pH 10

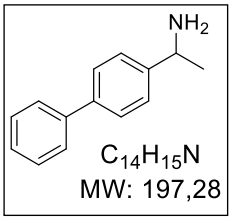
with aqueous 10 N NaOH and extracted with ethyl acetate (3 × 5 mL). The organic layer was washed with 10 mL of brine and dried over Na_2SO_4. Further concentration on a rotary evaporator afforded the corresponding biaryl amine **8a, f-i** (25-30% yield).



General procedure for the synthesis of racemic amine standards 8c-e:

Et_3N (2 eq) and MsCl (2 eq) were added to a solution of alcohol **6c-e** (0.4 mmol, beforehand obtained by reducing the corresponding ketone **5c-e** with $NaBH_4$) in 3.0 mL of dichloromethane and the mixture was stirred overnight at room temperature. Then, the solvent was removed and NaN_3 (10 eq) and anhydrous DMF (3 mL) were added, the resulting mixture being stirred at 70 °C for 24 h. Further evaporation of the solvent under reduced pressure afforded a crude which was purified by flash chromatography (EtOAc/hexane mixtures). Finally, to a suspension of the previous azido compound and Pd-C 10% (25 mg) in a round-bottom flask, a H_2 balloon was connected and deoxygenated MeOH (1.5 mL) was carefully added. The resulting mixture was stirred at room temperature for 24 h. After this time, the mixture was filtered through Celite® and the solvent evaporated under reduced pressure to afford the corresponding biaryl amine **8c-e** in high yield (70-75%).

5.7.1 Synthesis of rac-1-([1,1'-biphenyl]-4-yl)ethanamine (8a)



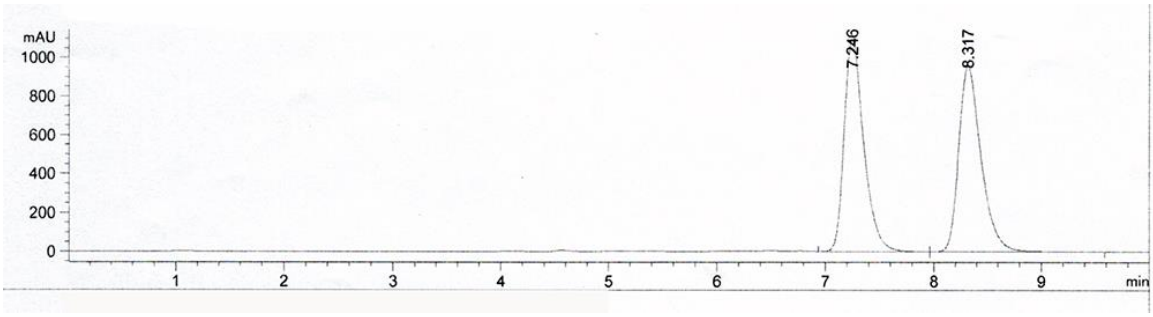
The title compound was obtained with the general procedure according to the substrate in question.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.47 (d, *J* 6.6 Hz, 3H), 1.85 (brs, NH_2), 4.21 (q, *J* 6.6 Hz, 1H), 7.29-7.41 (m, 1H), 7.43-7.49 (m, 4H), 7.53-7.65 (m, 4H). These spectroscopic data are in good agreement with those previously published.[179]

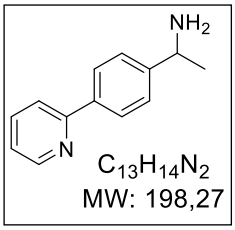
MS (APCI$^+$, m/z): 198 [$(M+H)^+$, 100%].

NP-HPLC (AD-H, hexane/isopropanol 90:10 v/v, 0.8 mL/min, 258 nm) t_R = 7.2 min (*R*), 8.3 min (*S*)

HPLC separation for both enantiomers of (±)-**8a** as Boc derivative



5.7.2 Synthesis of rac-1-(4-(pyridin-2-yl)phenyl)ethanamine (8c)



The title compound was obtained with the general procedure according to the substrate in question.

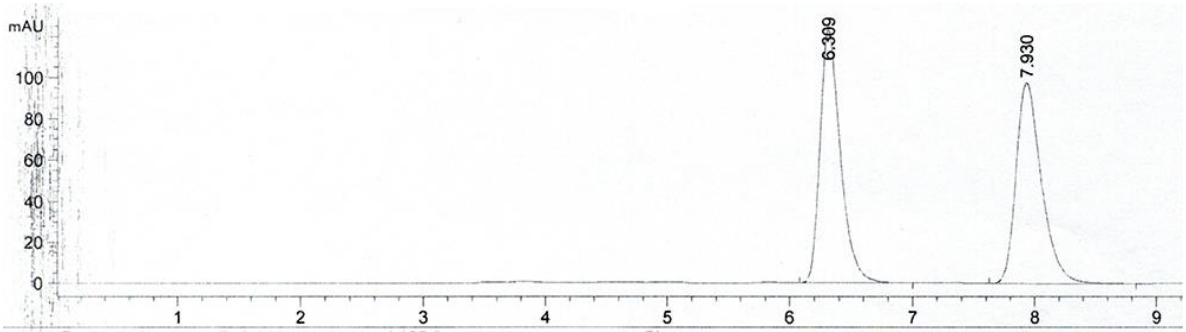
^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.42 (d, *J* 6.6 Hz, 3H), 2.66 (brs, NH_2), 4.16 (q, *J* 6.6 Hz, 1H), 7.16-7.21 (m, 1H), 7.45 (d, *J* 8.1 Hz, 2H), 7.70-7.76 (m, 2H), 7.95 (d, *J* 8.1 Hz, 2H), 8.68 (d, *J* 4.8 Hz, 1H).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ 25.25 (CH_3), 51.05 (CH), 120.39 (CH), 121.98 (CH), 126.17 (2CH), 127.07 (2CH), 136.70 (CH), 138.07 (C), 147.92 (C), 149.61 (CH), 157.20 (C).

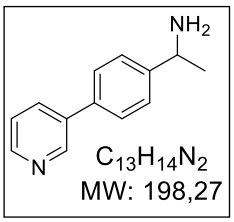
MS ($APCI^+$, m/z): 199 [$(M+H)^+$, 100%].

NP-HPLC (AD-H, hexane/isopropanol 75:25 v/v, 0.8 mL/min, 258 nm) t_R = 6.3 min (*R*), 7.9 min (*S*)

HPLC separation for both enantiomers of (±)-**8c** as Boc derivative



5.7.3 Synthesis of rac-1-(4-(pyridin-3-yl)phenyl)ethanamine (8d)



The title compound was obtained with the general procedure according to the substrate in question.

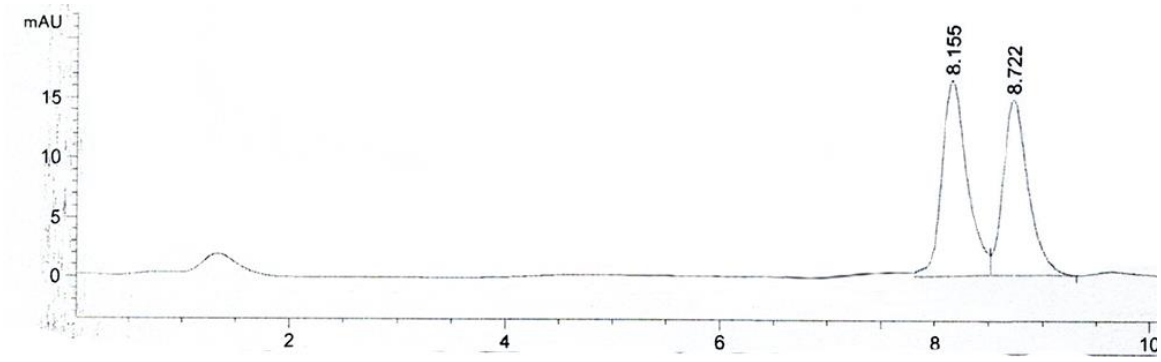
^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.43 (d, *J* 6.6 Hz, 3H), 2.11 (brs, NH_2), 4.18 (q, *J* 6.6 Hz, 1H), 7.34 (dd, *J* 8.1 and 4.8 Hz, 1H), 7.45 (d, *J* 8.1 Hz, 2H), 7.55 (d, *J* 8.1 Hz, 2H), 7.87 (dt, *J* 7.8 and 2.1 Hz, 1H), 8.56 (dd, *J* 4.5 and 1.5 Hz, 1H), 8.83 (d, *J* 1.2 Hz, 1H).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ 25.60 (CH_3), 50.98 (CH), 123.57 (CH), 126.49 (2CH), 127.24 (2CH), 134.29 (CH), 136.31 (C), 136.41 (C),147.63 (C), 148.11 (CH), 148.23 (CH).

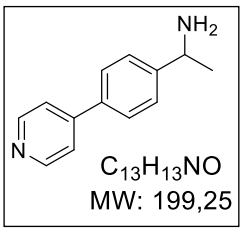
MS ($APCI^+$, m/z): 199 [$(M+H)^+$, 100%].

NP-HPLC (AD-H, hexane/isopropanol 75:25 v/v, 0.8 mL/min, 258 nm) t_R = 8.1 min (*S*), 8.7 min (*R*

HPLC separation for both enantiomers of (±)-**8d** as Boc derivative



5.7.4 Synthesis of rac-1-(4-(pyridin-4-yl)phenyl)ethanamine (8e)



The title compound was obtained with the general procedure according to the substrate in question.

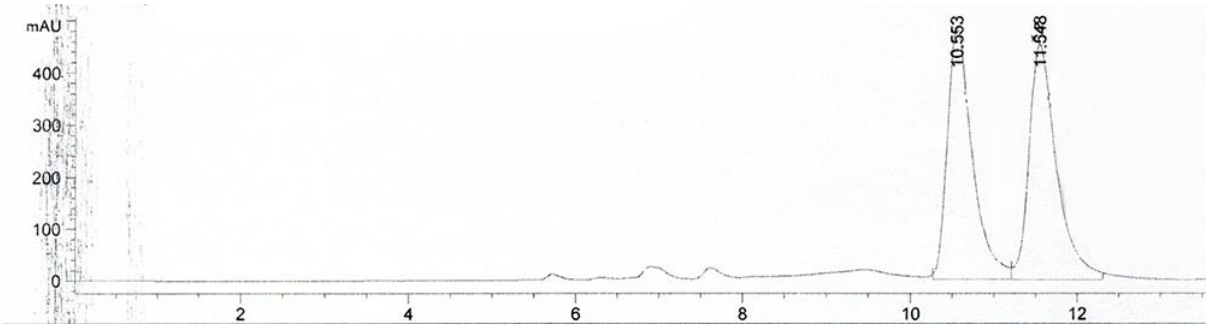
^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.41 (d, *J* 6.6 Hz, 3H), 1.85 (brs, NH_2), 4.18 (q, *J* 6.6 Hz, 1H), 7.40-7.48 (m, 4H), 7.60 (d, *J* 8.1 Hz, 2H), 8.63 (d, *J* 4.8 Hz, 1H).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ 25.70 (CH_3), 51.00 (CH), 121.48 (2CH), 126.55 (2CH), 127.10 (2CH), 136.62 (C), 148.05 (C), 148.78 (C), 150.22 (2CH).

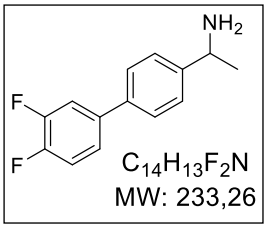
MS ($APCI^+$, m/z): 199 [$(M+H)^+$, 100%].

NP-HPLC (AD-H, hexane/isopropanol 75:25 v/v, 0.8 mL/min, 258 nm) t_R = 10.5 min (*R*), 11.5 min (*S*).

HPLC separation for both enantiomers of (±)-**8e** as Boc derivative



5.7.5 Synthesis of rac-1-(3',4'-difluoro-[1,1'-biphenyl]-4-yl)ethanamine (8f)



The title compound was obtained with the general procedure according to the substrate in question.

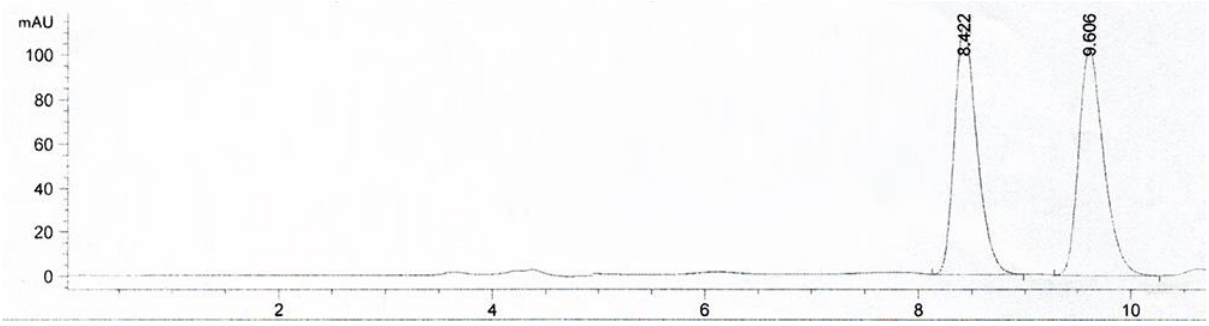
^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.59 (d, *J* 6.6 Hz, 3H), 3.78 (brs, NH_2), 4.31 (q, *J* 6.6 Hz, 1H), 7.26-7.73 (m, 9H).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ 24.69 (CH_3), 50.89 (CH), 115.84 (CH, d, J_{CF}= 17 Hz), 117.51 (CH, d, J_{CF}= 17 Hz), 122.83 (CH, dd, J_{CF}= 6 and 2 Hz), 126.55 (2CH), 127.08 (2CH), 129.35 (C, d, J_{CF}= 12 Hz), 137.95 (C), 145.63 (C), 148.52 (C, dd, J_{CF}= 45 and 12 Hz), 151.81 (C, dd, J_{CF}= 44 and 12 Hz).

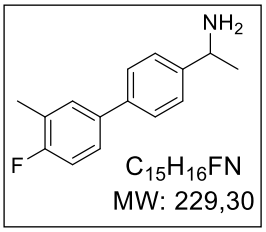
MS ($APCI^+$, m/z): 234 [$(M+H)^+$, 100%].

NP-HPLC (AD-H, hexane/isopropanol 95:5 v/v, 0.8 mL/min, 258 nm) t_R = 8.4 min (*R*), 9.6 min (*S*)

HPLC separation for both enantiomers of (±)-**8f** as Boc derivative



5.7.6 Synthesis of rac-1-(4'-fluoro-3'-methyl-[1,1'-biphenyl]-4-yl)ethanamine (8g)



The title compound was obtained with the general procedure according to the substrate in question.

