Universidad de Oviedo

Departamento de Química Orgánica e Inorgánica

Programa de doctorado: Síntesis y reactividad química

BIOCATALYTIC CASCADE REACTIONS FOR THE SYNTHESIS OF OPTICALLY ACTIVE COMPOUNDS

Doctoral Thesis

Elisa Liardo

2018

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Memoria para optar al grado de Doctor en Química

por Elisa Liardo

2018

RESUMEN DEL CONTENIDO DE TESIS DOCTORAL

1.- Título de la Tesis	
Español/Otro Idioma: Reacciones Biocatalíticas en cascada para la síntesis de compuestos optícamente activos	Inglés: Biocatalytic cascade reactions for the synthesis of optically active compounds

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RESUMEN (en español)

La biocatálisis ofrece enormes ventajas para generar compuestos quirales con altos rendimiento y pureza enantiomérica. La gran eficiencia catalítica de las enzimas, así como las condiciones suaves de reaccion que requieren, permiten el desarrollo de rutas nuevas y más económicas, que generan menos desechos que la síntesis orgánica convencional de tales productos ópticamente activos. Además, las propiedades biodegradables y altamente selectivas de los biocatalizadores se combinan con la capacidad de muchos de ellos de ser compatibles entre sí y con otros catalizadores de naturaleza diferente, dentro de cierto intervalo de condiciones operativas.

Por lo tanto, en los últimos años, diferentes grupos de investigación han realizado varios intentos para desarrollar nuevas reacciones multicatalíticas en cascada *in vitro*, emulando las rutas sintéticas de múltiples etapas que tienen lugar en los organismos vivos. Estas transformaciones en cascada han tenido una gran acogida en los sectores académico e industrial, para la síntesis de intermedios ópticamente activos de productos farmacéuticos.

Esta tesis doctoral consta de una introducción general y tres capítulos. Se han estudiado en ella varios tipos de catalizadores (biocatalizadores, organocatalizadores y catalizadores metálicos) de diversas reacciones químicas, como transaminación, reducción, oxidación, hidratación e hidrólisis de nitrilos. Se han desarrollado a continuación diferentes métodos multicatalíticos de forma concurrente o secuencial para la síntesis de interesantes compuestos ópticamente activos: ácidos 2-hidroxicicloalcanocarboxílicos, β-hidroxiamidas, derivados de β-aminoalcoholes, aminas, alcoholes y dioles; es decir, compuestos que pueden usarse para la síntesis asimétrica de productos de interés farmacéutico.

En el primer capítulo, se han utilizado enzimas purificadas como cetorreductasas y células enteras de *Rhodococcus rhodochrous* en un proceso de cascada concurrente para la síntesis de ácidos 2-hidroxicicloalcanocarboxílicos, con excelentes pureza óptica, relación diastereomérica y rendimiento. También se ha puesto a punto un nuevo protocolo para la síntesis de 2-hidroxiciclohexanocarboxamida ópticamente pura, cuyo rendimiento final resultó empobrecido debido a una inhibición incompleta de la actividad de la amidasa presente en el microorganismo.

Para superar esta limitación, en el capítulo 2 se ha desarrollado una metodología

multicatalítica para la síntesis de β-hidroxiamidas a partir de β-cetonitrilos. Por lo tanto, la acción tándem de un catalizador de Ru(IV) y cetorreductasas ha permitido la síntesis de los compuestos deseados con redimientos excelentes. También se han estudiado β-cetonitrilos con un centro quiral y, aprovechando una eficiente resolución cinética dinámica, los productos finales se han obtenido con relaciones distereoméricas muy altas. Esta contribución representa un ejemplo válido de compatibilidad entre metales y enzimas en agua, sin necesidad de separación física de ambos catalizadores.

Finalmente, se han descrito en el capítulo 3, nuevas metodologías híbridas para la síntesis de aminoalcoholes, aminas, alcoholes y dioles. Una de las claves de este proyecto ha sido el desarrollo de una oxidación de alcoholes racémicos catalizada por AZADO y en presencia de NaOCl en medio acuoso. De este modo, una vez obtenida la cetona proquiral de forma cuantitativa, la acción de una transaminasa o una cetorreductasa ha permitido la síntesis de aminas o alocholes ópticamente puros con rendimientos y relaciones diastereoméricas excelentes.

Parte del trabajo presentado se encuentra recogido en las siguientes publicaciones.

- E. Liardo, N. Ríos-Lombardía, F. Morís, J. González-Sabín, F. Rebolledo. "Developing a Biocascade Process: Concurrent Ketone Reduction-Nitrile Hydrolysis of 2-Oxocycloalkanecarbonitriles" *Org. Lett.*, **2016**, *18*, 3366-3369.

- E. Liardo, N. Ríos-Lombardía, F. Morís, F. Rebolledo, J. González-Sabín. "Hybrid Organo- and Biocatalytic Process for the Asymmetric Transformation of Alcohols into Amines in Aqueous Medium" *ACS Catalysis*, **2017**, *7*, 4768-4774.

- E. Liardo, N. Ríos-Lombardía, F. Morís, J. González-Sabín, F. Rebolledo. "A Straightforward Deracemisation of *sec*-Alcohols Combining Organocatalytic Oxidation and Biocatalytic Reduction" *Eur. J. Org. Chem*., accepted article. http://dx.doi.org/10.1002/ejoc.201800569.

RESUMEN (en Inglés)

Biocatalysis offers tremendous advantages to generate complex chiral compounds in high enantiomeric purity and yield. The exquisite catalytic efficiency, mild conditions, and regenerative production offered by enzymes is enabling the development of new and more step-economical routes that generate less waste than conventional organic synthesis. Moreover, the intrinsically biodegradable and highly selective properties of biocatalysts are combined with the fact that they are compatible with each other and catalysts of different nature, within certain range of operating conditions. Thus, various attempts have been made to develop new multi-catalytic cascade reactions *in vitro*, by emulating the multi-step synthetic routes built in living organisms. For these reasons, these cascade transformations have emerged as a good alternative in the academic and industrial sectors, for the synthesis of optically active compounds as building blocks for pharmaceuticals.

This doctoral thesis is presented as a main introduction and three chapters. Several kinds of catalysts (biocatalysts, organocatalysts and metal catalysts) have been studied in order to perform various chemical reactions, as transamination, reduction, oxidation,

nitrile hydration, and nitrile hydrolysis. Then, different multi-catalytic methods in concurrent or sequential fashion have been developed for the synthesis of interesting optically active compounds: 2-hydroxycycloalkanecarboxylic acids, β-hydroxy amides, β-amino alcohol derivatives, amines, alcohols, and diols; that is, compounds that can be used as building blocks for pharmaceuticals.

In Chapter 1, purified enzymes as ketoreductases and whole cells of *Rhodococcus rhodochrous* have been used in a genuine cascade process for the synthesis of optically pure 2-hydroxycycloalkanecarboxylic acids. In all the cases, the final compounds have been obtained in enantiomerically pure form and with excellent diastereomeric ratio and yield. Moreover, a new protocol for the synthesis of optically pure (1*R*,2*S*)-2-hydroxycyclohexanecarboxyamide has also been determined, but an incomplete inhibition of the amidase activity of the microorganism resulted in a detraction of the final yield.

In order to overcome this limitation, in Chapter 2 we developed a multi-catalytic methodology for the synthesis of optically pure β-hydroxy amides starting from easily accessible and commercially available β-ketonitriles. Thus, the tandem action of a Ru(IV) catalyst and a ketoreductase allowed the synthesis of the desired compounds in excellent yields. Also β-ketonitriles bearing a chiral centre have been investigated and an efficient dynamic reductive kinetic resolution yielded the final compounds with perfect diastereomeric ratios. This contribution represents a valid example of compatibility between metals and enzymes in water, without need of separation or compartmentalization.

Finally, in Chapter 3, a new hybrid methodology for the synthesis of interesting amino alcohols, amines, alcohols and diols has been investigated. The core of this project was the development of a highly efficient AZADO-catalyzed oxidation of easily available racemic alcohols using NaOCl in water. Thus, once the prochiral ketone was quantitatively obtained, the subsequent action of a transaminase or a ketoreductase allowed the synthesis of optically active amines or alcohols, respectively, with excellent enantiomeric excesses, yields and diastereomeric ratios (when applicable).

A good part of the work presented here has resulted in the following three publications.

• E. Liardo, N. Ríos-Lombardía, F. Morís, J. González-Sabín, F. Rebolledo. "Developing a Biocascade Process: Concurrent Ketone Reduction-Nitrile Hydrolysis of 2-Oxocycloalkanecarbonitriles" *Org. Lett.*, **2016**, *18*, 3366-3369.

• E. Liardo, N. Ríos-Lombardía, F. Morís, F. Rebolledo, J. González-Sabín. "Hybrid Organo- and Biocatalytic Process for the Asymmetric Transformation of Alcohols into Amines in Aqueous Medium" *ACS Catalysis*, **2017**, *7*, 4768-4774.

• E. Liardo, N. Ríos-Lombardía, F. Morís, J. González-Sabín, F. Rebolledo. "A Straightforward Deracemisation of *sec*-Alcohols Combining Organocatalytic Oxidation and Biocatalytic Reduction" *Eur. J. Org. Chem*., accepted article. http://dx.doi.org/10.1002/ejoc.201800569.

Acknowledgements

I warmly acknowledge funding from the European Union's Horizon 2020 MSCA ITN-EID program under grant agreement No 634200 (Project BIOCASCADES).