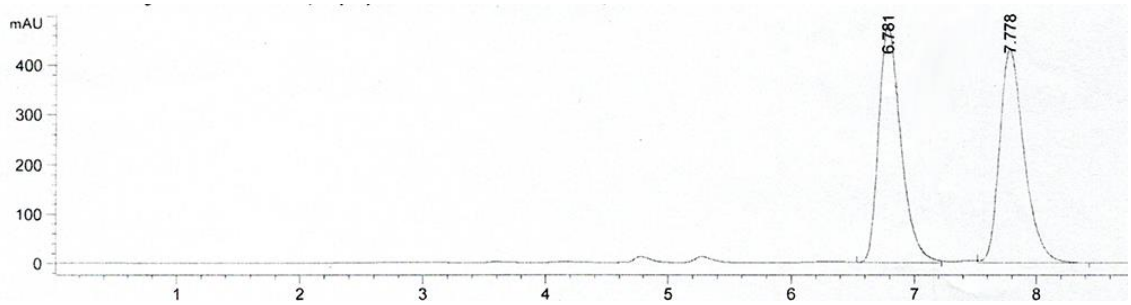
^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.45 (d, *J* 6.6 Hz, 3H), 1.84 (brs, NH_2), 2.36 (s, 3H), 4.19 (q, *J* 6.6 Hz, 1H), 7.08 (t, *J* 8.4 Hz, 1H), 7.31-7.48 (m, 4H), 7.52 (d, *J* 8.1 Hz, 1H).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ 14.69 (CH_3,d, J_{CF}= 3 Hz), 25.63 (CH_3), 51.03 (CH), 115.22 (CH, d, J_{CF}= 22 Hz), 125.52 (C, d, J_{CF}= 17 Hz), 126.18 (2CH), 127.09 (2CH), 130.11 (CH, d, J_{CF}= 5 Hz), 136.78 (C, d, J_{CF}= 4 Hz), 139.04 (C), 146.56 (C), 160.98 (C, d, J_{CF}= 245 Hz).

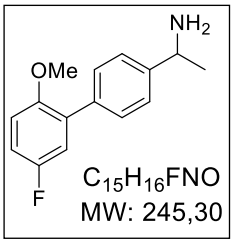
MS ($APCI^+$, m/z): 230 [$(M+H)^+$, 100%].

NP-HPLC (AD-H, hexane/isopropanol 90:10 v/v, 0.8 mL/min, 258 nm) t_R = 6.8 min (*R*), 7.8 min (*S*).

HPLC separation for both enantiomers of (±)-**8g** as Boc derivative



5.7.7 Synthesis of rac-1-(5'-fluoro-2'-methoxy-[1,1'-biphenyl]-4-yl)ethanamine (8h)



The title compound was obtained with the general procedure according to the substrate in question.

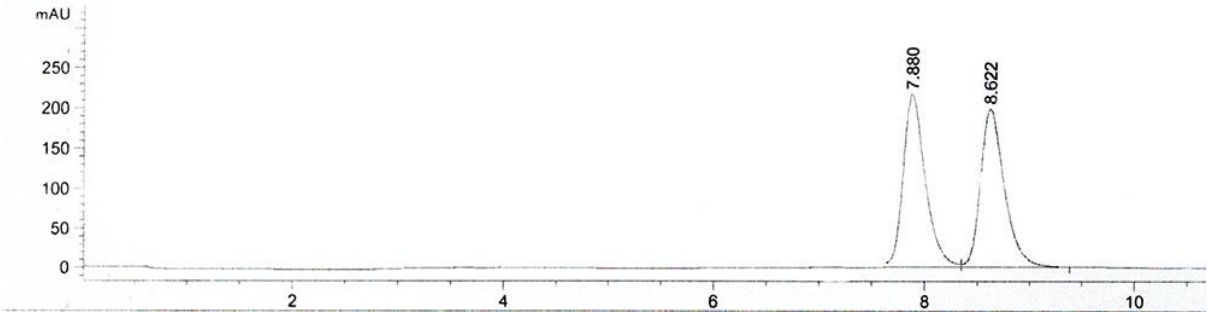
^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.59 (d, *J* 6.6 Hz, 3H), 3.80 (s, 3H), 4.67 (q, *J* 6.6 Hz, 1H), 6.92 (dd, *J* 9.0 and 4.5 Hz, 1H), 6.99-7.09 (m, 2H), 7.39 (d, *J* 8.1 Hz, 1H), 7.56 (d, *J* 8.1 Hz, 1H).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ 25.66 (CH_3), 56.28 (CH_3), 60.99 (CH), 112.43 (CH, d, J_{CF}= 7 Hz), 114.35 (CH, d, J_{CF}= 23 Hz), 117.45 (CH, d, J_{CF}= 23 Hz), 126.25 (2CH), 129.85 (2CH), 131.42 (C, d, J_{CF}= 7 Hz), 137.37 (C), 140.08 (C), 152.75 (C), 157.23 (C, d, J_{CF}= 239 Hz).

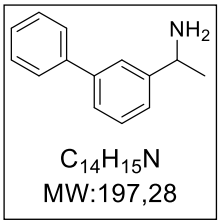
MS ($APCI^+$, m/z): 246 [$(M+H)^+$, 100%].

NP-HPLC (AD-H, hexane/isopropanol 90:10 v/v, 0.8 mL/min, 258 nm) t_R = 7.8 min (*S*), 8.6 min (*R*)

HPLC separation for both enantiomers of (±)-**8h** as Boc derivative



5.7.8 Synthesis of rac-1-([1,1'-biphenyl]-3-yl)ethanamine (8i)



The title compound was obtained with the general procedure according to the substrate in question.

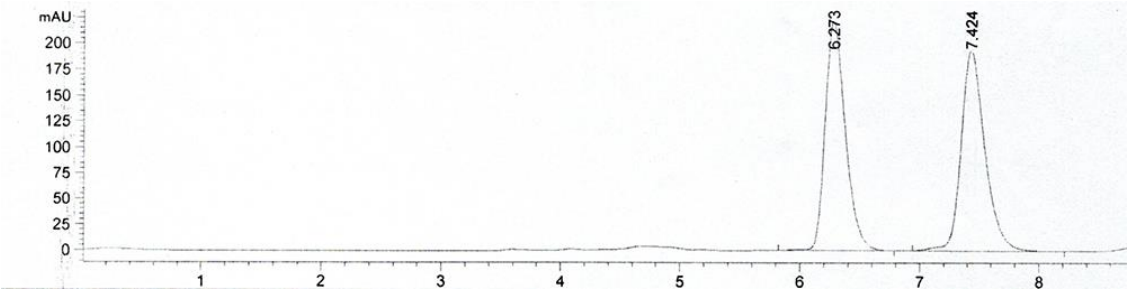
^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.47 (d, *J* 6.6 Hz, 3H), 1.78 (brs, NH_2), 4.21 (q, *J* 6.6 Hz, 1H), 7.35-7.43 (m, 2H), 7.44-7.54 (m, 4H), 7.60-7.68 (m, 2H).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ 25.86 (CH_3), 51.53 (CH), 124.73 (CH), 125.78 (C), 127.27 (2CH), 127.32 (2CH), 128.79 (2CH), 128.97 (2CH), 141.49 (C), 148.34 (C).

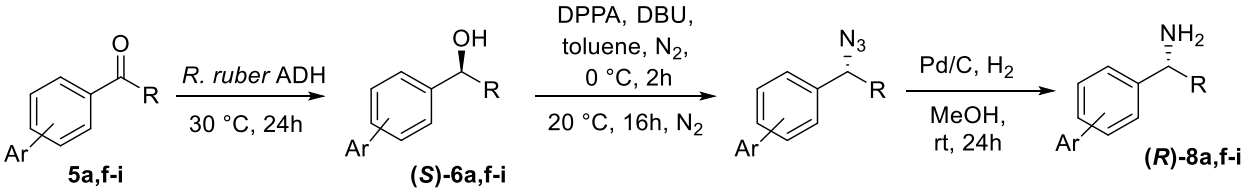
MS ($APCI^+$, m/z): 198 [$(M+H)^+$, 100%].

NP-HPLC (AD-H, hexane/isopropanol 90:10 v/v, 0.8 mL/min, 258 nm) t_R = 6.2 min (*R*), 7.4 min (*S*)

HPLC separation for both enantiomers of (±)-**8i** as Boc derivative



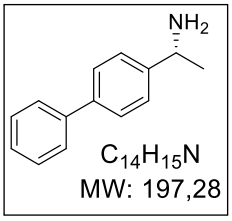
5.8 Synthesis of enantiopure amine standards



General procedure:

In a 15 mL reaction tube, ketone (**5a,f-i**, 0.25 mmol) and ADH from *R. ruber* (16 U) were added to a *i*-PrOH (5 mL)/50 mM KPi buffer pH 7.0 (5 mL) mixture (1 mM NAD^+). The reaction was shaken at 30 °C and 250 rpm for 24 h. The mixture was extracted with ethyl acetate (2 × 10 mL), the organic layers combined, dried over Na_2SO_4, filtered and concentrated under vacuum to provide the corresponding (*S*)-alcohol (**6a,f-i**, ee >99%). Then, the corresponding (*S*)-alcohol (**6a,f-i**, 0.25 mmol) and diphenyl phosphoryl azide (DPPA, 0.30 mmol) were dissolved in dry toluene (0.4 mL). The mixture was cooled to 0 °C under N_2, and DBU (0.30 mmol) was added. The reaction was stirred for 2 h at 0 °C and then at 20 °C for 16 h. The resulting mixture was washed with H_2O (2 × 5 mL) and aqueous 1N HCl (5 mL). The organic layer was concentrated under reduced pressure and purified by flash chromatography (EtOAc/hexane mixtures). Finally, to a suspension containing the resulting azide compound and Pd-C 10% (10 mg) in a 25 mL round-bottom flask a H_2 balloon was connected and deoxygenated MeOH (1.0 mL) carefully added. The resulting suspension was stirred at room temperature for 24 h. After this time, the reaction mixture was filtered through Celite® and the solvent evaporated under reduced pressure to afford the corresponding (*R*)-biaryl amine as a colorless oil (**8a,f-i**, *ee* = 97%, yield = 50-70%).

5.8.1 Synthesis of (*R*)-1-([1,1'-biphenyl]-4-yl)ethanamine (8a)

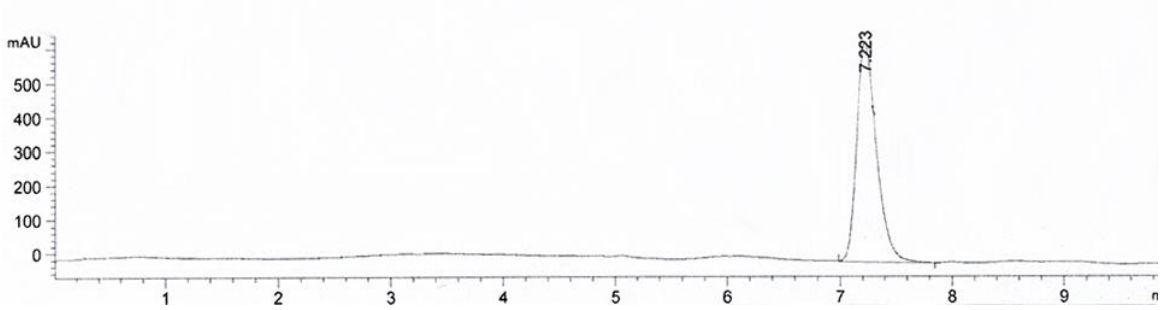


The title compound was obtained with the general procedure.

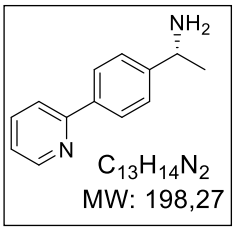
$[\alpha]_D^{18}$ +18.7 (*c* 0.5, $CHCl_3$), *ee* = 99% for (*R*)-**8a.**

NP-HPLC (AD-H, hexane/isopropanol 90:10 v/v, 0.8 mL/min, 258 nm) t_R = 7.2 min (*R*).

HPLC chromatogram of compound (*R*)-**8a** in >99% ee



5.8.2 Synthesis of (*R*)-1-(4-(pyridin-2-yl)phenyl)ethanamine (8c)

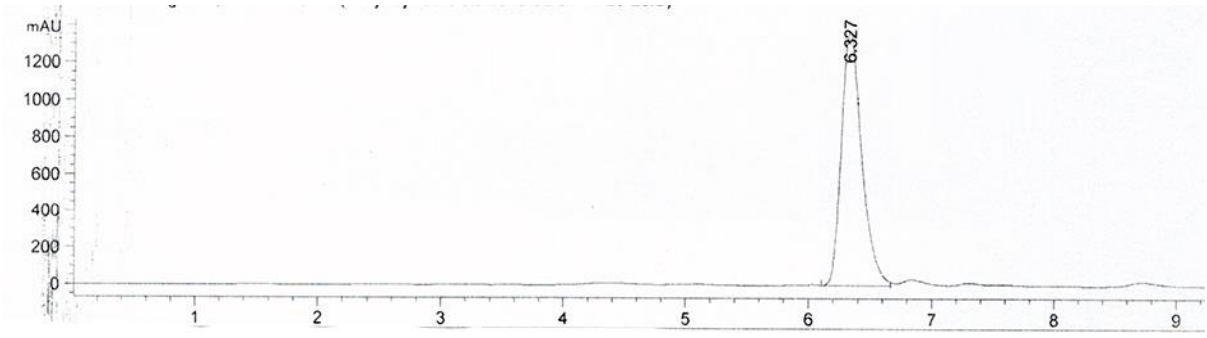


The title compound was obtained with the general procedure.

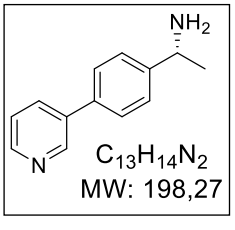
$[\alpha]_D^{18}$ +20.7 (*c* 1.3, $CHCl_3$), *ee* = 99% for (*R*)-**8c.**

NP-HPLC (AD-H, hexane/isopropanol 75:25 v/v, 0.8 mL/min, 258 nm) t_R = 6.3 min (*R*.

HPLC chromatogram of compound (*R*)-**8c** in >99% ee



5.8.3 Synthesis of (*R*)-1-(4-(pyridin-3-yl)phenyl)ethanamine (8d)

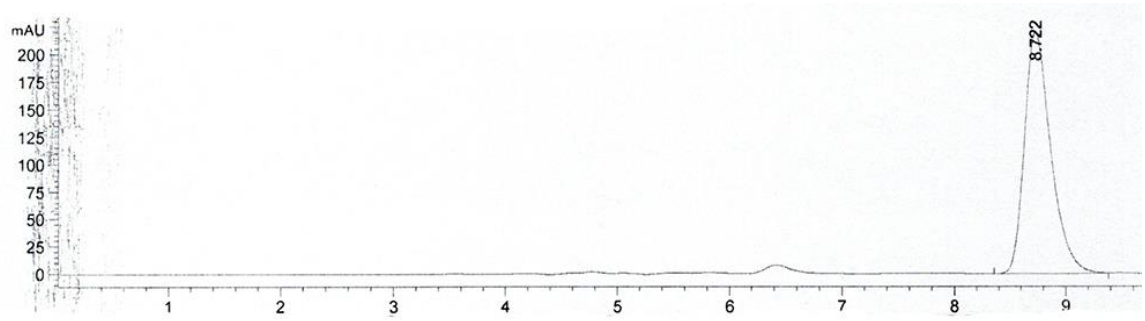


The title compound was obtained with the general procedure.

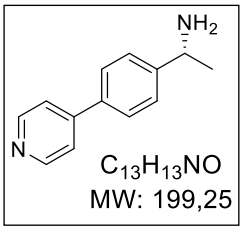
$[\alpha]_D^{18}$ +24.5 (*c* 1.0, $CHCl_3$), *ee* = 99% for (*R*)-**8d.**

NP-HPLC (AD-H, hexane/isopropanol 75:25 v/v, 0.8 mL/min, 258 nm) t_R = 8.7 min (*R*)

HPLC chromatogram of compound (*R*)-**8d** in >99% ee



5.8.4 Synthesis of (*R*)-1-(4-(pyridin-4-yl)phenyl)ethanamine (8e)

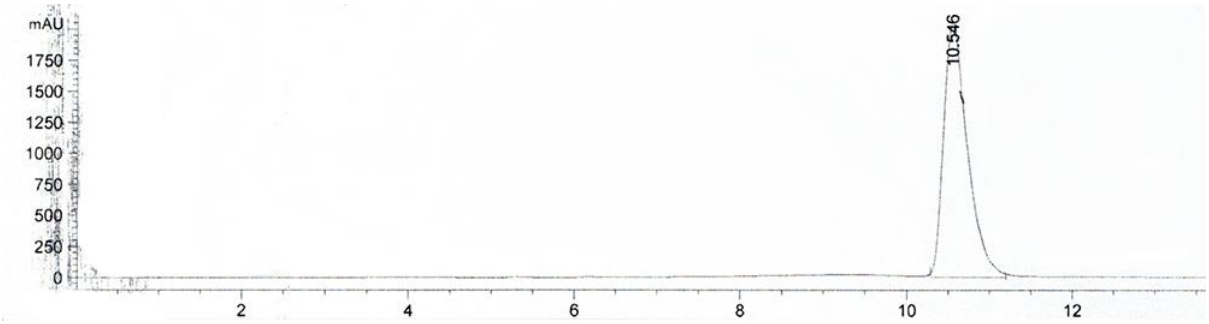


The title compound was obtained with the general procedure.

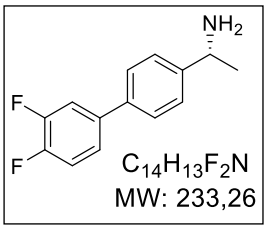
$[\alpha]_D^{18}$ +23.3 (*c* 1.0, $CHCl_3$), *ee* = 99% for (*R*)-**8e.**

NP-HPLC (AD-H, hexane/isopropanol 75:25 v/v, 0.8 mL/min, 258 nm) t_R = 10.5 min (*R*).

HPLC chromatogram of compound (*R*)-**8e** in >99% ee



5.8.5 Synthesis of (*R*)-1-(3',4'-difluoro-[1,1'-biphenyl]-4-yl)ethanamine (8f)

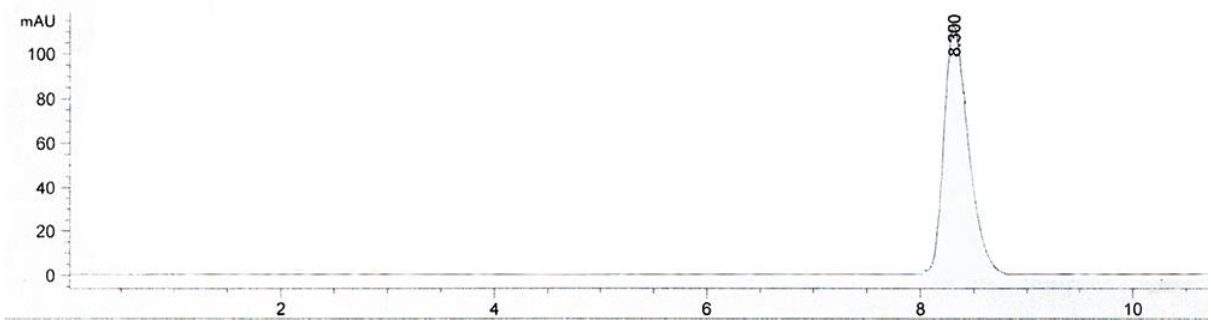


The title compound was obtained with the general procedure.

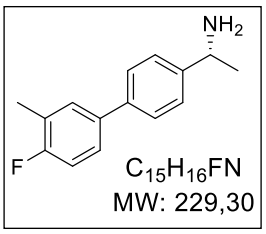
$[\alpha]_D^{18}$ +21.0 (*c* 1.1, $CHCl_3$), *ee* = 97% for (*R*)-**8f.**

NP-HPLC (AD-H, hexane/isopropanol 95:5 v/v, 0.8 mL/min, 258 nm) t_R = 8.4 min (*R*).

HPLC chromatogram of compound (*R*)-**8f** in >99% ee



5.8.6 Synthesis of (*R*)-1-(4'-fluoro-3'-methyl-[1,1'-biphenyl]-4-yl)ethanamine (8g)

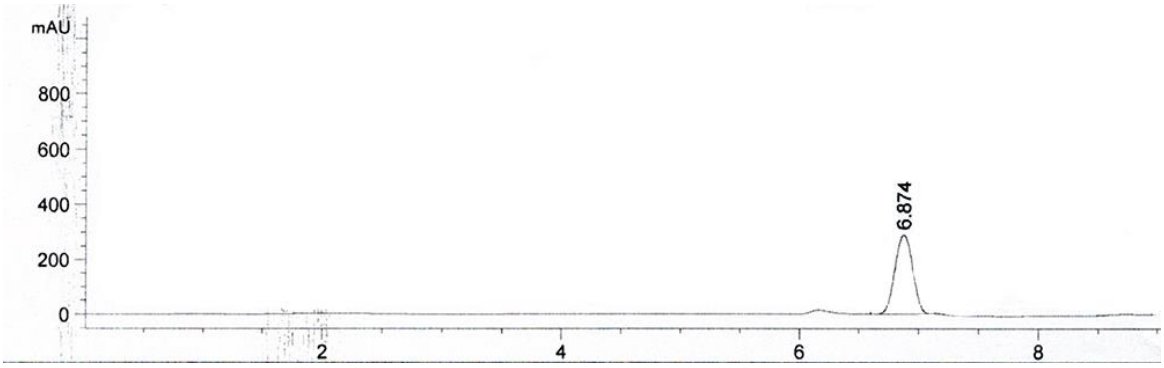


The title compound was obtained with the general procedure.

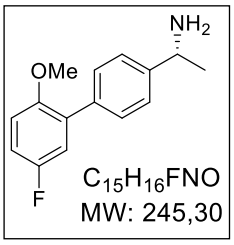
$[\alpha]_D^{18}$ +39.4 (*c* 0.8, $CHCl_3$), *ee* = 97% for (*R*)-**8g.**

NP-HPLC (AD-H, hexane/isopropanol 90:10 v/v, 0.8 mL/min, 258 nm) t_R = 6.8 min (*R*)

HPLC chromatogram of compound (*R*)-**8g** in >99% ee



5.8.7 Synthesis of (*R*)-1-(5'-fluoro-2'-methoxy-[1,1'-biphenyl]-4-yl)ethanamine (8h)



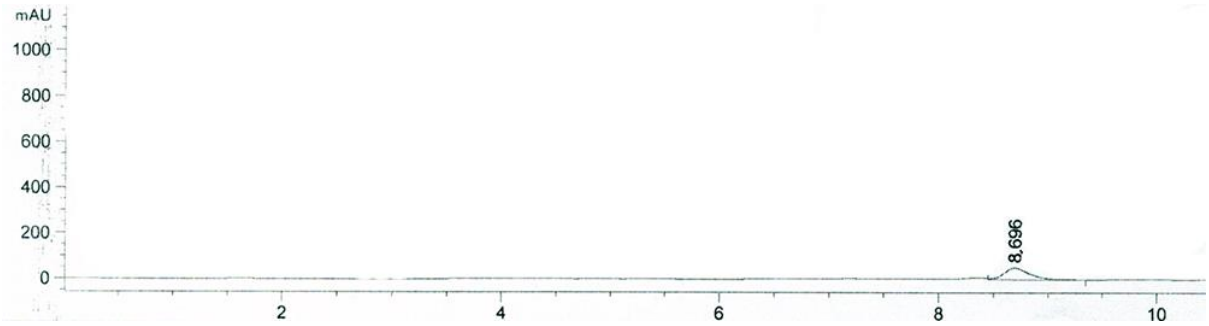
$C_{15}H_{16}FNO$
MW: 245,30

The title compound was obtained with the general procedure.

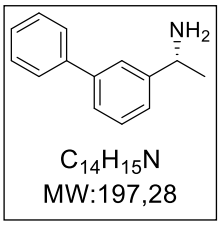
$[\alpha]_D^{18}$ +34.7 (*c* 1.1, $CHCl_3$), *ee* 97% for (*R*)-**8h.**

NP-HPLC (AD-H, hexane/isopropanol 90:10 v/v, 0.8 mL/min, 258 nm) t_R = 8.6 min (*R*)

HPLC chromatogram of compound (*R*)-**8h** in >99% ee



5.8.8 Synthesis of (*R*)-1-([1,1'-biphenyl]-3-yl)ethanamine (8i)



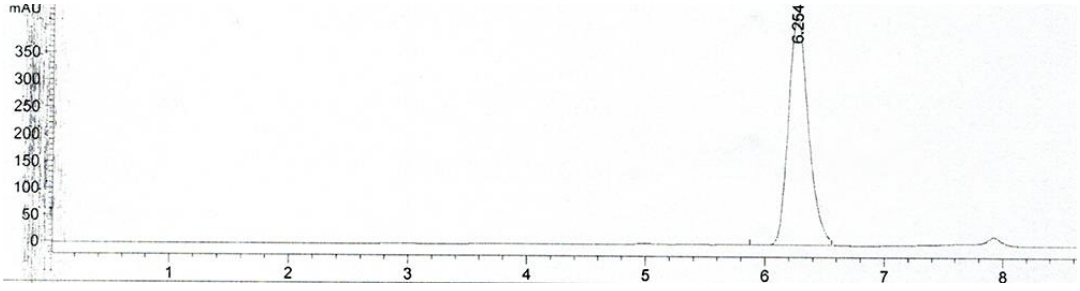
$C_{14}H_{15}N$
MW:197,28

The title compound was obtained with the general procedure.

$[\alpha]_D^{18}$ +25.2 (*c* 1.0, $CHCl_3$), *ee* >99% for (*R*)-**8i.**

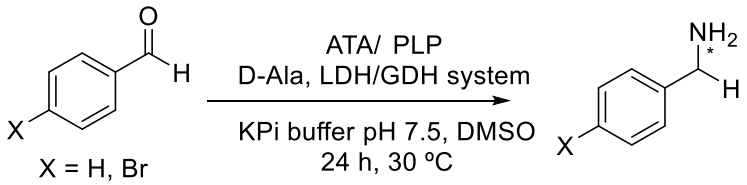
NP-HPLC (AD-H, hexane/isopropanol 90:10 v/v, 0.8 mL/min, 258 nm) t_R = 6.2 min (*R*)

HPLC chromatogram of compound (*R*)-**8i** in >99% ee



5.9 Enzymatic transamination of ketones

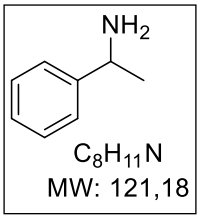
5.9.1 Screening of monoaryl ketones with alanine as amine donor



General procedure for the screening of monoaryl ketones using alanine as amine donor:

The ketone (20 mM) was first dissolved in DMSO (32 µL, 5% v/v) in a 2 mL reaction tube (Eppendorf). Then ATA lysate (8 U), potassium phosphate buffer (KPi) 100 mM pH 7.5, 1 mM PLP, 0.1 mM NAD^+, D-glucose (57 mM), D-alanine (130 mM), LDH (90 U) and GDH (30 U) were added. The reaction was shaken at 30 °C and 250 rpm for 24 h. To determine the conversion, 10 µL of the mixture were diluted with 90 µL of DMSO and analysed by achiral reverse phase with previous centrifuging and filtering of the sample. To determine the enantiomeric excess the reaction was quenched by addition of aqueous 10 N NaOH (400 µL). The mixture was then extracted with ethyl acetate (2 × 500 µL) and the organic layers were separated by centrifugation (90 s, 13000 rpm), combined and dried over Na_2SO_4.

5.9.1.1 *Synthesis of α-methylbenzylamine (7a)*



The title compound was obtained according to the general procedure.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.24 (d, J 6.5 Hz, 3H), 4.25 (q, J 6.5 Hz, 1H), 7.28 (m, 2H), 7.30 (m, 1H), 7.33-7.45 (m, 2H).

NP-HPLC (OJ-H, hexane/isopropanol 90:10 v/v, 0.8 mL/min, 258 nm) t_R = 7.54 min (*S*), 8.54 min (*R*).