Abbreviations and Acronyms

[α]	Specific rotation
TA	Transaminase
δ	Chemical shift
λ	Wavelength
µL	Microliter(s)
µmol	Micromol(s)
A_{650}	Absorbance at 650 nm
ABTS	2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
Ac	Acetyl
AcOEt	Ethyl acetate
ADH	Alcohol dehydrogenase
aq	Aqueous
ArR	(*R*)-*Arthrobacter*
ARMut11	Evolved variant of ArR
ArS	(*S*)-*Arthrobacter*
ATA	Amine transaminase
AZADO	2-Azaadamantane *N*-oxyl
Bn	Benzyl
Boc	*tert*-Butoxycarbonyl
br	Broad signal
°C	Degrees Celsius
C	Conversion
c	Concentration in g/100 mL

cat	Catalyst
Cbz	Benzyloxycarbonyl
^{13}C-NMR	Carbon-13 nuclear magnetic resonance
CAL-B	*Candida antarctica* lipase B
Cv	*Chromobacterium violaceum*
d	Doublet
dd	Doublet of doublets
ddd	Doublet of doublet of doublets
DEPA	Diethyl phosphoramidate
DKR	Dynamic kinetic resolution
DMAP	4-*N,N*-Dimethylaminopyridine
DMSO	Dimethyl sulfoxide
dr	Diastereomeric ratio
dt	Doublet of triplets
DYRKR	Dynamic reductive kinetic resolution
E	Enantioselectivity
E. coli	*Escherichia coli*
ee	Enantiomeric excess
equiv	Equivalents
ESI^+	Electrospray ionization in positive ion mode
EtOH	Ethanol
FDH	Formate dehydrogenase
GalOx	Galactose oxidase
GC	Gas chromatography

GDH	Glucose dehydrogenase
h	Hour(s)
HAA	3-Hydroxyanthranilic acid
^{1}H-NMR	Proton nuclear magnetic resonance
HIV	Human immunodeficiency virus
HLADH	*Horse liver* alcohol dehydrogenase
HMDS	1,1,1,3,3,3-Hexamethyldisilazane
HOBT	1-Hydroxybenzotriazole
HPLC	High performance liquid chromatography
HRMS	High resolution mass spectrometry
i-Pr	Isopropyl
IPA	Isopropyl alcohol
J	Coupling constant
KPi	Potassium phosphate
KRED	Ketoreductase
M	Molar
m	Multiplet
Me	Methyl
1-Me-AZADO	1-Methyl-2-azaadamantane *N*-oxyl
MeCN	Acetonitrile
MeOH	Methanol
mg	Milligram(s)
MHz	Megahertz(s)
min	Minutes

mL	Milliliter(s)
mM	Millimolar
mmol	Millimol(s)
mp	Melting point
N	Normal
NHase	Nitrile hydratase
NADH	β-Nicotinamide adenine dinucleotide (reduced)
NAD^+	β-Nicotinamide adenine dinucleotide (oxidized)
NADPH	β-Nicotinamide adenine dinucleotide phosphate (reduced)
$NADP^+$	β-Nicotinamide adenine dinucleotide phosphate (oxidized)
NHPI	*N*-Hydroxyphthalimide
NPs/FSG	Nanoparticles immobilized in fluorous silica gel
Ph	Phenyl
$PhCF_3$	α,α,α-Trifluorotoluene
PLP	Pyridoxal-5'-phosphate
PMP	Pyridoxamine-5'-phosphate
ppm	Parts per million
PSL	*Pseudomonas cepacia* lipase
Pyr	Pyridine
q	Quadruplet
rac	Racemic
rpm	Revolutions per minute

R.r.	*Rhodococcus rhodochrous*
R_S	Resolution factor
rt	Room temperature
s	Second(s)
s	Singlet
(*S*)-MTPA-Cl	*(S)*-α-Methoxy-α-(trifluoromethyl)phenylacetyl chloride
T	Temperature
t	Triplet
TBME	*tert*-Butyl methyl ether
td	Triplet of doublets
TEMPO	2,2,6,6-Tetramethylpiperidine *N*-oxyl
TeSADH	Secondary ADH from *Thermoanaerobacter ethanolicus*
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMSCl	Trimethylsilyl chloride
t_R	Retention time
Tris-HCl	Tris(hydroxymethyl)aminomethane hydrochloride
U	Enzyme unit
UV	Ultraviolet
VA	Violuric acid
v/v	Volume/volume

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Introduction

Our modern society has finally focused its attention on solving problems to enhance the quality of our lives. Waste in chemical industry is one of them. Growing awareness of the need for sustainable technology has stimulated the chemical community to streamline synthetic methodologies across academic and industrial fields.[1] A paradigm shift was clearly needed, from traditional concepts of reaction efficiency and selectivity, which focus largely on chemical yield, to one that assigns value to the minimization of waste and the avoidance of the use of toxic and hazardous substances.[2]

Traditional organic synthesis relies heavily on organic solvents for a multitude of tasks. Given that they are used in vast quantities compared to the reactants, solvents have been the focus of environmental concerns. The best solvent is no solvent but if a solvent is needed, it should be safe to use and there should be provisions for its efficient removal from the product and reuse.

The U.S. Pollution Prevention Act of 1990[3] focused attention on the need to reduce environmental pollution and recognized that waste prevention at the source not only eliminates the cost of waste treatment but

[1] T. Welton, *Proc. R. Soc. A*, **2015**, *471*, 20150502.
[2] R. A. Sheldon, *Chem. Soc. Rev.*, **2012**, *41*, 1437−1451.
[3] Summary of the Pollution Prevention Act http://www.epa.gov/laws-regulations/summary-pollution-prevention-act.

actually strengthens economic competitiveness through a more efficient use of raw materials. This act led to a fundamental shift in the strategy for environmental protection from "end-of-pipe" waste treatment to waste prevention and led to the emergence of the term "green chemistry" coined by the U.S. Environmental Protection Agency (EPA) in the early 1990s.

Green chemistry efficiently utilizes (preferably renewable) raw materials, eliminates waste, and avoids the use of toxic and/or hazardous reagents and solvents in the manufacture and application of chemical products.[4] Green chemistry is primarily pollution prevention rather than waste remediation.

According to Graedel,[5] to be sustainable, a technology must fulfil two conditions: i) natural resources should be used at rates that do not unacceptably deplete supplies over the long term, and ii) residues should be generated at rates no higher than can be readily assimilated by the natural environment. Hence, a balance needs to be found among environmental impact and economic development. If a technology is not economically competitive, it will not be sustainable in the long term.

Industrial Biotechnology, also known as *White Biotechnology*, is one of the pillars that support sustainable chemistry nowadays and that is expected to support it even more in the future. It is based on "the use of enzymes and micro-organisms as catalysts in order to make efficient and sustainable products." The aqueous medium inherent to the biocatalysis field is one of its maximal attractive. A water based procedure is synonymous of cost-efficiency and environmental compatibility. It has been significant the number of studies where its employment has contributed to improve reactivities and selectivities and simplify the work-up procedure. This fact, combined with the continuous demand of asymmetric compounds

[4] R. A. Sheldon. *C. R. Acad. Sci., Ser. IIc: Chim.,* **2000**, *3*, 541−551.
[5] T. E. Graedel, Green chemistry and sustainable development. In *Handbook of Green Chemistry and Technology*; J. Clark, D. J. Macquarrie, Eds.; Blackwell Science Ltd.: Oxford, U.K., **2002**; Chapter 4, pp 56−61.

as fine chemicals, chiral intermediates or pharmaceutical ingredients enhance the potential of biocatalysis.

I.1. Biocatalysis

Biocatalysis is the point in which nature and development meet to improve the quality of human being. It is defined as the application of biological systems in chemical reactions, in form of isolated/immobilized enzymes, whole cells, or cell-free extracts.[6]

Compared to conventional synthetic methods, biocatalysis offers a tremendous advantage to generate complex chiral compounds in high enantiomeric purity and yield. In addition, enzymes exhibit exquisite catalytic efficiency, mild conditions and regenerative production, often without the need for functional-group activation, protection and deprotection steps. This affords routes that are more step-economical and generates less waste than conventional organic synthesis.[2] Modern biocatalysis originates from the huge interest in the production of chiral molecules as single enantiomers that arose in the 1970s as a consequence of the discovery of the dramatic impact that chirality has on biological activity of drugs.[7] With the need to find greener alternatives for chemical processes, research in biocatalysis has been in turn highly encouraged and stimulated.

Until the late 1980s, when gene technology evolved to become a standard tool, biocatalysis had to rely on the isolation of enzymes from natural resources (microbes, animal tissue, and plants). The properties of these enzymes could not really be altered, and only immobilization could be used to try to enhance their stability and recycling. Later, the ability to identify and clone genes to subsequently express the enzyme of interest recombinantly in a host organism, not only allowed the production of enzymes of reliable quality on a larger scale, but most importantly granted

[6] T. Hudlicky, J. W. Reed, *Chem. Soc. Rev.,* **2009**, *38*, 3117−3132.
[7] G. W. Huisman, A. Krebber, *Curr. Opin. Chem. Biol.,* **2010**, *4*, 122−129.

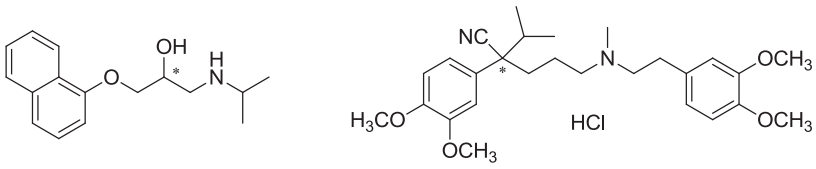
the opportunity to perform protein engineering by rational design. Nowadays, advanced bioinformatic programs allow the design of completely novel enzymes from "scratch", thus broadening the scope of reactions to be catalyzed by proteins, as well as advances in understanding the enzymatic mechanistic details.[8]

I.2. Chiral Drugs

The importance of chirality and selectivity in the chemical and pharmaceutical industry has become quite clear when racemic products are involved.[9] The body, with its numerous homochiral compounds, will interact with each enantiomer of the racemic drug differently and metabolize each one of them by a separate pathway to generate different pharmacological activity.

The majority of racemic pharmaceuticals have one major bioactive enantiomer (eutomer), the other being inactive, less active (distomer), or toxic. β-Adrenergic blocking agents, calcium channel antagonists and angiotensin-converting enzyme inhibitors could be cited as an example. Levorotatory isomer of all β-blockers is more potent in blocking the β-adrenoreceptors than its corresponding dextrorotatory-isomer. Thus, the β-blocker (*S*)-(–)-propranolol is 100 times more active than its *R*-(+)-antipode[10] and also for the calcium channel antagonist verapamil, the (S)-(–)-isomer is more active than its counterpart[9] (Figure I.2.1).