HPLC separation for both enantiomers of compound (±)-**7a**

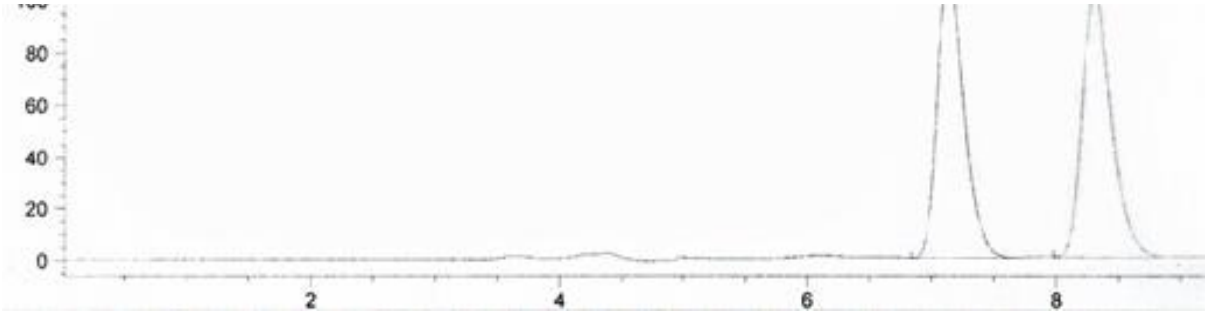
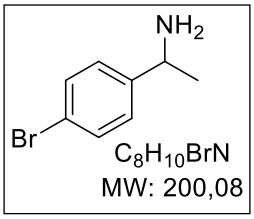


Table 25. Screening of various ATAs to obtain the monoaryl amine **7a**

Entry	Enzyme	*c* (%)	*ee* (%)
1	Vf	≥ 99	≥99 (*S*)
2	Cv	90	≥99 (*S*)
3	Ate	98	98 (*R*)
4	Ate-TA_T274S	≥ 99	≥99 (*R*)
5	Esi	5	≥99 (*R*)
6	EX-wt	15	≥99 (*R*)
7	Pac	42	≥99 (*R*)
8	Shi	51	≥99 (*R*)
9	Tja	10	≥99 (*R*)

5.9.1.2 *Synthesis of 4-bromo-α-methylbenzylamine (7b)*



The title compound was obtained according to the general procedure.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.20 (d, J 6.6 Hz, 3H), 4.32 (q, J 6.6 Hz, 1H), 7.30 (ddd, J 8.3, 2H), 7.45 (ddd, J = 8.3, 2H).

NP-HPLC (OJ-H, hexane/isopropanol 90:10 v/v, 0.8 mL/min, 258 nm) t_R = 8.4 min (*S*), 8.89 min (*R*).

HPLC separation for both enantiomers of compound (±)-**7b**

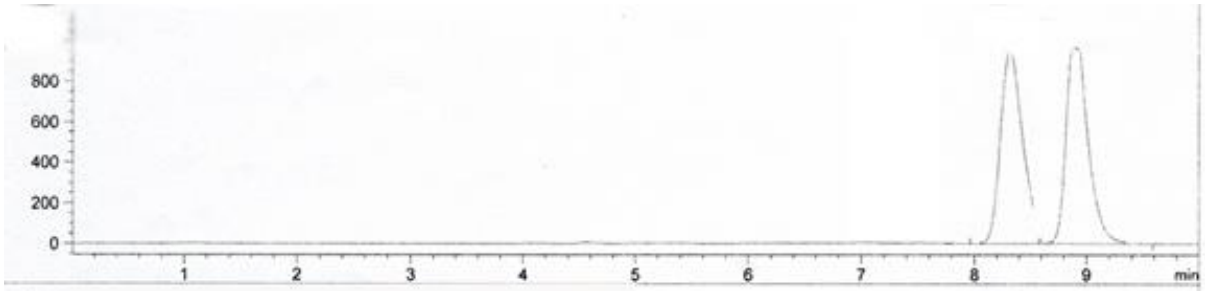
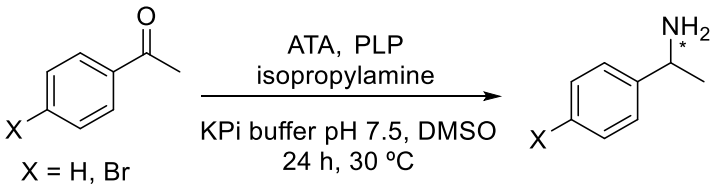


Table 26. Screening of various ATAs to obtain the monoaryl amine **7b**

Entry	Enzyme	*c* (%)	*ee* (%)
1	Vf	90	≥99 (*S*)
2	Cv	68	≥99 (*S*)
3	Ate	93	≥99 (*R*)
4	Ate-TA_T274S	95	≥99 (*R*)
5	Esi	18	≥99 (*R*)
6	EX-wt	>99	≥99 (*R*)
7	Pac	45	≥99 (*R*)
8	Shi	95	≥99 (*R*)
9	Tja	13	≥99 (*R*)

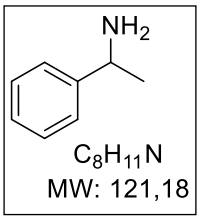
5.9.2 Screening of monoaryl ketones with isopropylamine as amine donor



General procedure for the synthesis of monoaryl amines with isopropylamine as amine donor:

In a 2 mL reaction tube, ketone (20 mM) was first dissolved in DMSO (32 µL, 5% v/v) and then ATA lysate (8 U), KPi buffer 100 mM pH 7.5, 1 M isopropylamine and 1 mM PLP were added. The reaction was shaken at 30 °C and 250 rpm for 24 h. To determine the conversion, 10 µL of the mixture were diluted with 90 µL DMSO and analysed by achiral reverse phase with previous centrifuging and filtering of the sample. To determine the enantiomeric excess the reaction was quenched by addition of aqueous 10 N NaOH (400 µL). The mixture was then extracted with ethyl acetate (2 × 500µL) and the organic layers were separated by centrifugation (90 s, 13000 rpm), combined and dried over Na_2SO_4.

5.9.2.1 *Synthesis of α-methylbenzylamine (7a)*

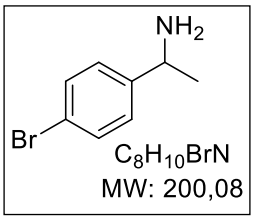


The title compound was obtained according to the general procedure.

Table 27. Screening of various ATAs to obtain the monoaryl amine **7a**

Entry	Enzyme	*c* (%)	*ee* (%)
1	Esi	<5	n.d.
2	EX-wt	0	n.d.
3	Pac	23	n.d.
4	Shi	<5	n.d.
5	Tja	<5	n.d.

5.9.2.2 *Synthesis of 4-bromo-α-methylbenzylamine (7b)*

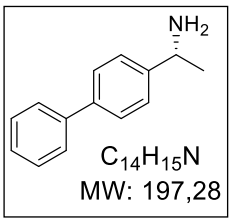


The title compound was obtained according to the general procedure.

Table 28. Screening of various ATAs to obtain the monoaryl amine **7b**

Entry	Enzyme	*c* (%)	*ee* (%)
1	Esi	16	n.d.
2	EX-wt	0	n.d.
3	Pac	32	n.d.
4	Shi	20	n.d.
5	Tja	0	n.d.

5.9.3 Synthesis of (*R*)-1-([1,1'-biphenyl]-4-yl)ethanamine (8a) with alanine as amine donor

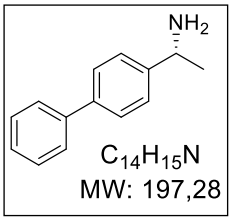


The ketone (**5a**, 20 mM) was first dissolved in DMSO (32 µL, 5% v/v) in a 2 mL reaction tube (Eppendorf). Then EX-ωTA lysate (8 U), potassium phosphate buffer (KPi) 100 mM pH 7.5, 1 mM PLP, 0.1 mM NAD^+, D-glucose (57 mM), D-alanine (130 mM), LDH (90 U) and GDH (30 U) were added. The reaction was shaken at 30 °C and 250 rpm for 24 h. To determine the conversion, 10 µL of the mixture were diluted with 90 µL of DMSO and analysed by achiral reverse phase with previous centrifuging and filtering of the sample. To determine the enantiomeric excess the reaction was quenched by addition of aqueous 10 N NaOH (400 µL). The mixture was then extracted with ethyl acetate (2 × 500 µL) and the organic layers were separated by centrifugation (90 s, 13000 rpm), combined and dried over Na_2SO_4. The enantiomeric excess of amines was measured by chiral HPLC.

Table 29. Screening of different transaminases to obtain **5a**

Entry	Enzyme	*c* (%)	*ee* (%)
1	Vf-ATA	0	n.d.
2	Cv-ATA	0	n.d.
3	Ate-ATA	0	n.d.
4	Ate-ATA_T274S	0	n.d.
5	Esi	0	n.d.
6	EX-wt	83	≥99 (*R*)
7	Pac	0	n.d.
8	Shi	17	≥99 (*R*)
9	Tja	0	n.d.

5.9.4 Synthesis of (*R*)-1-([1,1'-biphenyl]-4-yl)ethanamine (8a) with isopropylamine as amine donor

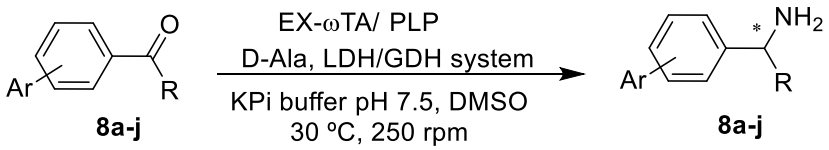


In a 2 mL reaction tube, ketone (**5a**, 20 mM) was first dissolved in DMSO (32 µL, 5% v/v) and then ATA lysate (8 U), KPi buffer 100 mM pH 7.5, 1 M isopropylamine and 1 mM PLP were added. The reaction was shaken at 30 °C and 250 rpm for 24 h. To determine the conversion, 10 µL of the mixture were diluted with 90 µL DMSO and analysed by achiral reverse phase with previous centrifuging and filtering of the sample. To determine the enantiomeric excess the reaction was quenched by addition of aqueous 10 N NaOH (400 µL). The mixture was then extracted with ethyl acetate (2 × 500µL) and the organic layers were separated by centrifugation (90 s, 13000 rpm), combined and dried over Na_2SO_4. The enantiomeric excess of amines was measured by chiral HPLC.

Table 30. Screening of various ATAs to obtain **8a**

Entry	Enzyme	*c* (%)	*ee* (%)
1	Vf	0	n.d.
2	Cv	0	n.d.
3	Ate	0	n.d.
4	Ate-TA_T274S	0	n.d.
5	Esi	0	n.d.
6	EX-wt	83	≥99 (*R*)
7	Pac	0	n.d.
8	Shi	17	≥99 (*R*)
9	Tja	0	n.d.

5.9.5 Synthesis of biaryl amines 8a-j with alanine as amine donor

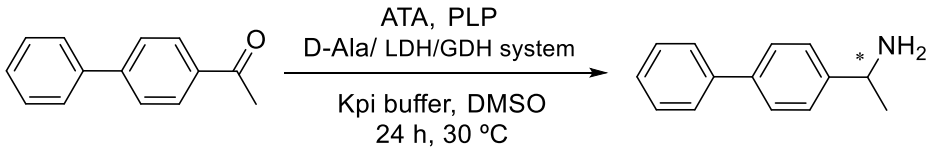


General procedure for the screening of biaryl ketones (8a-j) with alanine as amine donor:

The ketone (20 mM) was first dissolved in DMSO (32 µL, 5% v/v) in a 2 mL reaction tube (Eppendorf). Then ATA lysate (8 U), potassium phosphate buffer (KPi) 100 mM pH 7.5, 1 mM PLP, 0.1 mM NAD^+, D-glucose (57 mM), D-alanine (130 mM), LDH (90 U) and GDH (30 U) were added. The reaction was shaken at 30 °C and 250 rpm for 24 h. To determine the conversion, 10 µL of the mixture were diluted with 90 µL of DMSO and analysed by achiral reverse phase with previous centrifuging and filtering of the sample. To determine the enantiomeric excess the reaction was quenched by addition of aqueous 10 N NaOH (400 µL). The mixture was then extracted with ethyl acetate (2 × 500 µL) and the organic layers were separated by centrifugation (90 s, 13000 rpm), combined and dried over Na_2SO_4.

5.9.5.1 *Enzymatic transamination of 1-(biphenyl-4-yl)ethanone (5a) using ATAs*

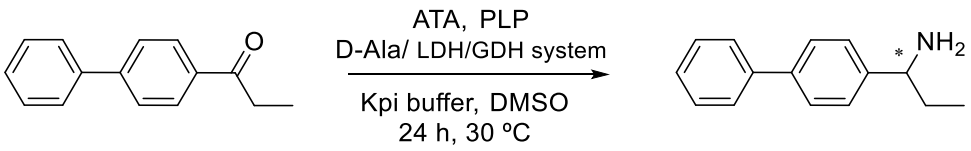
Table 31. Enzymatic transamination of 1-(biphenyl-4-yl)ethanone (**5a**) using ATAs



Entry	Enzyme	*c* (%)	*ee* (%)
1	EX-wt	83	>99 (*R*)
2	EX-X2	20	n.d.
3	EX-X3	25	n.d.
4	EX-STA	45	>99 (*R*)
5	EX-SSA	5	n.d.
6	EX-SSG	0	n.d.
7	EX-STA 5	45	n.d.
8	EX5	15	n.d.

5.9.5.2 *Enzymatic transamination of 1-([1,1'-biphenyl]-4-yl)propan-1-one (5b) using ATAs*

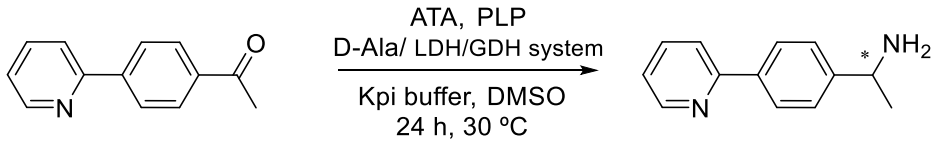
Table 34. Enzymatic transamination of 1-([1,1'-biphenyl]-4-yl)propan-1-one (**5b**) using ATAs



Entry	Enzyme	*c* (%)	*ee* (%)
1	EX-wt	0	n.d
2	EX-X2	0	n.d
3	EX-X3	0	n.d.
4	EX-STA	0	n.d
5	EX-SSA	0	n.d.
6	EX-SSG	0	n.d.

5.9.5.3 *Enzymatic transamination of 1-(4-(pyridin-2-yl)phenyl)ethanone (5c) using ATAs*

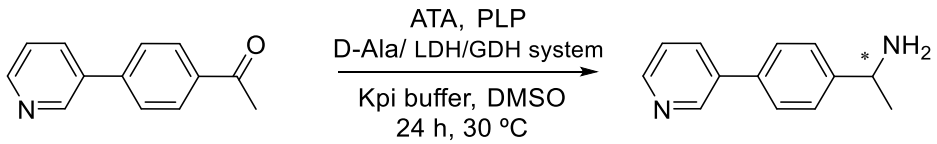
Table 38. Enzymatic transamination of 1-(4-(pyridin-2-yl)phenyl)ethanone (**5c**) using ATAs



Entry	Enzyme	*c* (%)	*ee* (%)
1	EX-wt	72	>99 (*R*)
2	EX-STA	99	>99 (*R*)
3	EX-SSA	10	n.d.
4	EXSSG	10	n.d.
5	EX-STA5	80	n.d.
6	EX-X5	40	n.d.