[8] U. T. Bornscheuer, *Angew. Chem. Int. Ed*., **2016**, *55*, 4372−4373.
[9] L. A. Nguyen, H. He, C. Pham-Huy, *Int. J. Biomed. Sci*., **2006**, *2*, 85−100.
[10] K. Stoschitzky, W. Lindner, G. Zernig, *J. Clin. Basic Cardiol*., **1998**, *1*, 15−19.



Propranolol Verapamil

Figure I.2.1

For many drugs, industrial companies need to provide very high enantiomeric purities as required by the US Food and Drug Administration and the European Medicines Agency. Drugs previously granted patent protection and marketed as racemates are candidates for a "chiral switch" (development as single or paired enantiomers in the case of diasteromic mixtures), which permits additional years of market exclusivity. Economic forces and favorable clinical profiles have driven successful chiral switches such as esomeprozole, levofloxacin, and escitalopram, also racemic veterinarian compounds (medetomidine) have been adapted for human use as (*S*)-dexmedetomidine (Fig I.2.2).[11]

[11] S. W. Smith, *Toxicol. Sci.*, **2009**, *110*, 4−30.

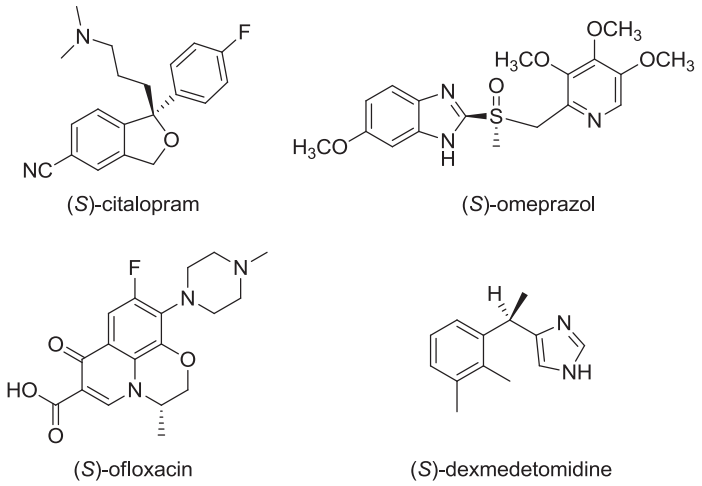


Figure I.2.2

With this scenario in mind, the high selectivity of biocatalytic processes emerges as a new alternative to conventional methods.

I.3. Multi-catalytic Cascade Reactions

Living organisms do not use enzymes in isolation. However, they build up the living system by applying multi-step synthesis strategies catalyzed by enzymes acting cooperatively. In that way, complex molecules are built from simple elements through multi-step synthetic routes. Taking as inspiration the cooperative action of enzymatic reactions, chemists and biochemists have tried to mimic nature and to develop multi-step catalysis for selective synthesis.[12]

If the intrinsically biodegradable and highly selective properties of biocatalysts are combined with the fact that they are compatible with each others within certain range of operating conditions, biocatalysis appears as the answer for the development of cleaner, environmentally benign and

[12] Y. Wang, H. Zhao, *Catalysts*, **2016**, *6*, 194−215.

sustainable chemical processes. Consequently, numerous attempts have been made to reproduce such enzymatic cascade reactions *in vitro* and to apply them in organic synthesis. Cascade reactions do not only help to save time and reduce waste in multi-step synthesis through elimination of downstream processing step, they also offer advantages when unstable or toxic intermediates are involved, since these are not accumulated but are transformed into final product. This leads to safer processes, reduced side reactions and better yields.[13]

The same basic principle of cascade reactions can also be applied when biocatalysis is used in combination with chemocatalysis. The proven fact that many enzymes are compatible with metal catalysts has opened the door to a multitude of possibilities for the synthesis of precursors of complex molecules. In this sense, the identification of reaction conditions that are mutually suitable to both catalytic steps is a key area of focus in the research community[14] and, particularly, the combination of metal catalysts and biocatalysts working together in water appears as the main objective of several research groups.

In this thesis, different multi-catalytic cascade transformations have been successfully combined in concurrent or sequential one-pot processes. The main objective has been the development of efficient methods for the synthesis of interesting optically pure compounds, which can be used as building blocks for pharmaceuticals.

[13] E. Ricca, B. Brucher, J. H. Schrittwieser, *Adv. Synth. Catal*., **2011**, *353*, 2239−2262.
[14] F. Rudroff, M. D. Mihovilovic, H. Gröger, R. Snajdrova, H. Iding, U. T. Bornscheuer, *Nat. Catal.*, **2018**, *1*, 11−22.

Objectives

This thesis is the result of the study and development of different cascade reactions. Two main objectives have been the driving force of this PhD project.

The first one has been the study of different kinds of catalysts: biocatalysts, organocatalysts and metal catalysts in order to perform various chemical reactions, as transamination, reduction, oxidation, nitrile hydration, and nitrile hydrolysis. Firstly, the optimal conditions for the different catalysts must be determined and then, the viability to couple them in a concurrent or sequential fashion has to be demonstrated.

The second main objective of this project has been the development of different multi-catalytic methods for the synthesis of interesting optically pure compounds: 2-hydroxycycloalkanecarboxylic acids, β-hydroxy amides, β-amino alcohol derivatives, amines, alcohols, and diols; that is, compounds that can be used as building blocks for pharmaceuticals.

Chapter 1

Biocascade processes involving purified ketoreductases and whole cells of **Rhodococcus rhodochrous** *for the synthesis of optically pure 2-hydroxycycloalkanecarboxylic acids*

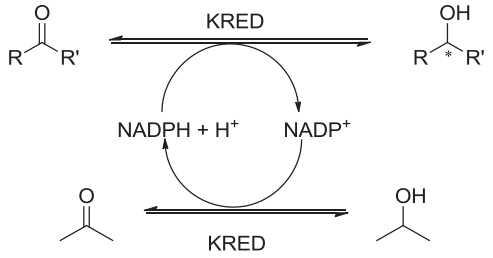
1.1. Bibliographic Background

Oxidative-reductive transformations are one of the most important reactions in organic synthesis. The enzymes employed in redox reactions are classified into three categories: dehydrogenases, oxygenases, and oxidases. Among them, dehydrogenases have been widely used for the reduction of carbonyl groups of aldehydes and ketones. These enzymes require redox cofactors, which donate or accept the chemical equivalents for reduction or oxidation. For the majority of redox enzymes (in particular the ones that have been used in this contribution), nicotinamide adenine dinucleotide (NADH) or its respective phosphate analogue (NADPH) are required.[15]

These redox cofactors are relatively unstable molecules and prohibitively expensive if used in stoichiometric amounts. For this reason, cofactor recycling appears as a mandatory solution. Formate dehydrogenase (FDH) in combination with formate is a good option for recycling NADH. This method has the advantage that both the auxiliary substrate –formic salt– and the resulting product –CO_2– are innocuous to enzymes and they are easily removed from the reaction medium.

[15] K. Faber, Biotransformations in Organic Chemistry, p 139–145, 6th ed. Springer-Verlag Berling Heidelberg New York, **2011**.

Another useful method for recycling NAD(P)H is based on the oxidation of glucose, catalyzed by glucose dehydrogenase (GDH). In this case, the equilibrium is shifted towards the product because the gluconolactone formed is spontaneously hydrolyzed to give gluconic acid. In other cases, isopropyl alcohol (IPA) can be used to recycle the catalyst. This method doesn't require an additional redox enzyme, but a high amount of the co-substrate IPA is necessary in order to drive the equilibrium towards the desired product. With no need of purification problems, this method is attractive for laboratory use and it is a convenient way to regenerate NAD(P)H.



Scheme 1.1.1

Among the possibilities to combine several enzyme classes in vitro facilitating a one-pot cascade, the use of redox enzymes has been successfully shown in the last years.[16] Due to their high stereoselectivity and ability to work under mild reaction conditions, KREDs have been the selected enzymes, in this chapter, in order to investigate the multi-catalytic transformation of several 2-oxocycloalkanecarbonitriles.

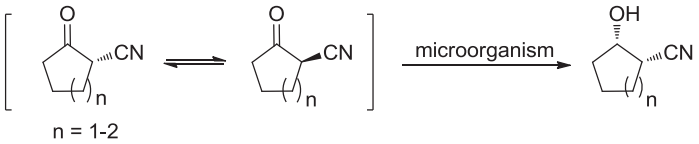
Cyclic β-ketonitriles are compounds of great interest as they are precursors of optically active β-hydroxyacids, β-hydroxyamides and γ-

[16] a) J. H. Schrittwieser, S. Velikogne, M. Hall, W. Kroutil, *Chem. Rev*., **2018**, *118*, 270–348; b) S. P. France, L. J. Hepworth, N. J. Turner, S. L. Flitsch, *ACS Catal*., **2017**, *7*, 710–724.

aminoalcohols. The preparation of these interesting building blocks has received considerable attention due to their importance in both medicinal chemistry and asymmetric synthesis.

The bioreduction of α-monosubstituted β-ketonitriles has been scarcely reported in literature. Itoh *et al.*[17] described that baker's yeast-mediated bioreduction of acyclic substrates resulted in a nearly equimolar mixture of diastereomers in most cases. On the other hand, Azerad *et al.*[18] studied the microbial reduction of a couple of 2-cyano-1-tetralones mediated by *Saccharomyces montanus* CBS 6772 and *R. arrhizus* ATCC 11145 to get both enantiomers of the *cis*-2-cyano-1-tetralols with high enantiomeric purity.

Some years ago, the bioreduction of two cyclic β-ketonitriles was accomplished by fungi and yeasts, leading to the corresponding *cis*-(1*S*,2*S*)-β-hydroxynitriles in high enantio- and diastereomeric excesses (Scheme 1.1.2).[19] In particular, the bioreduction of the five-membered ring yielded the *cis*-(1*S*,2*S*)-2-hydroxycyclopentanecarbonitrile with a complete conversion, 95:5 *dr*, and 90% *ee* when baker's yeast was used. With *Saccharomyces montanus* NRRL 6772 the results were slightly improved and the same enantiomer was obtained with 96:4 *dr* and 93% *ee*.

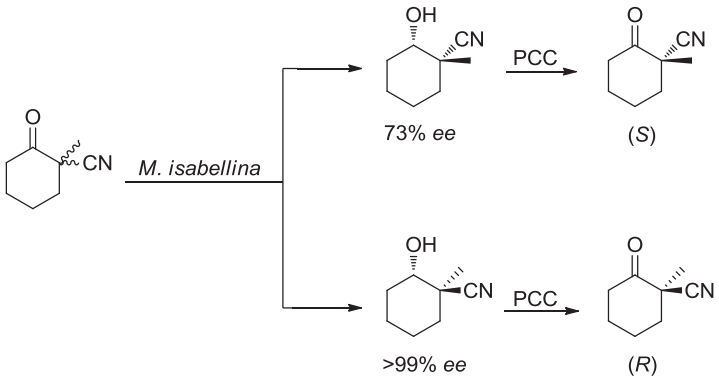


Scheme 1.1.2

[17] T. Itoh, T. Fukuda, T. Fujisawa, *Bull. Chem. Soc. Jpn.,* **1989**, *62*, 3851–3855.
[18] M. Mehmandoust, D. Buisson, R. Azerad, *Tetrahedron Lett.,* **1995**, *36*, 6461–6462.
[19] J. R. Dehli, V. Gotor, *J. Org. Chem*., **2002**, *67*, 6816–6819.

These interesting results have been obtained thanks to the intrinsic ability of the selected β-ketonitriles to racemize in water that depends on the acidity of their α-hydrogen (pK_a = 7.84 for **1**),[19] thus allowing an efficient dynamic kinetic resolution (DKR). The dynamic kinetic resolution process is a powerful strategy for the transformation of a racemic mixture into an optically pure compound with a 100% theoretical yield. It relies on a racemisation step of the substrate enantiomers combined with a selective transformation of one single enantiomer through a kinetic resolution process. The high selectivity shown by the enzymes when reacting with racemic mixtures makes this kind of chemo-enzymatic process even more powerful.

The same authors also developed the preparation of enantiopure ketones and alcohols containing a quaternary stereocentre through a parallel kinetic resolution process.[20] They studied the bioreduction of two selected β-ketonitriles where a parallel kinetic resolution took place, so that each enantiomer yielded a different diastereomer β-hydroxynitrile (Scheme 1.1.3 includes the results obtained in the reaction with the six-membered ring).

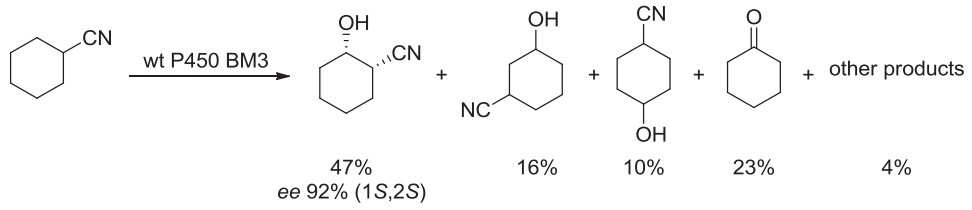


Scheme 1.1.3

[20] J. R. Dehli, V. Gotor, *J. Org. Chem*., **2002**, *67*, 1716–1718.

The subsequent oxidation under mild conditions of each diastereomer yielded the corresponding optically active ketones . The iterative enzymatic reduction and conventional oxidation of the (*S*)-ketone (obtained with a moderate 73% *ee*) allowed a significant improved of its *ee* (96%).

More recently, Reetz *et al.* [21] reported the desymmetrization of prochiral monosubstituted cyclohexane derivatives by means of oxidative hydroxylation catalyzed by wild type of the monooxygenase cytocrome P450 BM3 (Scheme 1.1.4). This process was regio-, diastereo-, and enantioselective, thus leading to the creation of two centres of chirality in a single C-H activation event. The regioselective formation of the cis distereomers appeared to be the rule, with high enantioselectivity in many cases.

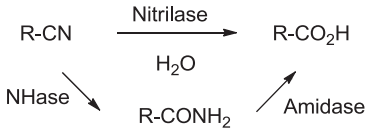


Scheme 1.1.4

On the other hand, nitriles are immediate precursors of carboxylic acids, but the demanded harsh conditions of the conventional hydrolytic methods are often incompatible with other functional groups present in the molecule. [22] However, the enzymatic route for this transformation has proven to be an efficient and mild alternative.[23] Nitrile biotransformation can be carried out through two pathways: a direct hydrolysis by means of

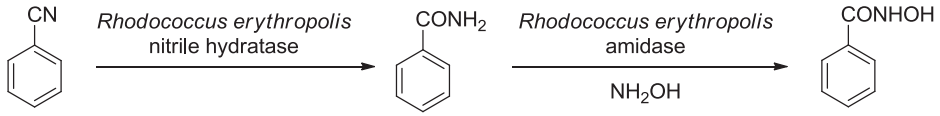
[21] G. D. Roiban, R. Agudo, M. T. Reetz, *Angew. Chem. Int. Ed*., **2014**, *83*, 8659–8663.
[22] E. Hann, A. E. Sigmund, S. K. Fager, F. B. Cooling, J. E. Gavagan, A. Ben-Bassat, S. Chauhan, M. S. Payne, S. M. Hennessey, R. Di Cosimo, *Adv. Synth. Catal.*, **2003**, *345*, 775–782.
[23] L. Martinkova, V. Kren, *Biocatal. Biotrans.,* **2002**, *20*, 73–93.

nitrilases or a hydratation with a lyase, nitrile hydratase, followed by hydrolysis of the resulting amide by means of an amidase (Scheme 1.1.5). Usually the microorganisms present only one of the two possible pathways.



Scheme 1.1.5

Beside amide hydrolysis, some of the amidases have also been used for catalysing the acyl transfer from amides to hydroxylamines. As example, in a recent contribution,[24] the authors examined a method, which made use of the same type of enzymes (*Rhodococcus erythropolis*) but a different nucleophile (hydroxylamine). This method was demonstrated with aromatic and aliphatic nitriles as substrates and catalysts (nitrile hydratases, amidase) from various sources (Scheme 1.1.6).



Scheme 1.1.6

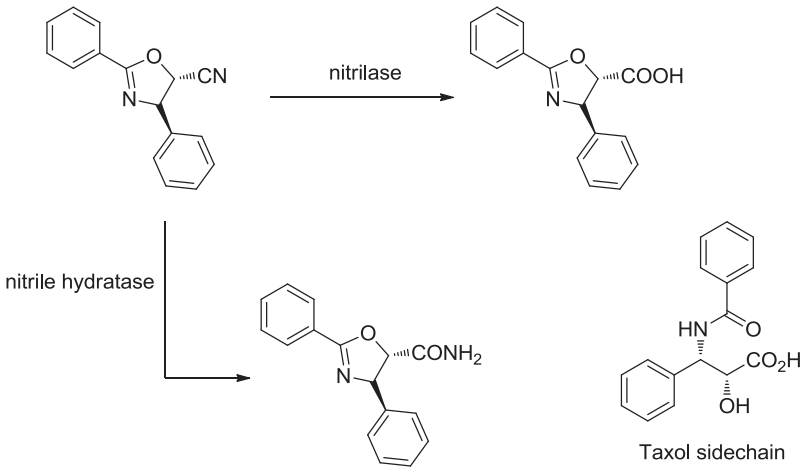
Regarding β-substituted cycloalkanecarbonitriles, several *N*-protected β-amino nitriles were previously transformed enantioselectively into β-

[24] V. Vejvoda, L. Martinkova, A. B. Veselá, O. Kaplan, S. Lutz-Wahl, L. Fischer, B. Uhnáková, *J. Mol. Catal. B: Enzym.*, **2011***, 71*, 51–55.

amino amides and β-amino acids by whole cells of *Rhodoccocci*.[25] In this work, they reported the stereoselective transformation of *cis*- and *trans*-2-aminocyclopentane(or hexane)nitriles, finding dependence of ring size and relative configuration of the 1,2-positions. They observed that the biotransformation of five-membered cyclic 2-amino nitriles proceeded significantly faster than the six-membered compounds. More specific, the products proceeding from the *trans*-2-amino nitriles (amides and acids) were formed considerably faster than the products of the *cis*-counterparts. In the same way, the enantioselectivities achieved for these compounds were strongly dependent on the structure. Thus, the *trans*-five-membered 2-amino nitriles gave exclusively the amides in excellent enantiopurities, while in the case of the *trans*-six-membered nitriles only the corresponding acids were converted with excellent enantioselectivity.

Due to their powerful viability, nitrilases, nitrile hydratases and amidases have also been investigated for the chemoenzymatic synthesis of the taxol sidechain (Scheme 1.1.7),[26] being taxol widely used in the treatment of cancer. This work was the first investigation of nitrilases as tools for the chemo-enzymatic synthesis of this compound. A number of nitrilases and nitrile hydratases converted two sidechain precursors: an openchain α-hydroxy-β-amino nitrile and the cyanohydrooxazole of the Scheme 1.1.6 as well as their epimers, but the enantioselective properties of these enzymes were not investigated.

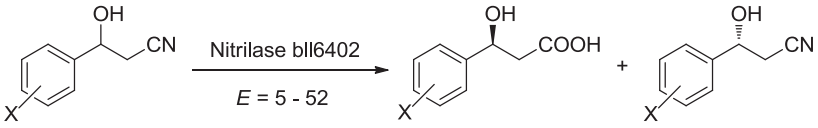
[25] M. Winkler, L. Martínková, A. C. Knall, S. Krahulec, N. Klempier, *Tetrahedron*, **2005**, *61*, 4249–4260.
[26] B. Wilding, A. B. Veselá, J. J. B. Perry, G. W. Black, M. Zhang, L. Martínková, N. Klempier, *Org. Biomol. Chem*., **2015**, *13*, 7803–7812.