5.9.5.4 *Enzymatic transamination of 1-(4-(pyridin-3-yl)phenyl)ethanone (5d) using ATAs*

Table 39. Enzymatic transamination of 1-(4-(pyridin-3-yl)phenyl)ethanone (**5d**) using ATAs

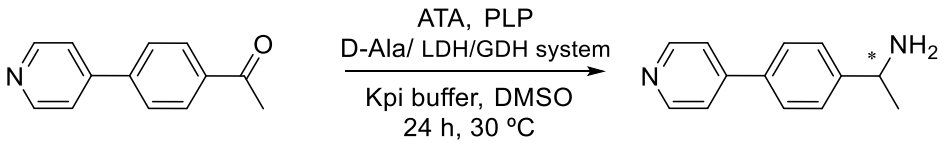


Entry	Enzyme	*c* (%)	*ee* (%)
1	EX-wt	82	>99 (*R*)
2	EX-STA	99	>99 (*R*)

Entry	Enzyme	*c* (%)	*ee* (%)
3	EX-SSA	10	n.d.
4	EX-SSG	10	n.d.
5	EX-STA5	95	n.d.
6	EX-X5	50	n.d.

5.9.5.5 *Enzymatic transamination of 1-(4-(pyridin-4-yl)phenyl)ethanone (5e) using ATAs*

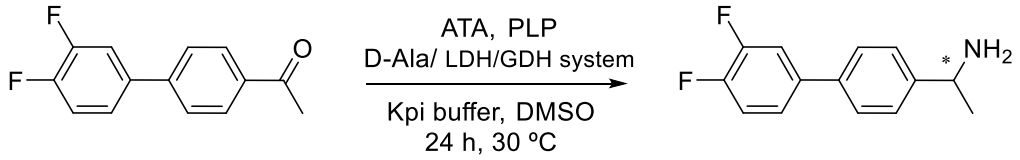
Table 40. Enzymatic transamination of 1-(4-(pyridin-4-yl)phenyl)ethanone (**5e**) using ATAs



Entry	Enzyme	*c* (%)	*ee* (%)
1	EX-wt	75	>99 (*R*)
2	EX-STA	99	>99 (*R*)
3	EX-SSA	10	n.d.
4	EX-SSG	10	n.d.
5	EX-STA5	70	n.d.
6	EX-X5	82	n.d.

5.9.5.6 *Enzymatic transamination of 1-(3',4'-difluoro-[1,1'-biphenyl]-4-yl)ethanone (5f) using ATAs*

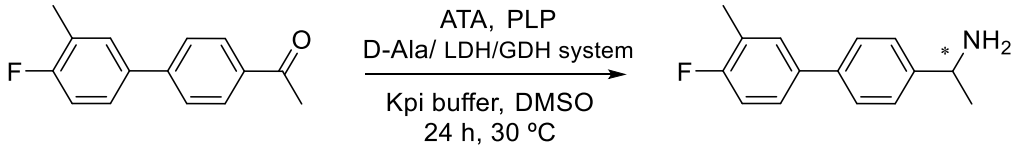
Table 35. Enzymatic transamination of 1-(3',4'-difluoro-[1,1'-biphenyl]-4-yl)ethanone (**5f**) using ATAs



Entry	Enzyme	*c* (%)	*ee* (%)
1	EX-wt	25	n.d.
2	EX-X2	5	n.d.
3	EX-X3	10	n.d.
4	EX-STA	40	>99 (*R*)
5	EXSSA	5	n.d.
6	EX-SSG	0	n.d.
7	EX-STA5	30	n.d.
8	EX-X5	30	n.d.

5.9.5.7 *Enzymatic transamination of 1-(4'-fluoro-3'-methyl-[1,1'-biphenyl]-4-yl)ethanone (5g) using ATAs*

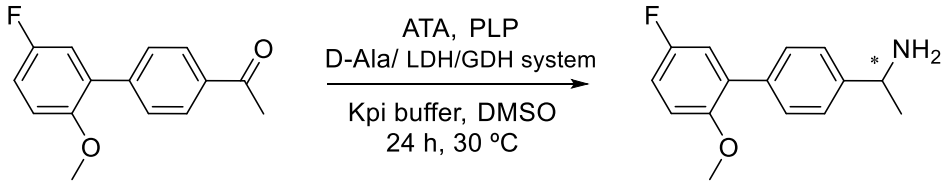
Table 36. Enzymatic transamination of 1-(4'-fluoro-3'-methyl-[1,1'-biphenyl]-4-yl)ethanone (**5g**) using ATAs



Entry	Enzyme	*c* (%)	*ee* (%)
1	EX-wt	21	n.d.
2	EX-X2	10	n.d.
3	EX-X3	12	n.d.
4	EX-STA	72	>99 (*R*)
5	EX-SSA	5	n.d.
6	EX-SSG	0	n.d.
7	EX-STA5	55	n.d.
8	EX-X5	84	n.d.

5.9.5.8 *Enzymatic transamination of 1-(5'-fluoro-2'-methoxy-[1,1'-biphenyl]-4-yl)ethanone (5h) using ATAs*

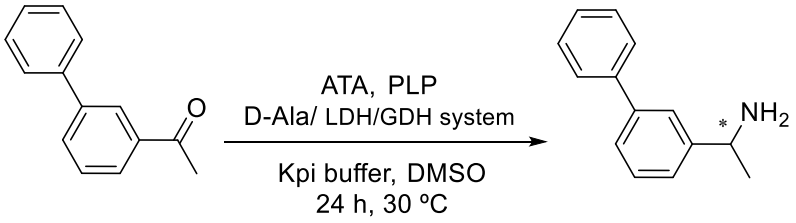
Table 37. Enzymatic transamination of 1-(5'-fluoro-2'-methoxy-[1,1'-biphenyl]-4-yl)ethanone (**5h**) using ATAs



Entry	Enzyme	*c* (%)	*ee* (%)
1	EX-wt	25	n.d.
2	EX-X2	5	n.d.
3	EX-X3	10	n.d.
4	EX-STA	30	>99 (*R*)
5	EX-SSA	5	n.d.
6	EX-SSG	5	n.d.
7	EX-STA5	24	n.d.
8	EX-X5	30	n.d.

5.9.5.9 *Enzymatic transamination of 1-([1,1'-biphenyl]-3-yl)ethanone (5i using ATAs*

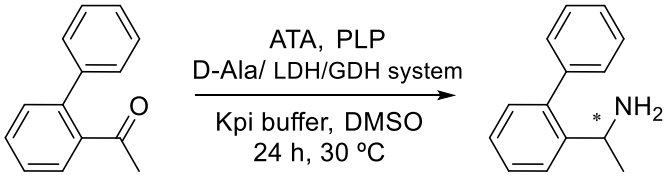
Table 32. Enzymatic transamination of 1-([1,1'-biphenyl]-3-yl)ethanone (**5i**) using ATAs



Entry	Enzyme	*c* (%)	*ee* (%)
1	EX-wt	38	n.d.
2	EX-X2	23	n.d.
3	EX-X3	30	n.d.
4	EX-STA	85	>99 (*R*)
5	EX-SSA	17	n.d.
6	EX-SSG	5	n.d.
7	EX-STA5	85	n.d.
8	EX-X5	28	n.d.

5.9.5.10 *Enzymatic transamination of 1-([1,1'-biphenyl]-2-yl)ethanone (5j) using ATAs*

Table 33. Enzymatic transamination of 1-([1,1'-biphenyl]-2-yl)ethanone (**5j**) using ATAs



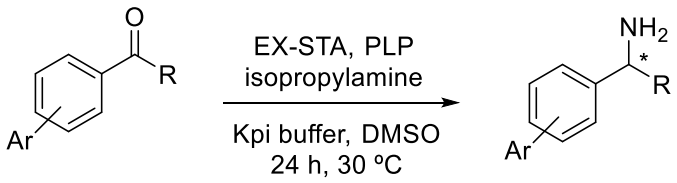
Entry	Enzyme	*c* (%)	*ee* (%)
1	EX-wt	0	n.d.
2	EX-X2	8	n.d.
3	EX-X3	6	n.d.
4	EX-STA	5	n.d.
5	EX-SSA	0	n.d.
6	EX-SSG	0	n.d.

5.9.6 Synthesis of biaryl amines 8a-j using EX-STA with isopropylamine as amine donor

General procedure:

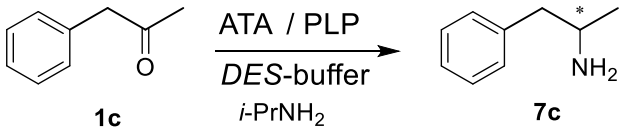
In a 2 mL reaction tube, ketone (20 mM) was first dissolved in DMSO (32 µL, 5% v/v) and then EX-STA lysate (8 U), KPi buffer 100 mM pH 7.5, 1 M isopropylamine and 1 mM PLP were added. The reaction was shaken at 30 °C and 250 rpm for 24 h. To determine the conversion, 10 µL of the mixture were diluted with 90 µL DMSO and analysed by achiral reverse phase with previous centrifuging and filtering of the sample. To determine the enantiomeric excess the reaction was quenched by addition of aqueous 10 N NaOH (400 µL). The mixture was then extracted with ethyl acetate (2 × 500µL) and the organic layers were separated by centrifugation (90 s, 13000 rpm), combined and dried over Na_2SO_4.

Table 41. ATA-catalysed transamination of biaryl ketones using EX-STA and isopropylamine as amine donor



Entry	Ketone	*c* (%)	*ee* (%)
1	8a	0	n.d.
2	8b	0	n.d.
3	8c	0	n.d.
4	8d	0	n.d.
5	8e	0	n.d.
6	8f	0	n.d.
7	8g	0	n.d.
8	8h	0	n.d.
9	8i	0	n.d.
10	8j	0	n.d.

5.10 Bioamination of phenylacetone (1c)



General procedure:

Reactions were carried out in a 2.0 mL Eppendorf tube. The corresponding ATA (2.0 mg for Codexis enzymes or 5.0 mg for Cv-TA, ArR-TA, ArS-TA and ArRmut11-TA) was added to 500 μL of the corresponding mixture of *DES* and buffer 100 mM phosphate buffer pH 7.5, containing propan-2-amine (1.0 M) and the cofactor PLP (1.0 mM). Then, a solution of **1c** (2.0 mg) was added and the resulting mixture was shaken at 250 rpm and 30 °C for 24 h. After this time, a 50 μL aliquot was removed by the determination of the degree of conversion by HPLC (see section 6.2). On the other hand, aqueous 10 N NaOH (100 μL) was added to the reaction mixture, which was extracted with ethyl acetate (2 × 500 μL). The organic layers were separated by centrifugation (90 s, 13000 rpm), combined, and finally dried over Na_2SO_4. The enantiomeric excess of the resulting amine was determined by chiral HPLC after conventional derivatization of the sample using acetic anhydride (2 μL / mg of substrate).

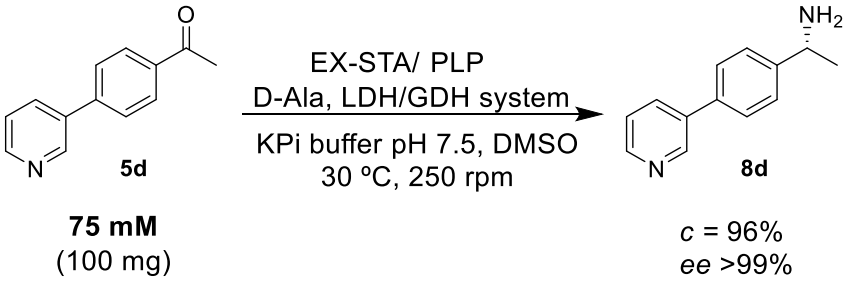
Table 42. Effect of different *DES*s-buffer media on the conversion of the ATA-catalysed bioamination of phenylacetone (**1c**)

Entry	Enzyme	Buffer	1*ChCl*/2*Gly*			1*ChCl*/2H_2O		1*ChCl*/1*Sorb*		1*ChCl*/2Urea	
			25% *DES*	50% *DES*	75% *DES*	25% *DES*	50% *DES*	25% *DES*	50% *DES*	25% *DES*	50% *DES*
1	ATA-237	95	98	93	93	90	90	93	88	91	88
2	ATA-251	95	97	92	92	93	90	>99	91	95	86
3	ATA-256	57	95	95	95	91	92	85	88	90	85
4	ATA-P1-G06	95	95	95	92	95	90	96	91	95	90
5	Cv	91	94	90	85	5	30	12	15	<5	<5
6	ArS	64	45	42	40	10	25	10	35	<5	<5

7	ArR	91	55	60	72	50	80	85	90	70	65
8	ArRmut11	>99	95	95	95	90	80	73	95	85	90

NP- HPLC (OD-H, hexane/isopropanol 96:4 v/v, 0.8 mL/min, 258 nm) t_R = 21.7 min (*S*), 23.9 min (*R*)

5.11 Preparative-scale synthesis of (*R*)-1-(4-(pyridin-3-yl)phenyl)ethanamine (8d)



75 mM
(100 mg)

c = 96%
ee >99%

General procedure:

After dissolving **5d** (75 mM, 100 mg) in DMSO (319 µL, 5% v/v) in a 50 mL reaction tube, lyophilised EX-STA (320 U, 700 mg), 100 mM KPi buffer pH 7.5 (5.8 mL, 1 mM PLP, 0.1 mM NAD+), D-glucose (114 mM, 293 mg), alanine (260 mM, 332 mg), LDH (3604 U, 682 µL) and GDH (1201 U, 400 mg) were added. The reaction was shaken at 30 °C and 250 rpm for 24 h. The reaction was quenched by addition of aqueous 10 N NaOH (10 mL) to adjust the pH to 14. The mixture was then extracted with ethyl acetate (2 × 20 mL) and the organic layers were separated by centrifugation (90 s, 13000 rpm), combined and dried over Na_2SO_4. Further evaporation under reduced pressure provided the compound (*R*)-**8d** as a white solid in 96% yield (ee >99%).

5.12 Spectrophotometric activity assay

5.12.1 Measurement of the enzymatic activity

The activity of amine transaminases is usually determined with a photometric assay. The substrate phenylethylamine is converted to acetophenone, which can be detected spectrophotometrically at 300 nm. The absorbance increases with rising product concentration. To measure the activities of the identified ATAs, reactions were set up in 1 mL volume with 50 mM KPi (pH 7.5) buffer supplemented with 0.1 mM PLP, 5 mM phenylethylamine and 5 mM pyruvic acid. For the reaction, 50 µg to 250 µg lysate protein or purified protein were used. The production of acetophenone was measured at 300 nm at 30°C for several minutes. The volumetric activity in U/mL and the specific activity in U/mg total protein of the enzyme samples is calculated using the law of Lambert-Beer with the molar extinction coefficient of acetophenone $\varepsilon_{300} = 0.28\ cm^2/\mu mol$.