Scheme 1.1.7

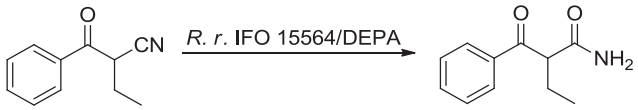
Additionally, an isolated nitrilase (bll6402) from *Bradyrhizobium japonicum* has been used in a kinetic resolution process of aromatic β-hydroxynitriles to the corresponding enantiomerically enriched (*S*)-β-hydroxycarboxylic acids with recovery of (*R*)-enriched β-hydroxynitriles, as shown in Scheme 1.1.8.[27] The substituents of the benzene ring of β-hydroxynitriles did not significantly affect the enzyme activity, but exerted some effect on the enantioselectivity. Although the *E* values obtained were moderate-low, it was significant because the same nitrilase showed no enantioselectivity towards the analogues α-hydroxynitriles. The authors propose that an hydrogen bond between the β-hydroxyl group of the β-hydroxynitriles with an amino or hydroxyl group of the enzyme could determine a better fitting of one of the two enantiomers in the active site of the enzyme, thus explaininig the observed stereopreference in the hydrolysis reaction.

[27] S. Kamila, D. Zhu, E. R. Biehl, L. Hua, *Org. Lett.*, **2006**, *8*, 4429–4431.



Scheme 1.1.8

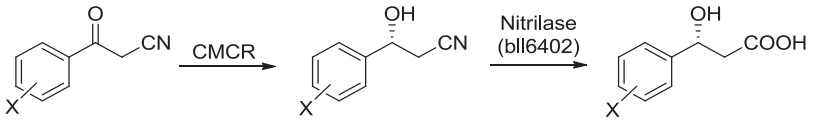
Moreover, the bacterium *Rhodococcus rhodochrous* IFO 15564, with nitrile hydratase and amidase activity, has been employed for the synthesis of a varied set of *N*-unsubstituted β-ketoamides, hardly obtainable or non-accessible by non-enzymatic methods.[28] In this work, an amidase inhibitor, namely diethyl phosphoramidate (DEPA), has been employed in order to suppress the amidase activity of the bacterium, leading to the desired final compounds with excellent yields (Scheme 1.1.9).



Scheme 1.1.9

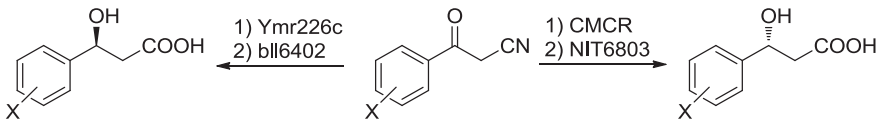
More recently, enantiopure (*R*)-β-hydroxycarboxylic acids (Scheme 1.1.10) have been obtained from unsubstituted β-ketonitriles with the use of a carbonyl reductase, from *Candida magnoliae* (CMCR), followed by a nitrilase (bll6402).[29] In this process, the intermediate (*R*)-β-hydroxynitrile required isolation prior to the nitrilase-catalyzed step.

[28] V. Gotor, R. Liz, A. Testera, *Tetrahedron*, **2004**, *60*, 607–618.
[29] D. Zhu, H. Ankati, C. Mukherjee, Y. Yang, E. R. Biehl, L. Hua, *Org. Lett*., **2007**, *9*, 2561–2563.



Scheme 1.1.10

Subsequently, the same research group developed a one-pot two-step methodology based on an enzymatic reduction with two different reductases (CMCR or Ymr226c) and subsequent hydrolysis catalyzed by two nitrilases (NIT6803 or bll6402). Thus, both enantiomers of several β-hydroxycarbocylic acids with excellent optical purity and yields were obtained (Scheme 1.1.11).[30]



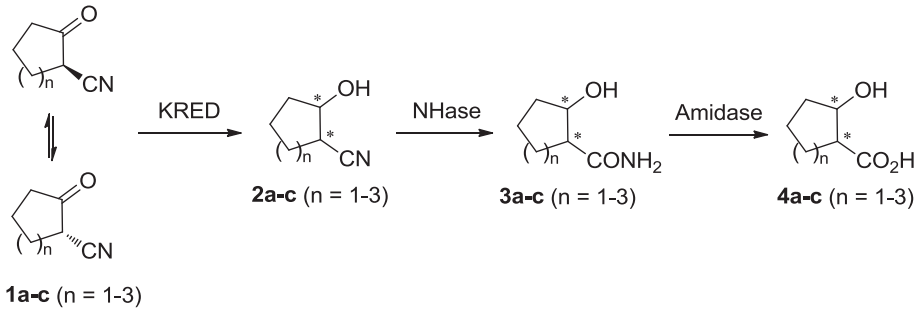
Scheme 1.1.11

Based on this background and to broaden the scope of multistep biocatalysis, we planned to investigate the possibility to carry out the concurrent bioreduction of β-ketonitriles and enzymatic hydrolysis of the cyano group.

[30] H. Ankati, D. Zhu, Y. Yang, E. R. Biehl, L. Hua, *J. Org. Chem*., **2009**, *74*, 1658–1662.

1.2. Results and Discussion

β-Oxocycloalkanecarbonitriles are a direct source of interesting optically pure building blocks. They contain two functional groups susceptibles to be transformed in a biocascade process. A ketoreductase could not only catalyze the asymmetric reduction of the ketonic group but also carry out an efficient dynamic kinetic resolution as consequence of the easily epimerizable C-1 of the β-oxonitrile **1a-c** (Scheme 1.2.1). In addition, the successive action of a nitrile hydratase (NHase) and an amidase could promote the transformation of the cyano group in the β-hydroxynitrile stage to finally give the β-hydroxycarboxylic acids **4a-c** with high enantio- and diastereomeric ratios. The proposed order of the steps in this sequence has a significant advantage because the dynamic kinetic resolution step would be associated to the ketonitrile stage, that is, the compound with the most acidic proton in the C-1 position.



Scheme 1.2.1. Proposal of biocascade transformation of 1a-c

The three selected cyclic β-ketonitriles **1a-c** have been easily obtained through a Thorpe-Ziegler reaction[19] (NaH/THF at 80°C followed by acidic hydrolysis for compounds **1a** and **1b**, or in the presence of *N*-methylaniline

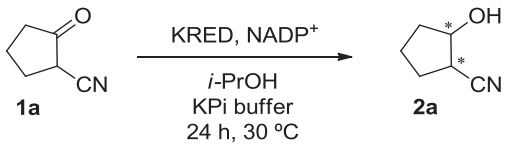
for compound **1c**).[31] Firstly, the bioreduction step of every substrate **1a-c** was essayed by using the ketoreductases from the Codex KRED screening kit, with the use of IPA for cofactor recycling and as co-solvent. Initially, the corresponding screening was performed under the standard conditions at pH 7.0, in buffer phosphate (125 mM) containing $MgSO_4$ (1.25 mM) and NADPH (1.0 mM), with a substrate concentration of 20 mM, and 19% (v/v) of co-solvent. Once analyzed the degree of conversion as well as the enantiomeric excess (*ee*) and the diastereomeric ratio (*dr*) of the β-hydroxycycloalkanecarbonitrile **2a-c** formed in each reaction, bioreductions were also tested at pH 5.0 and 10.0 with some selected KREDs in order to improve the diastereomeric ratios previously achieved.

Most of the KREDs investigated were very active and reached complete conversion after 24 h. In addition, excellent *ee*s were obtained in almost all cases, which joined to the high diastereomeric ratio obtained for the produced β-hydroxynitriles resulted in a very efficient dynamic reductive kinetic resolution (DYRKR).[32]

A selection of the best results obtained for **2a** is included in the following Table 1.2.1.

[31] L. Hsing-Jang, W. L. Tai, T. Chia-Liang, W. Jen-Dar, L. Jinn-Kwei, G. Jiunn-Cheh, T. Nai-Wen, S. Kak-Shan, *Tetrahedron*, **2003**, *59*, 1209–1226.
[32] G. A. Applegate, D. B. Berkowitz, *Adv. Synth. Catal.,* **2015**, *357*, 1619–1632.

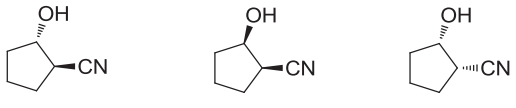
Table 1.2.1. KRED-catalyzed dynamic reductive kinetic resolution of 1a



Substrate	pH	KRED	2a *cis:trans* ratio	*ee* (%) for *cis*-2a[a]	*ee* (%) for *trans*-2a[a]
1a	7.0	P2-D11	10:90	-	>99 (1*R*,2*S*)
1a	5.0	P2-D11	6:94	-	>99 (1*R*,2*S*)
1a	7.0	P1-C01	12:88	-	>99 (1*R*,2*S*)
1a	5.0	P1-C01	7:93	-	>99 (1*R*,2*S*)
1a	7.0	P1-B12	83:17	98 (1*R*,2*R*)	80 (1*R*,2*S*)
1a	5.0	P1-B12	88:12	>99 (1*R*,2*R*)	83 (1*R*,2*S*)
1a	7.0	P2-G03	96:4	90 (1*S*,2*S*)	-
1a	5.0	P2-G03	98:2	95 (1*S*,2*S*)	-
1a	5.0	NADH-101	97:3	>99 (1*S*,2*S*)	-

[a] Configuration of the major stereoisomer between brackets.

For the five-membered ring the best results have been obtained at pH 5.0, enabling the synthesis of three of the four possible stereoisomers with good *dr* and excellent *ee*s (Figure 1.2.1). In particular, KRED-P2-D11 gave access to the *trans* isomer (1*R*,2*S*)-2-hydroxycyclopentanecarbonitrile **2a** in a 16:1 *dr* and >99% *ee*. KRED-P1-B12 provided enantiopure (1*R*,2*R*)-**2a** with 7:1 *dr*, while KRED-NADH-101 yielded the *cis* isomer (1*S*,2*S*)-**2a** with almost perfect *dr* and >99% *ee*.

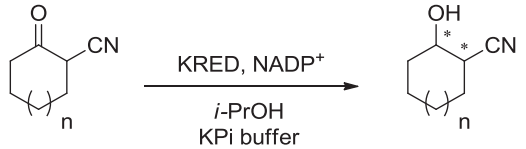


(1*R*,2*S*)-**2a** (1*R*,2*R*)-**2a** (1*S*,2*S*)-**2a**

Figure 1.2.1. Enantiopure 2-hydroxycyclopentanecarbonitriles

For compound **1b** and **1c**, pH 7.0 was enough to promote a perfect racemization of the chiral C-1 center and both enantiomers of the *cis*-diastereomer have been isolated with perfect enantio- and diastereomeric ratios. A selection of the best results is shown in the following Table 1.2.2.

Table 1.2.2. KRED-catalyzed dynamic reductive kinetic resolution of 1b,c



1b-c (n = 1-2) **2b-c** (n = 1-2)

Substrate	KRED	2b,c *cis:trans* ratio	*ee* (%) for *cis*-2b,c[a]
1b	P1-A04	>99:<1	>99 (1*S*,2*S*)
1b	P2-H07	>99:<1	>99 (1*S*,2*S*)
1b	P1-H10	>99:<1	97 (1*S*,2*S*)
1b	P2-C11	>99:<1	95 (1*S*,2*S*)
1b	P3-B03	99:1	99 (1*S*,2*S*)
1b	P1-B10	94:6	>99 (1*R*,2*R*)
1b	P1-B12	>99:<1	>99 (1*R*,2*R*)
1c	P1-A04	>99:<1	>99 (1*S*,2*S*)
1c	P1-B10	98:2	>99 (1*R*,2*R*)
1c	P1-H10	97:3	90 (1*S*,2*S*)

[a] Configuration of the major stereoisomer between brackets.

KRED-P1-A04 exhibited total selectivity towards (1*S*,2*S*)-**2b** and (1*S*,2*S*)-**2c**. The enantiomer (1*R*,2*R*)-**2b** was obtained by using KRED-P1-B12, while KRED-P1-B10 afforded (1*R*,2*R*)-**2c** in enantiopure form. It is of note that the method enabled the preparation for the first time of both enantiomers of *cis*-2-hydroxycyloheptanenitrile **2c** (Figure 1.2.2).

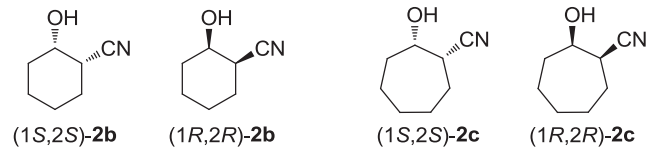
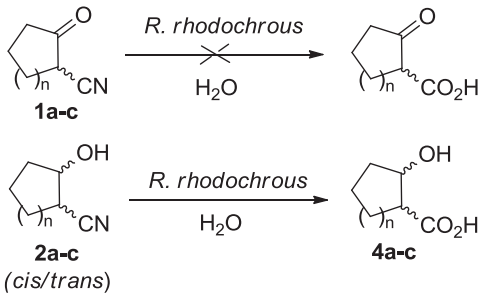


Figure 1.2.2. Enantiopure β-hydroxynitriles 2b and 2c

Once the bioreduction has been fully studied, we turned our attention towards the nitrile hydrolysis catalysed by whole-cells of commercially available bacterium *Rhodococcus rhodochrous* IFO 15564. Based on previous findings of our research group,[28] the preliminary study was performed with a standard cell concentration of $A_{650} = 1.0$, in aqueous 0.10 M phosphate buffer at pH 8.0, using 1% (v/v) EtOH as a co-solvent. In these conditions, both *cis* and *trans* isomers of **2a-c** were transformed into the corresponding β-hydroxyacids **4a-c**, while the β-ketonitriles **1a-c** remained untouched (Scheme 1.2.2). Moreover, the enantioselective properties of *R. r*. cells were also checked finding that both enantioenriched samples of **2a-c** as well as the corresponding racemic mixtures were similarly transformed.



Scheme 1.2.2. Preliminary study of *R. rhodochrous* activity

Luckily, the previous facts allow the design of a concurrent process because the starting β-ketonitrile is substrate only for the KREDs whereas the NHase-amidase system is active towards the intermediate β-hydroxynitrile independently of its absolute configuration.

As soon as both steps of the cascade have been deeply studied, we focused all the efforts in combining them to perform a real concurrent reaction, where both catalysts could be added at the beginning of the process.

Comparison of both steps reveals that two different co-solvents are used. For this reason, IPA (essential for KREDs) and EtOH (typical with *R. rhodochrous*) were checked in different ratios (1-15% v/v) in both enzymatic processes, using the buffer required for KRED at pH 7.0 as the reaction medium. The most significant outcomes were the following:

1. EtOH is not accepted for KREDs.

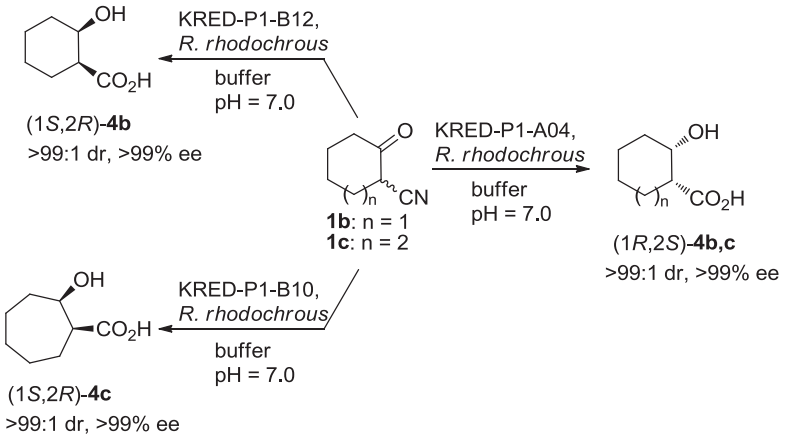
2. The use of 15% v/v of IPA inhibits the activity of *R. rhodochrous* at A_{650} = 1-4.

3. Both *R. rhodochrous* (A_{650} = 3-4) and KRED are active when 5% v/v of IPA is used.

So far, a plausible reaction conditions in which all biocatalysts are active involves the use of 5% IPA, a higher concentration of whole cells

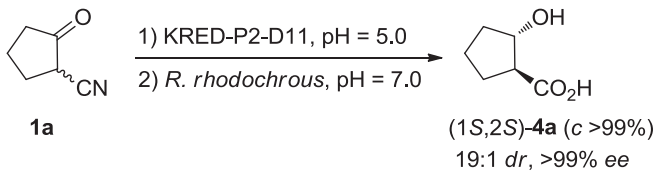
(A_{650} = 3-4), and pH 7.0. Nevertheless, as some KRED-catalyzed reactions happened at pH 5.0, the activity of *R. rhodochrous* was also checked at this pH value, but a significant decrease of the activity was observed.

On the lights of these findings, the concurrent biotransformation of β-ketonitriles was designed using phosphate buffer pH 7.0, IPA 5% v/v and whole cells of A_{650} = 4. Under these conditions both catalysts displayed perfect activity and the final β-hydroxyacids were isolated in high yields. By choosing the right KRED under the described standard conditions, the β-ketonitriles **1b** and **1c** underwent efficient bioconversion into the desired final products **4b** and **4c**, respectively, as shown in Scheme 1.2.3.



Scheme 1.2.3. Biocascades towards stereoisomers of 4b and 4c

Regarding the five-membered ring **1a**, a concurrent enzymatic cascade remained challenging due to the pH 5.0 inhibition of *R. r.* In this case, a stepwise approach was applied. Once the bioreduction at pH 5.0 was completed, a 3.0 M NaOH aq solution was added until pH 7.0 followed by a whole cells suspension of *R. r* in such amount to attain A_{650} = 4. As a result, the desired β-hydroxyacid **4a** was isolated with very high *dr* and *ee*, as shown in Scheme 1.2.4.



Scheme 1.2.4. One-pot sequential synthesis of (1*S*,2S)-4a

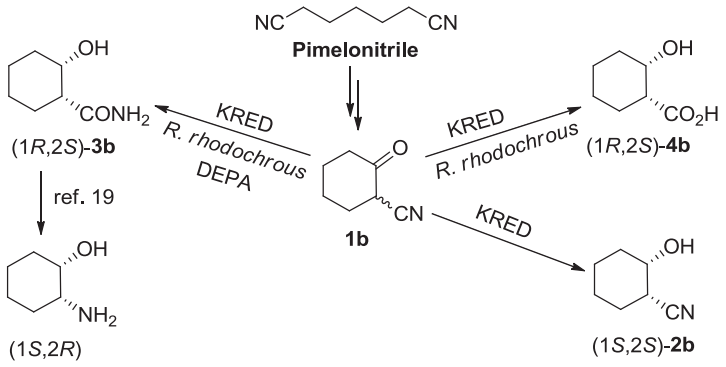
It should be noted that this easy methodology enabled the synthesis of the desired optically pure β-hydroxyacids in very high yields. The compounds were isolated by continuous flow extraction and no need of further purification was required.

Finally, to underline the synthetic utility of the enzymatic platform, a protocol for the synthesis of β-hydroxyamides was also investigated. For this, whole cells of *Rhodococcus rhodochrous* were grown in the presence of diethyl phosphoramidate (DEPA), an inhibitor of the amidase activity of the microorganism. Thus, under the only action of the NHase could allow access to the mentioned β-hydroxyamides (see Scheme 1.2.1).