Table 43. Measurement of the enzymatic volumetric activity of the identified new ATAs

Entry	Enzyme	Activity (U/ml lysate)
1	Esi	16.9
2	EX-wt	6.42
3	EX-STA	23.35
4	Pac	4.2
5	Shi	13.21
6	Tja	3.04

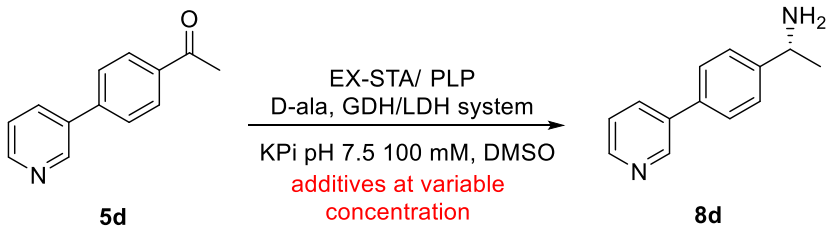
5.12.2 Measurement of the kinetics of EX-STA

Table 44. Measurement of the kinetics of EX-STA at different time intervals for a total of 4 hours

Entry	Time (h)	*c* (%)
1	0.5	35
2	1	50
3	2	75
4	3	>99
5	4	>99

5.13 Inhibition studies

5.13.1 Inhibitory effect of the cross-coupling components on the biocatalytic activity



General procedure:

The ketone (20 mM) was first dissolved in DMSO (32 µL, 5% v/v) in a 2 mL reaction tube (Eppendorf). Then ATA lysate (8 U), potassium phosphate buffer (KPi) 100 mM pH 7.5, 1 mM PLP, 0.1 mM NAD^+, D-glucose (57 mM), D-alanine (130 mM), LDH (90 U) and GDH (30 U) were added. Depending on the type of the study to this mixture the appropriate additive was added. The reaction was then shaken at 30 °C and 250 rpm for 24 h. To determine the conversion, 10 µL of the mixture were diluted with 90 µL of DMSO and analysed by achiral reverse phase with previous centrifuging and filtering of the sample. To determine the enantiomeric excess the reaction was quenched by addition of aqueous 10 N NaOH (400 µL). The mixture was then extracted with ethyl acetate (2 × 500

µL) and the organic layers were separated by centrifugation (90 s, 13000 rpm), combined and dried over Na_2SO_4. According to the study in question the reaction was repeated several times changing the parameters of the additive added.

Table 45. Measurement of the activity of EX-wt at different concentrations of the [Pd]/ligand complex

Entry	Additive conc. [mM]	Additive [mol%]	*c* (%)
1	0.18	1	81
2	0.36	2	70
3	0.54	3	67

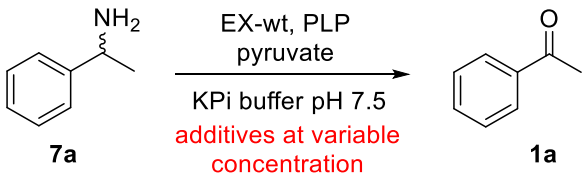
Table 46. Measurement of the activity of EX-wt at different concentrations of phenylboronic acid

Entry	Additive conc. [mM]	Additive [mol%]	*c* (%)
1	10	54	95
2	20	100	75
3	30	160	72

Table 47. Measurement of the activity of EX-wt at different concentrations of TPPTS

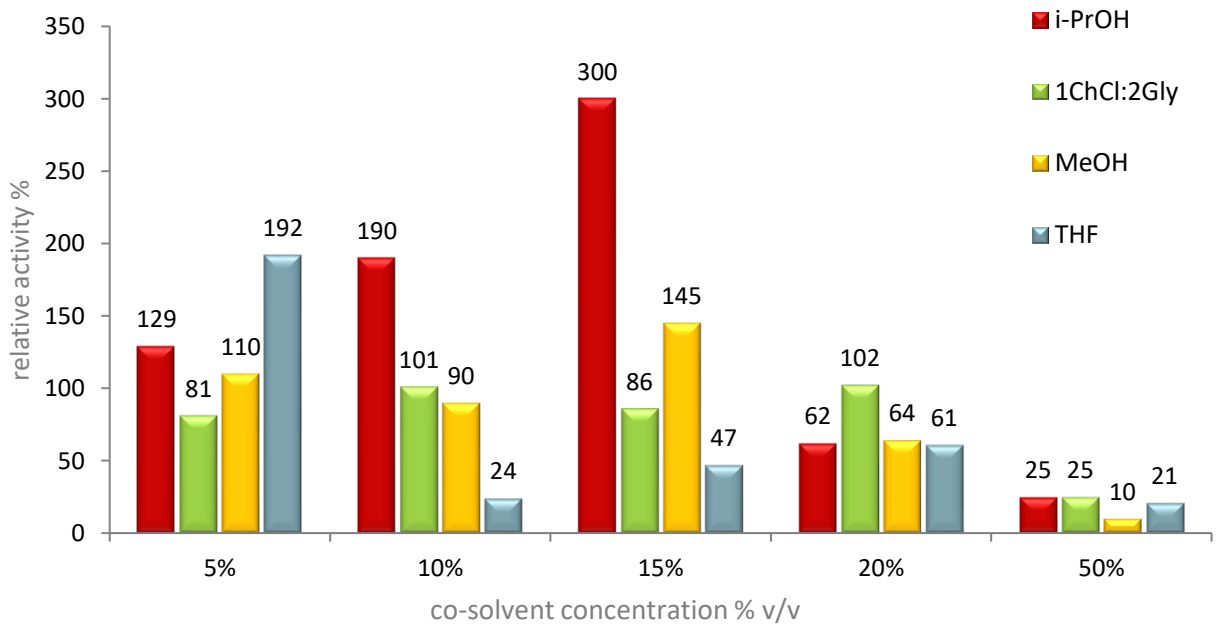
Entry	Additive conc. [mM]	Additive [mol%]	*c* (%)
1	2	11	95 %
2	3	17	74 %
3	4	22	45 %

5.13.2 Spectrophotometrical measurements of the effect of co-solvents on the biocatalytic activity



General procedure:

To measure the activities of the identified ATAs, reactions were set up in 1 mL volume with 50 mM KPi (pH 7.5) buffer supplemented with 0.1 mM PLP, 5 mM phenylethylamine, 5 mM pyruvic acid and supplemented with a predefined amount of co-solvent in question. For the reaction, 50 µg to 250 µg lysate protein or purified protein were used. The production of acetophenone was measured at 300 nm at 30°C for several minutes. To measure the absorbance a blank cuvette was placed in the spectrophotometer containing all the components except for the enzyme. The volumetric activity in U/mL and the specific activity in U/mg total protein of the enzyme samples is calculated using the law of Lambert-Beer with the molar extinction coefficient of acetophenone $\varepsilon_{300} = 0.28\ cm^2/\mu mol$.



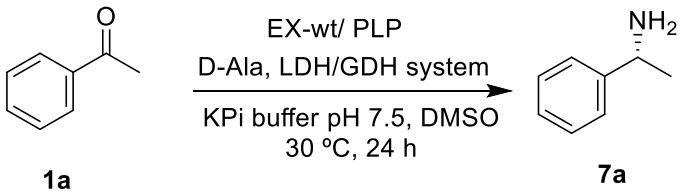
Scheme 58. Effect of co-solvents at different concentrations

5.14 Study of the effect of co-solvents on the bioamination of mono- and biaryl ketones

General procedure:

The ketone (20 mM) was first dissolved in predefined amount of the co-solvent of choice (2.5-50% v/v) in a 2 mL reaction tube (Eppendorf). Then ATA lysate (8 U), potassium phosphate buffer (KPi) 100 mM pH 7.5, 1 mM PLP, 0.1 mM NAD^+, D-glucose (57 mM), D-alanine (130 mM), LDH (90 U) and GDH (30 U) were added. The reaction was shaken at 30 °C and 250 rpm for 24 h. To determine the conversion, 10 µL of the mixture were diluted with 90 µL of DMSO and analysed by achiral reverse phase with previous centrifuging and filtering of the sample. To determine the enantiomeric excess the reaction was quenched by addition of aqueous 10 N NaOH (400 µL). The mixture was then extracted with ethyl acetate (2 × 500 µL) and the organic layers were separated by centrifugation (90 s, 13000 rpm), combined and dried over Na_2SO_4.

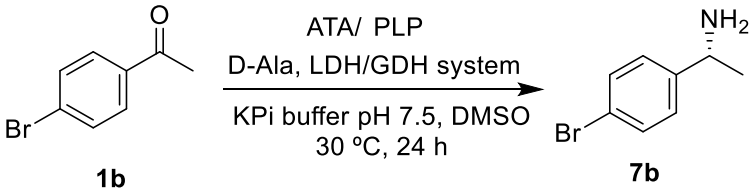
Table 48. Effect of DMSO on the EX-wt-catalysed bioamination of acetophenone **1a**



1a **7a**

Entry	Co-solvent (%)	*c* (%)	*ee* (%)
1	DMSO (2.5%)	51	>99 (*R*)
2	DMSO (5%)	43	>99 (*R*)
3	DMSO (10%)	33	>99 (*R*)

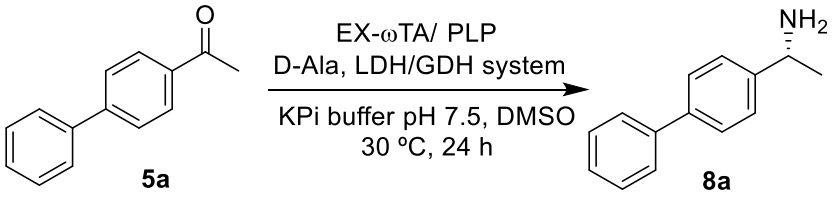
Table 49. Effect of co-solvent on the conversion of the ATA-catalysed bioamination of 4'-bromoacetophenone **1b.**



1b **7b**

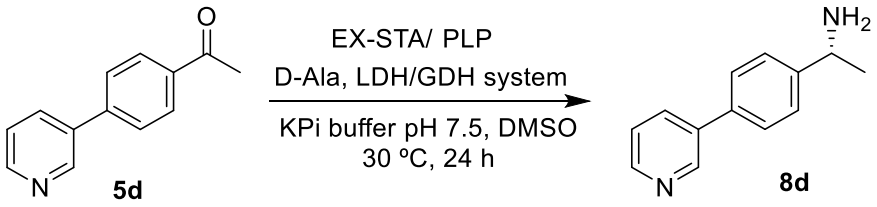
Entry	Enzyme	Co-solvent (%)	*c* (%)	*ee* (%)
1	Shi	DMSO (2.5%)	95	>99 (*R*)
2	Shi	DMSO (5%)	93	>99 (*R*)
3	Shi	DMSO (10%)	87	>99 (*R*)
4	Shi	*i*-PrOH (15%)	20	n.d.
5	EX-wt	DMSO (5%)	95	>99 (*R*)
6	EX-wt	*i*-PrOH (15%)	22	>99 (*R*)
7	EX-wt	1*ChCl*/2*Gly* (25%)	55	>99 (*R*)
8	EX-STA	1*ChCl*/2*Gly* (25%)	93	>99 (*R*)
9	Esi	DMSO (2.5%)	18	n.d.
10	Esi	*i*-PrOH (15%)	0	n.d.
11	Pac	DMSO (2.5%)	45	>99 (*R*)
12	Pac	*i*-PrOH (15%)	20	n.d.
13	Tja	DMSO (2.5%)	13	n.d.
14	Tja	*i*-PrOH (15%)	5	n.d.

Table 50. Effect of co-solvent on the ATA-catalysed bioamination of biaryl ketone **5a**



Entry	Enzyme	Co-solvent (%)	*c* (%)	*ee* (%)
1	EX-wt	DMSO (5%)	83	>99 (*R*)
2	EX-wt	DMSO (5%)	45	>99 (*R*)
3	EX-STA	1*ChCl*/2*Gly* (25%)	5	n.d.
4	EX-STA	1*ChCl*/2*Gly* (25%)	25	n.d.

Table 51. Effect of co-solvent on the EX-STA-catalysed bioamination of biaryl ketone **5d**.



Entry	Co-solvent (%)	*c* (%)	*ee* (%)
1	THF (5%)	8	>99 (*R*)
2	THF (15%)	n.d.	n.d.
3	*i*-PrOH (5%)	64	>99 (*R*)
4	*i*-PrOH (15%)	<5	n.d.
5	DMSO (5%)	>99	>99 (*R*)
6	DMSO (15%)	>99	>99 (*R*)

Entry	Co-solvent (%)	*c* (%)	*ee* (%)
7	1ChCl/2Gly (5%)	>99	>99 (*R*)
8	1*ChCl*/2*Gly* (15%)	95	>99 (*R*)
9	1*ChCl*/2*Gly* (25%)	60	>99 (*R*)
10	1*ChCl*/2*Gly* (50%)	<5	n.d.

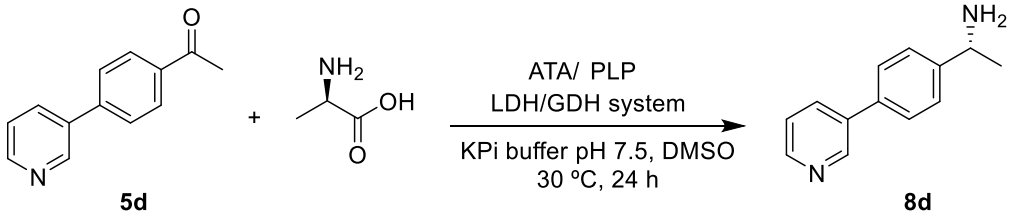
5.15 Investigation of process parameters

5.15.1 Amine donor optimisation

General procedure:

The ketone (20 mM) was first dissolved in DMSO (5 % v/v) in a 2 mL reaction tube (Eppendorf). Then ATA lysate (8 U), potassium phosphate buffer (KPi) 100 mM pH 7.5 and predefined amounts of D-ala, PLP (0.1 mM), NAD^+ (1 mM), LDH (90 U) and GDH (30 U) were added. The reaction was shaken at 30 °C and 250 rpm for 24 h. To determine the conversion, 10 µL of the mixture were diluted with 90 µL of DMSO and analysed by achiral reverse phase with previous centrifuging and filtering of the sample. To determine the enantiomeric excess the reaction was quenched by addition of aqueous 10 N NaOH (400 µL). The mixture was then extracted with ethyl acetate (2 × 500 µL) and the organic layers were separated by centrifugation (90 s, 13000 rpm), combined and dried over Na_2SO_4.

Table 52. Effect of D-alanine concentration on the ATA-catalysed bioamination of biaryl ketone **5d**.