The protocol was applied to **1b** and under these conditions (1*R*,2*S*)-2-hydroxycyclohexanecarboxamide (**3b**) was isolated from the cascade using KRED-P1-A04 as biocatalyst of the reduction step. However, the amidase activity was not totally inhibited by DEPA and the undesired β-hydroxyacid **4b** was also detected in the crude of reaction. In this case, a purification step was then mandatory and the desired compound was finally obtained with 65% yield. Although with moderate yield, this procedure has interest because this kind of β-hydroxyamides are highly valuable compounds for being direct precursors, among other compounds, of β-amino alcohols.[19]

As a summary, and to underline the synthetic possibilities of the cascade here developed, we have included in Scheme 1.2.5 the variety of interesting optically pure compounds which has been easily obtained from

the inexpensive pimelonitrile. Moreover, a simple Hofmann rearrangement also allows the transformation of **3b** into the optically active *cis*-(1*S*,2*R*)-2-aminocyclohexanol.[19]



Scheme 1.2.5. Valuable enantiopure compounds from pimelonitrile

Assignment of the absolute configuration

The absolute configuration of the optically active *cis*-**2a,b** and *trans*-**2a** was assigned after comparison of the sign of the optical rotation of each sample with the reported data.

The absolute configuration (1*S*,2*S*) for the optically active *cis*-(−)-**2c**, which was obtained from the bioreduction of **1c** with KRED-P1-A04, was assigned by applying the Mosher's method. Thus, treatment of *cis*-(−)-**2c** with (*S*)-MTPA-Cl gave the (*R*)-MTPA-ester derivative **5**, whose ^{1}H-NMR spectrum ($CDCl_3$, 600 MHz) is included in Figure 1.2.3

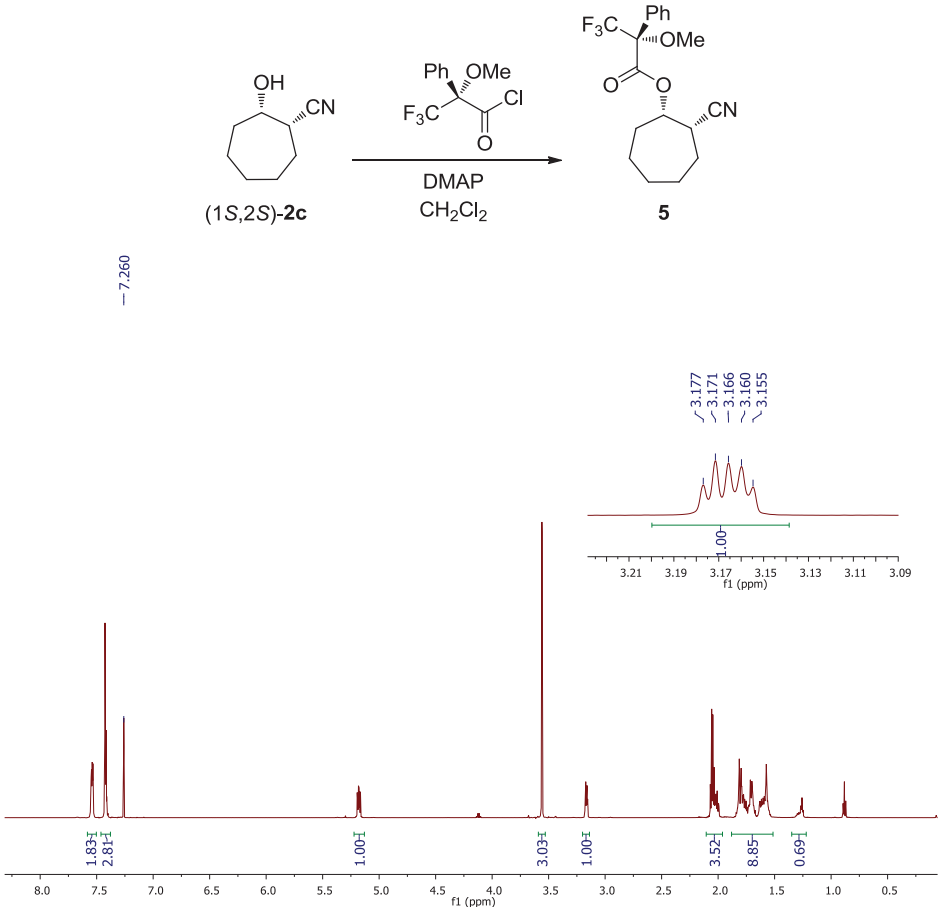


Figure 1.2.3. ^{1}H-NMR spectrum of the (*R*)-MTPA ester derivative 5

Similarly, a sample of *cis*-(±)-**2c** was prepared by conventional reduction of **1c** with $NaBH_4$ in methanol and subsequent separation of the resulting *cis*/*trans* mixture by flash chromatography (hexane-ethyl acetate 5:1 as eluent). The treatment of *cis*-(±)-**2c** with (*S*)-MTPA-Cl yielded the mixture of diastereomers named in the following Figure 1.2.4 as (*R*)-(*R,R*)-**5** and (*R*)-(*S,S*)-**5**. The portion of the ^{1}H-NMR spectrum corresponding to the resonance of proton H-1 of this diastereomeric mixture is also shown in the figure.

Taking the usual working models for these diastereomeric (*R*)-MTPA esters into account,[33] and analyzing the chemical shift for H-1 of each diastereomer, we have assigned the lowest δ value for H-1 of the diastereomer named as (*R*)-(*S,S*)-**5** due to the shielding effect that the phenyl group exerts on this *syn* placed proton.

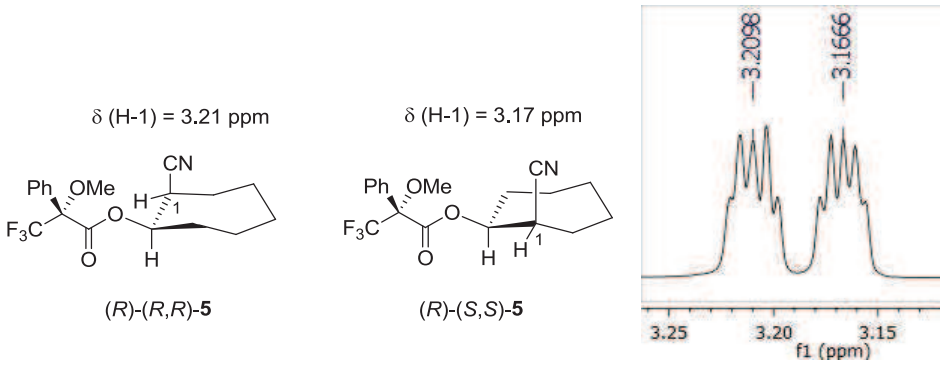


Figure 1.2.4. Working models for the diastereomeric (*R*)-MTPA ester derivatives and partial ^{1}H-NMR spectrum ($CDCl_3$, 600 MHz)

From this analysis, we conclude that the optically active *cis*-(−)-**2c** obtained in the bioreduction of **1c** with KRED-P1-A04 has the (1*S*,2*S*)

[33] J. M Seco, E. Quiñoá, R. Riguera, *Chem. Rev.*, **2004**, *104*, 17–118.

configuration. This is in agreement with the enantiopreference shown for this enzyme in the bioreduction of the cyclohexylic analogue **1b**.

Finally, we have assigned to the optically active 2-hydroxycycloalkanecarboxylic acids **4a-c**, obtained from the reduction-hydratation-hydrolysis biocascade, the same absolute configuration as that of its optically active precursor 2-hydroxycycloalkanecarbonitrile **2a-c** proceeding from the bioreduction with the same ketoreductase. This is based on the fact that each optically active carboxylic acid **4a-c** and its precursor nitrile **2a-c** are obtained in both processes (biocascade and simple bioreduction) with the same *ee* and also with the same *dr*.

1.3. Conclusions

As result of this first chapter, a new enzymatic concurrent process has been developed in aqueous medium, thanks to the direct combination of purified ketoreductases and whole cells of *Rhodococcus rhodochrous*.

The starting materials –cyclic β-ketonitriles– have been easily synthesized from inexpensive and commercially available alkanedinitriles. With their intrinsic ability to racemize in the reaction conditions, the selected β-ketonitriles have been quantitatively converted into the desired β-hydroxyacids with very high diastereomeric ratios and excellent enantiomeric excesses.

The selectivity of *Rhodococcus rhodochrous* towards the intermediate β-hydroxynitrile allowed a real one-pot three-step reaction.

The high enantio- and diastereoselectivities exhibited by the KREDs gave access to both enantiomers of the *cis*-diastereomers of all the cyclic β-hydroxyacids and β-hydroxynitriles of this study, as well as to one of the enantiomers *trans* for the five-membered ring.

Compounds prepared in this contribution can be used as building blocks for the synthesis of other interesting optically active products.

1.4. Experimental Section

1.4.1. General information

Enzymes

Codex® KRED Screening Kit was purchased from Codexis. Cultures of *Rhodococcus rhodochrous* IFO 15564 were prepared according to the described procedure.[34]

General methods

^{1}H-NMR and proton-decoupled ^{13}C-NMR spectra ($CDCl_3$, CD_3OD) 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. The ^{1}H-NMR spectra of the Mosher's derivative were obtained using a Bruker AV-600 (^{1}H, 600 MHz). Calibration was made on the signal of the solvent (^{13}C: $CDCl_3$, 76.95; ^{1}H: $CDCl_3$, 7.26; ^{13}C: CD_3OD, 49.00; ^{1}H: CD_3OD, 3.31).[35] High resolution mass spectra were recorded on a Bruker Impact II instrument. Optical rotations were measured using a Perkin-Elmer 241 polarimeter and are quoted in units of 10^{-1}deg cm^2 g^{-1}. Gas chromatography (GC) analyses were performed on a Hewlett Packard 6890 Series II chromatograph, with the following columns: CP-ChiraSil-DEX CB 25 m × 0.25 mm × 0.25 μm or Rt®-βDEXse 30 m × 0.25 mm × 0.25 μm.