5d **8d**

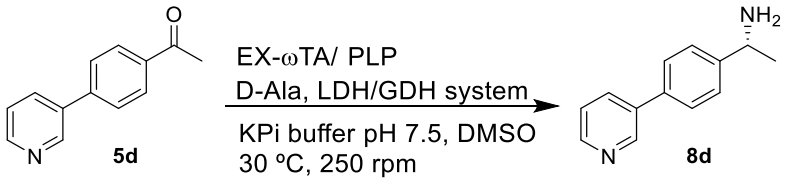
Entry	Enzyme	Amine donor (mM)	*c* (%)	*ee* (%)
1	EX-wt	250	>99	>99 (*R*)
2	EX-wt	130	>99	>99 (*R*)
3	Shi	250	>99	>99 (*R*)
4	Shi	130	>99	>99 (*R*)

5.15.2 Study of the biocatalyst formulation

General procedure:

The ketone (**5d**, 20 mM) was first dissolved in DMSO (5 % v/v) in a 2 mL reaction tube (Eppendorf). Then ATA lyophilisate (8 U), potassium phosphate buffer (KPi) 100 mM pH 7.5 and D-Ala (130 mM), PLP (0.1 mM), NAD^+ (1 mM), LDH (90 U) and GDH (30 U) were added. The reaction was shaken at 30 °C and 250 rpm for 24 h. To determine the conversion, 10 µL of the mixture were diluted with 90 µL of DMSO and analysed by achiral reverse phase with previous centrifuging and filtering of the sample. The mixture was then extracted with ethyl acetate (2 × 500 µL) and the organic layers were separated by centrifugation (90 s, 13000 rpm), combined and dried over Na_2SO_4.

Table 53. Enzymatic transamination of 5**d** catalysed by lyophilised EX-ωTA and its variants upon optimised reaction conditions



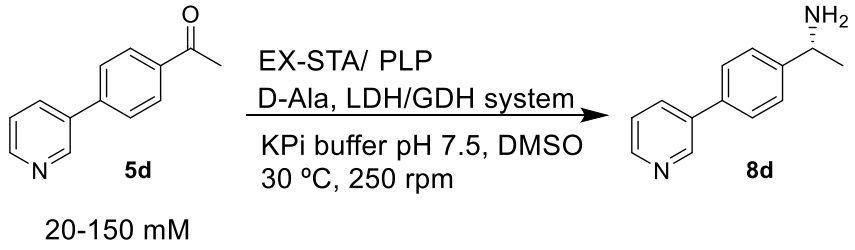
Entry	Enzyme	c (%)
1	EX-wt	7
2	EX-STA	>99
3	EX-5	55
4	EX-STA5	98

5.15.3 Substrate loading study

General procedure:

The ketone (**5d**, 20-150 mM) was first dissolved in DMSO (32 µL, 5% v/v) in a 2 mL reaction tube (Eppendorf). Then EX-STA lyophilisate (8 U), potassium phosphate buffer (KPi) 100 mM pH 7.5, 1 mM PLP, 0.1 mM NAD^+, D-glucose (57 mM), D-alanine (130 mM), LDH (90 U) and GDH (30 U) were added. The reaction was shaken at 30 °C and 250 rpm for 24 h. To determine the conversion, 10 µL of the mixture were diluted with 90 µL of DMSO and analysed by achiral reverse phase with previous centrifuging and filtering of the sample. To determine the enantiomeric excess the reaction was quenched by addition of aqueous 10 N NaOH (400 µL). The mixture was then extracted with ethyl acetate (2 × 500 µL) and the organic layers were separated by centrifugation (90 s, 13000 rpm), combined and dried over Na_2SO_4.

Table 54. Measurements of conversion at different concentrations of **5d** for the enzymatic transamination catalysed by EX-STA



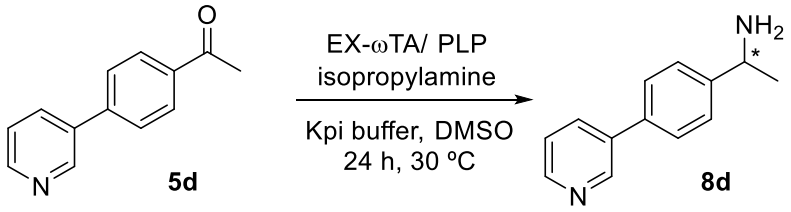
Entry	Ketone concentration (mM)	c (%)
1	20	99
2	40	99
3	75	95
4	100	60
5	150	47

5.15.4 Enzymatic transamination catalysed by lyophilised EX-ωTA using isopropylamine

General procedure:

In a 2 mL reaction tube, ketone (**5d**, 20 mM) was first dissolved in DMSO (32 µL, 5% v/v) and then ATA lyophilisate (8 U), KPi buffer 100 mM pH 7.5, 1 M isopropylamine and 1 mM PLP were added. The reaction was shaken at 30 °C and 250 rpm for 24 h. To determine the conversion, 10 µL of the mixture were diluted with 90 µL DMSO and analysed by achiral reverse phase with previous centrifuging and filtering of the sample. The reaction was quenched by addition of aqueous 10 N NaOH (400 µL). The mixture was then extracted with ethyl acetate (2 × 500µL) and the organic layers were separated by centrifugation (90 s, 13000 rpm), combined and dried over Na_2SO_4. The enantiomeric excess of amines was measured by chiral HPLC.

Table 55. Enzymatic transamination of **5d** catalysed by lyophilised EX-ωTA and its variants using isopropylamine as amino donor



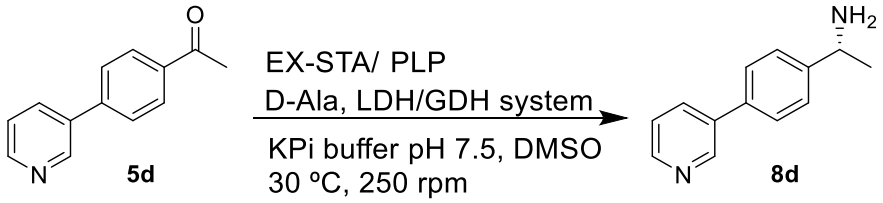
Entry	Enzyme	c (%)
1	EX-wt	5
2	EX-STA	50
3	EX-5	30
4	EX-STA5	35

5.15.5 Enzyme loading studies

General procedure:

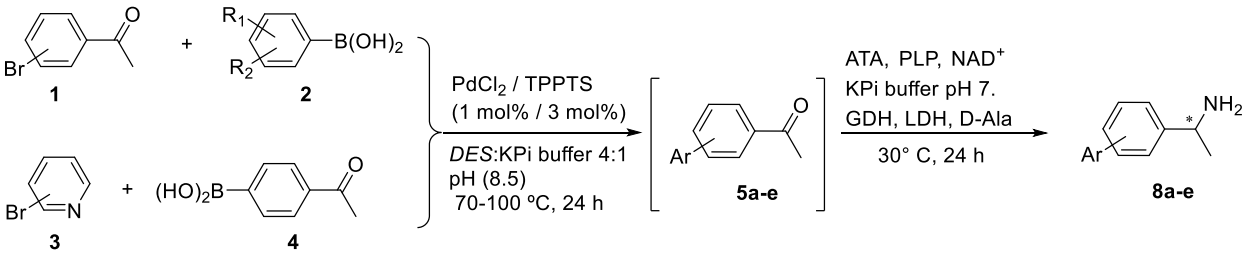
In a 2 mL reaction tube, ketone (**5d**, 20 mM) was first dissolved in DMSO (32 µL, 5% v/v) and then ATA lyophilisate (1-4 U/mg of substrate), potassium phosphate buffer (KPi) 100 mM pH 7.5, 1 mM PLP, 0.1 mM NAD^+, D-glucose (57 mM), D-alanine (130 mM), LDH (90 U) and GDH (30 U) were added. The reaction was shaken at 30 °C and 250 rpm for 24 h. To determine the conversion, 10 µL of the mixture were diluted with 90 µL of DMSO and analysed by achiral reverse phase with previous centrifuging and filtering of the sample. The reaction was quenched by addition of aqueous 10 N NaOH (400 µL). The mixture was then extracted with ethyl acetate (2 × 500 µL) and the organic layers were separated by centrifugation (90 s, 13000 rpm), combined and dried over Na_2SO_4.

Table 56. Enzymatic transamination of **5d** catalysed by lyophilised EX-STA upon optimised reaction conditions at different enzyme loadings



Entry	U/mg	c (%)
1	4	99
2	3	91
3	2	60
4	1	17

5.16 Synthesis of biaryl amines in a one-pot sequential process



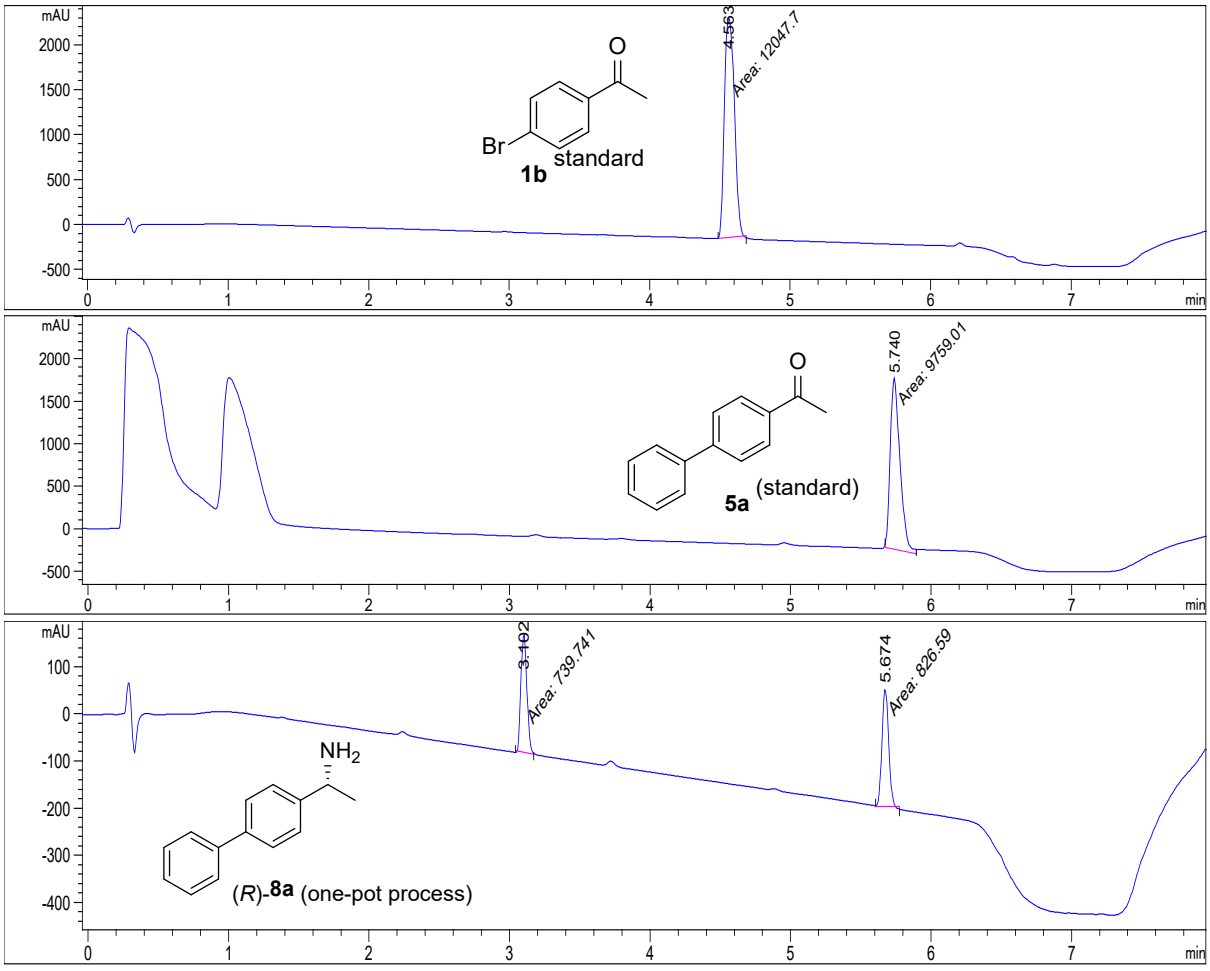
R_1, R_2 = H, Me, OMe, F
R_3 = Me,Et

General procedure:

At first, a suspension of $PdCl_2$ (0.086 mg; 1 mol %) and TPPTS (8.32 mg; 3 mol %) in degassed and deionized water was prepared (0.25 mL). After 30 min the resulting catalyst solution was added to a mixture consisting of aryl bromide (**1** or **3**, 0.5 mmol), arylboronic acid (**2** or **4**, 0.5 mmol), sodium carbonate (132 mg, 1.25 mmol), *DES* (2.0 mL) and degassed and deionized water (0.25 mL). The

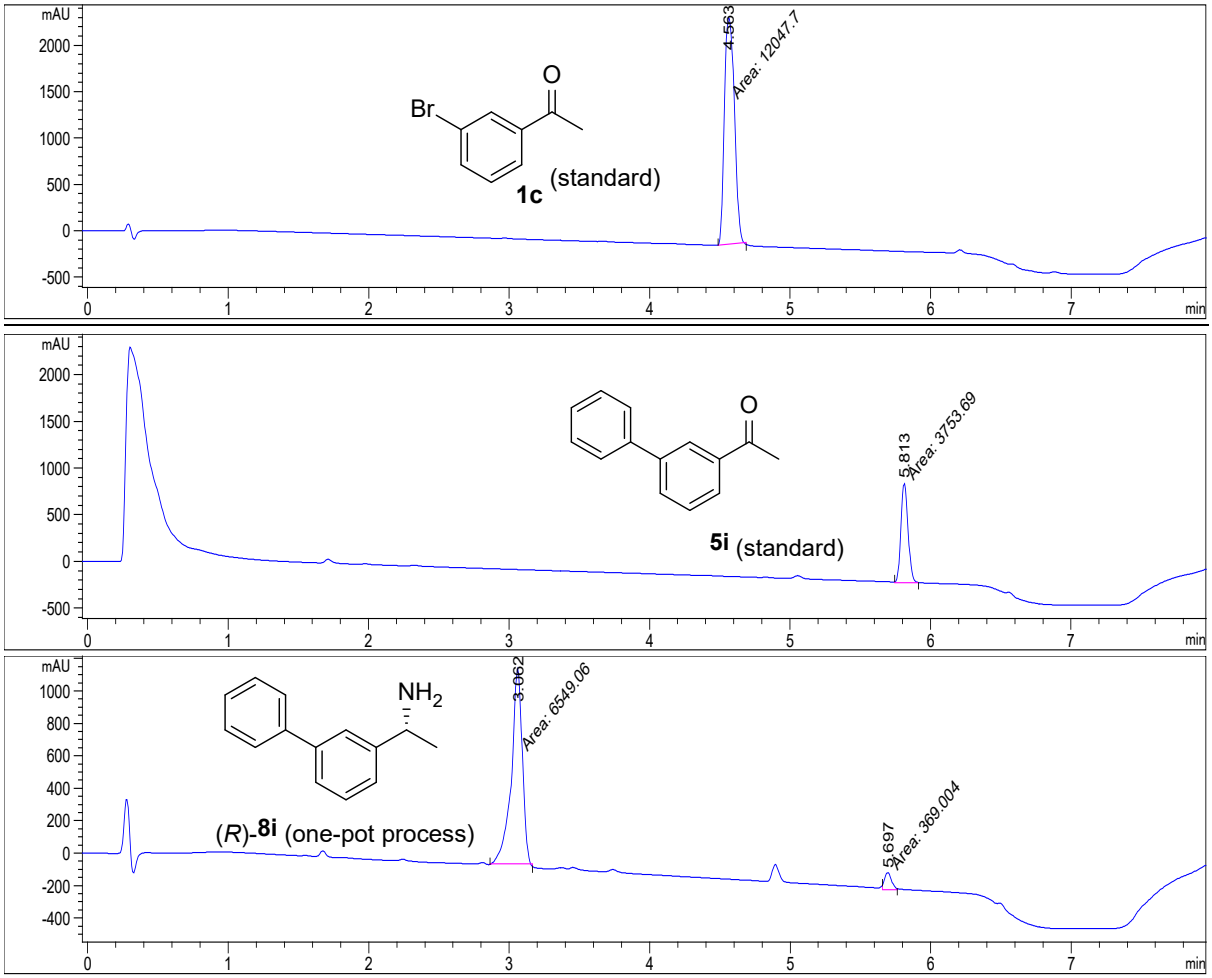
reaction mixture was heated to 100 °C during 24 h. After cooling to room temperature, EX-STA lysate (11 mL, 257 U), KH_2PO_4 buffer 100 mM pH 7.5 (6.5 mL), PLP (1 mM), NAD^+ (0.1 mM), D-glucose (57 mM), D-alanine (130 mM), LDH (3600 U, 680 µL) and GDH (1200U, 400 mg) were added and the reaction was stirred for 24 h at 30 °C. Then, the reaction was quenched by addition of aqueous 10 N NaOH (10 mL) to adjust the pH to 14. The mixture was then extracted with ethyl acetate (2 × 25 mL) and the organic layers were separated by centrifugation (90s, 13000 rpm), combined and dried over Na_2SO_4 to provide the crude product. Further filtration by flash chromatography (silica gel 60 Å, ethyl acetate) yielded the corresponding (*R*)-biaryl amines **8a-e**. The enantiomeric excess of the resulting amines was determined by chiral HPLC after conventional derivatization of the sample using acetic anhydride (2 µL / mg of substrate).

5.16.1 Synthesis of (*R*)-1-([1,1'-biphenyl]-4-yl)ethanamine (8a)



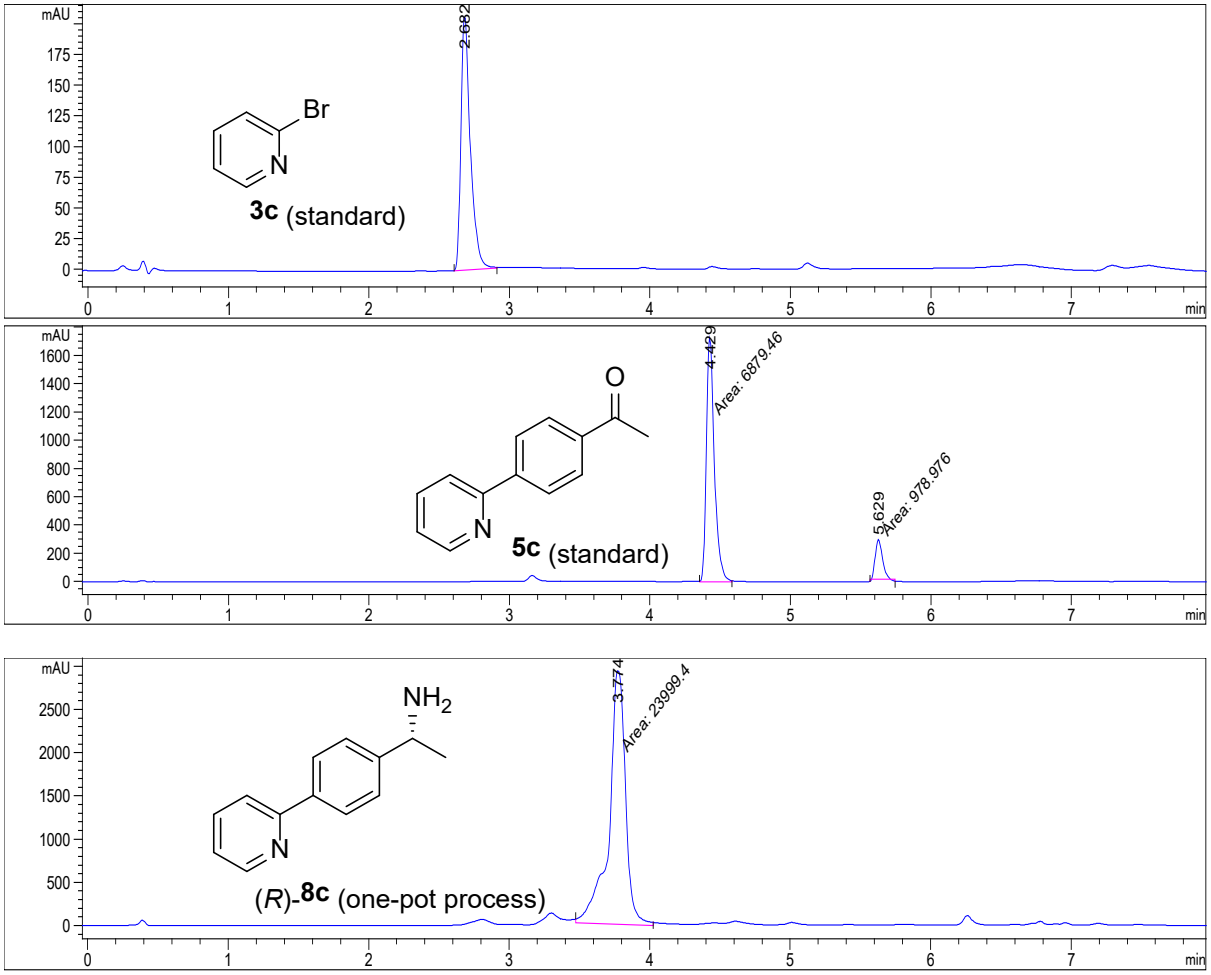
Scheme 59. In process HPLC monitoring for the preparation scale synthesis of **8a**

5.16.2 Synthesis of (*R*)-1-([1,1'-biphenyl]-3-yl)ethanamine (8i)



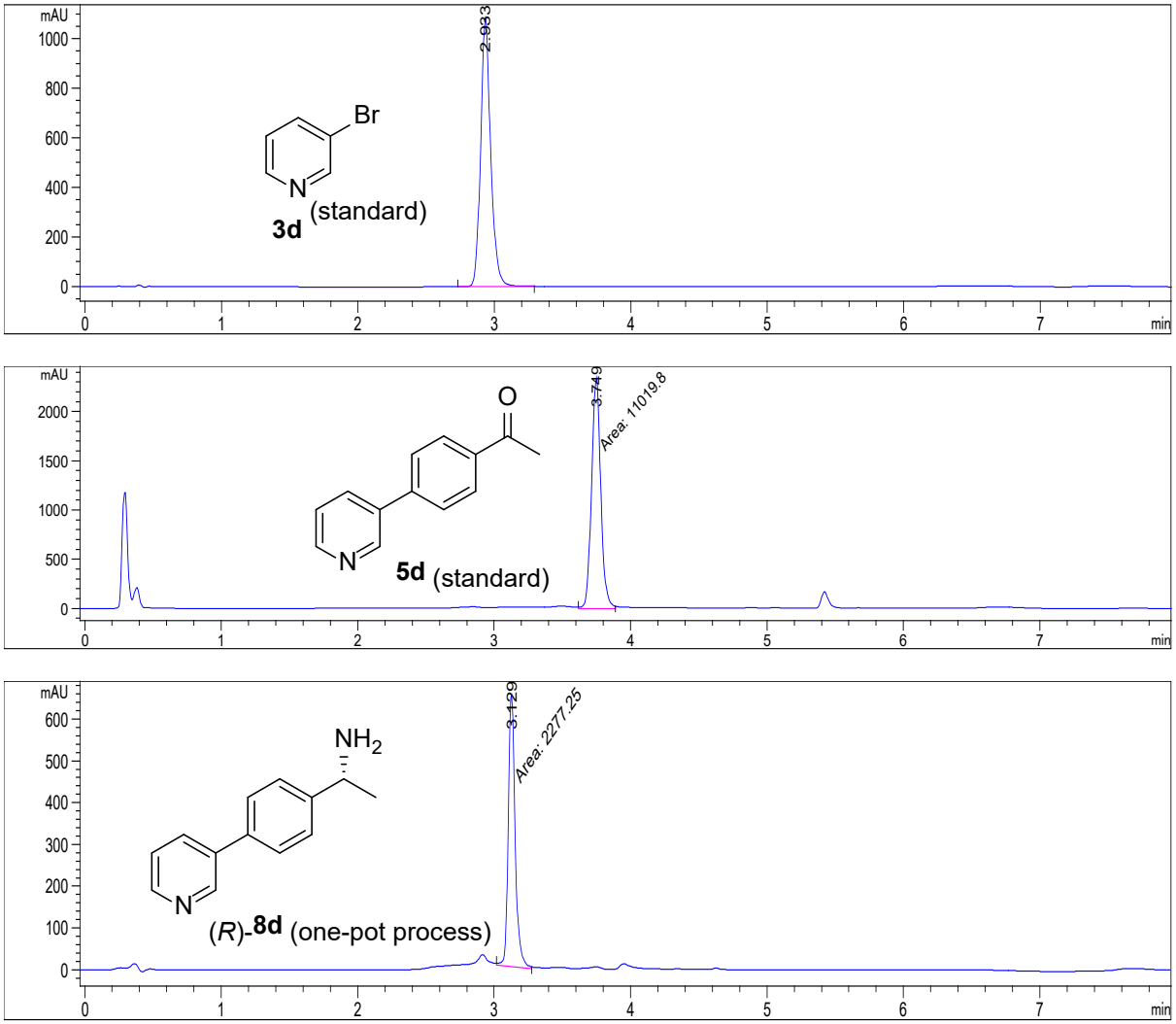
Scheme 60. In process HPLC monitoring for the preparation scale synthesis of **8i**

5.16.3 Synthesis of (*R*)-1-(4-(pyridin-2-yl)phenyl)ethanamine (8c)



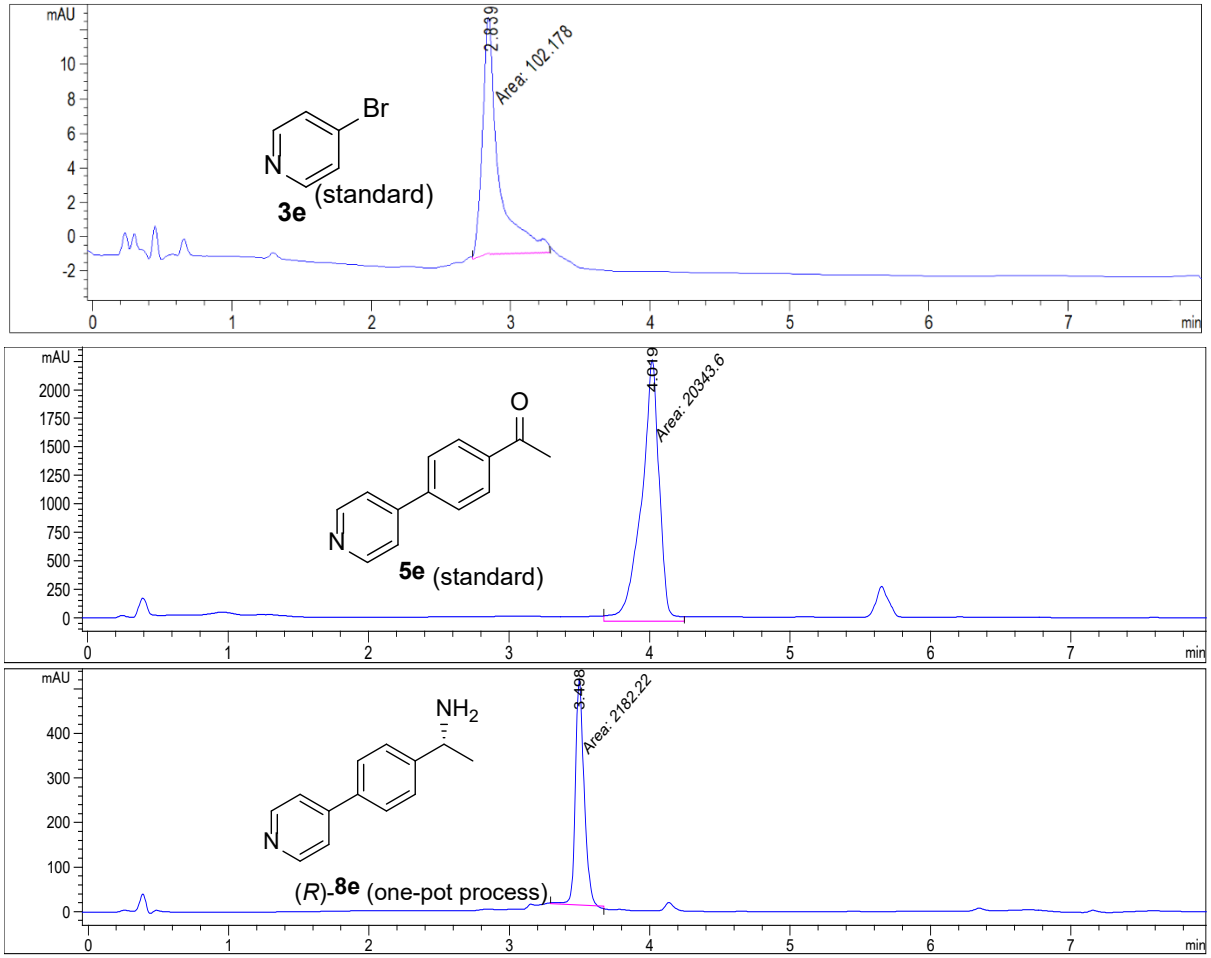
Scheme 61. In process HPLC monitoring for the preparation scale synthesis of **8c**

5.16.4 Synthesis of (*R*)-1-(4-(pyridin-3-yl)phenyl)ethanamine (8d)



Scheme 62. In process HPLC monitoring for the preparation scale synthesis of **8d**

5.16.5 Synthesis of (*R*)-1-(4-(pyridin-4-yl)phenyl)ethanamine (8e)



Scheme 63. In process HPLC monitoring for the preparation scale synthesis of **8e**

6 Literature

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