Racemic substrates

Racemic 2-oxocycloalkanecarbonitriles **1a-c** were prepared as previously described for **1a-b**[19] and **1c**.[31] Obtained yields: **1a** (92%), **1b** (93%), and **1c** (80%).

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[34] R. Morán-Ramallal, R. Liz, V. Gotor, *Org. Lett*., **2007**, *9*, 521–524.

[35] G. R. Fulmer, A. J. M. Miller, N. H. Sherden, H. E. Gottlieb, A. Nudelman, B. M. Stoltz, J. E. Bercaw, K. I. Goldberg, *Organometallics*, **2010**, *29*, 2176–2179.

1.4.2. Analytical scale biotransformations

*General procedure for the bioreduction of **1a-c** at pH 7.0.*

In a 2.0 mL eppendorf tube, KRED (2.0 mg), ketone (20 mM) and *i*-PrOH (190 μL) were added to 900 μL of 125 mM phosphate buffer, pH 7.0. This buffer also contains $MgSO_4$ (1.25 mM) and the cofactor $NADP^+$ (1.0 mM). The resulting reaction mixture was shaken at 250 rpm and 30 ºC for 24 h. Then, the mixture 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 and the diastereomeric ratio of the resulting optically active 2-hydroxycycloalkanecarbonitriles **2a-c** were determined by GC equipped with a chiral column, after conversion into the corresponding *O*-trimethylsilyl derivative. In all the cases, the GC analysis revealed the absence of the starting material.

*General procedure for the bioreduction of **1a-b** at pH ≠ 7.0.*

The general procedure described above was followed, but the pH of the buffer solution was previously changed from 7.0 to the required one by addition of 3 N aq NaOH or 3 N aq HCl. In both cases, once the reaction was completed, the pH of the resulting mixture was adjusted to pH 7.0 before the extraction with ethyl acetate.

*Hydrolytic activity assay of R.r. IFO-15564 towards **2a-c**. General method.*

In a 2.0 mL eppendorf tube, racemic *cis*- and/or *trans*-**2a-c** (2.0 mg) and *i*-PrOH (50 μL) were added to 1000 μL of a suspension of the microorganism with A_{650} = 4.0 in 100 mM phosphate buffer pH 7.0. The reaction was shaken at 200 rpm and 28 °C for 5-24 h. An aliquot was periodically analyzed by TLC (hexane:ethyl acetate 1:1). Once the starting material was consumed completely, the mixture was acidified until pH 2 and extracted with ethyl acetate (2 x 450 μL). The organic layers were separated by centrifugation (90 s, 13000 rpm), combined and finally dried

over Na_2SO_4. The GC analysis of the resulting mixtures revealed the formation of the 2-hydroxycycloalkanecarboxylic acids almost racemic and with the same diastereomeric ratio of the starting **2a-c**.

General procedure for the concurrent one-pot synthesis

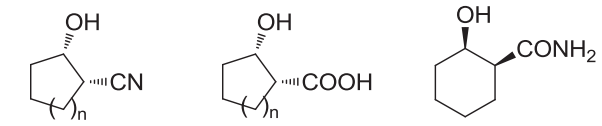
In a 2.0 mL eppendorf tube, a suspension of harvested cells of *R.r.* IFO 15564 in 125 mM phosphate buffer pH 7.0 of a high absorbance value (A_{650} = 22-16) was mixed with the adequate amount of 125 mM phosphate buffer pH 7.0 containing $MgSO_4$ (1.25 mM) and the cofactor $NADP^+$ (1.0 mM) to get a final A_{650} = 4.0. Then, substrate **1a-c** (20 mM), KRED (2 mg), and *i*-PrOH (50 μL) were added. The reaction was shaken at 200 rpm and 28 °C until disappearance of the intermediate β-hydroxynitrile **2a-c** (TLC control, 5-48h). Then, the procedure described above was followed. The diastereomeric ratio and enantiomeric excess of each optically active 2-hydroxycycloalkanecarboxylic acid were determined by GC equipped with a chiral column after the appropriate derivatization.

General procedure for the sequential one-pot synthesis: bioreduction at pH 5.0 and subsequent biohydrolysis at pH 7.0.

First, the general procedure of the bioreduction at pH 5.0 was followed. After 24 h of reaction, the pH was adjusted at 7.0 using 3 N aq NaOH and the adequate amount of a suspension of *R.r.* IFO 15564 of high A_{650} value was added to get a final A_{650} = 4.0. As a consequence a 20-25% degree of dilution occurred. Then, the procedure described above was followed.

*1.4.3. Preparative scale reactions and characterization of optically active **2a-c**, **4a-c**, and **3b**.*

For a better overview, a selection of compounds included in this section is reported in the following Figure 1.4.3.1.



(1*S*,2*S*)-**2a-c** (1*R*,2*S*)-**4a-c** (1*S*,2*R*)-**3b**

a: n = 1; **b**: n = 2; **c**: n = 3

Figure 1.4.3.1. A selection of optically active 2a-c, 4a-c, and 3b

*1.4.3.1. (1S,2S)-2-Hydroxycylopentanecarbonitrile [(1S,2S)-**2a**]*

In a 100 mL Erlenmeyer flask, which contains 30 mL of 125 mM phosphate solution at pH 5.0 (also containing 1.25 mM $MgSO_4$ and 1.0 mM $NADP^+$), a solution of 2-oxocyclopentanecarbonitrile (30 mg) in isopropylic alcohol (3.8 mL), and KRED-NADH-101 (30 mg) were added. The solution was shaken at 250 rpm and 30°C. After 12 h, 10 mg of the enzyme were added and the solution left shaken for another 12 h. Then the mixture was extracted with ethyl acetate (2 × 30 mL), the organic layers separated by centrifugation (3 min, 5000 rpm), combined and finally dried over Na_2SO_4. The crude material was purified by flash chromatography (dichloromethane/hexane/diethyl ether 2:2:1) to yield the pure compound as a colourless oil. Yield, 92% (28 mg); $[\alpha]_D^{20}$ +7.2 (*c* 1.1, CH_2Cl_2), *ee* >99%, *dr* 97:3. Lit.[19] for (1*S*,2*S*)-**2a** $[\alpha]_D^{20}$ +6.0 (*c* 1.0, CH_2Cl_2), *ee* = 97%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.57-2.13 (m, 7H), 2.70-2.78 (m, 1H, H-1), 4.41-4.45 (m, 1H, H-2); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 21.9 (CH_2), 27.8 (CH_2), 33.6 (CH_2), 36.7 (C-1), 73.3 (C-2), 120.2 (CN). These spectroscopic data are in agreement with published data.[19]

*1.4.3.2. (1R,2S)-2-Hydroxycylopentanecarbonitrile [(1R,2S)-**2a**]*

In a 100 mL Erlenmeyer flask, which contains 40 mL of 125 mM phosphate solution at pH 5.0 (also containing 1.25 mM $MgSO_4$ and 1.0 mM $NADP^+$), a solution of 2-oxocyclopentanecarbonitrile (40 mg) in isopropylic alcohol (5.0 mL), and KRED-P2-D11 (50 mg) were added. The solution was shaken at 250 rpm and 30°C. After 24 h, the pH of the solution was adjusted at 7.0 and the mixture was extracted with ethyl acetate (2 × 30 mL), the organic layers separated by centrifugation (3 min, 5000 rpm), combined and finally dried over Na_2SO_4. The crude material was purified by flash chromatography (dichloromethane/hexane/diethyl ether 4:4:1) to yield the pure compound as a colourless oil. Yield, 84% (34 mg). $[\alpha]_D^{20}$ +58.8 (*c* 1.3, CH_2Cl_2), *ee* >99%, *dr* 94:6. Lit.[36] for diastereomerically pure (1*R*,2*S*)-**2a**: $[\alpha]_D^{20}$ +78.9 (*c* 2.5, CH_2Cl_2), *ee* = 99%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.60-1.71 (m, 1H), 1.73-1.95 (m, 3H), 1.99-2.11 (m, 1H), 2.13-2.25 (m, 1H), 2.46 (br s, 1H, OH), 2.66-2.73 (m, H-1), 4.45-4.50 (q, 1H, *J* 5.7 Hz, H-2); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 22.1 (CH_2), 28.7 (CH_2), 34.0 (CH_2), 37.4 (C-1), 76.6 (C-2), 121.8 (CN). These spectroscopic data are in agreement with published data.[36]

*1.4.3.3. (1S,2S)-2-Hydroxycylohexanecarbonitrile [(1S,2S)-**2b**]*

In a 100 mL Erlenmeyer flask, which contains 45 mL of 125 mM phosphate buffer pH 7.0 (also containing 1.25 mM $MgSO_4$ and 1.0 mM $NADP^+$), a solution of 2-oxocyclohexanecarbonitrile (45 mg) in isopropylic alcohol (5.7 mL), and KRED-P1-A04 (60 mg) were added. The solution was shaken at 250 rpm and 30°C for 24 h. Then the mixture was extracted with ethyl acetate (2 × 40 mL), the organic layers separated by centrifugation (3 min, 5000 rpm), combined and finally dried over Na_2SO_4. The crude material was purified by flash chromatography (hexane/ethyl acetate 5:1) to yield the pure compound as a colourless oil. Yield, 96% (43 mg); $[\alpha]_D^{20}$ –27.4 (*c* 1.0, CH_2Cl_2), *ee* >99%. Lit.[19] for (1*S*,2*S*)-**2b**: $[\alpha]_D^{20}$

[36] E.Forró, K. Lundell, F. Fülöp, L. T. Kanerva, *Tetrahedron: Asymmetry*, **1997**, *8*, 3095–3099.

–26.9 (*c* 1.0, CH_2Cl_2), *ee* = 93%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.24-1.41 (m, 1H), 1.48-2.06 (m, 8H), 3.00-3.03 (m, 1H, H-1), 3.76-3.82 (m, 1H, H-2); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 21.9 (CH_2), 22.5 (CH_2), 26.4 (CH_2), 31.8 (CH_2), 36.2 (C-1), 68.5 (C-2), 120.2 (CN). These spectroscopic data are in agreement with published data.[19]

*1.4.3.4. (1R,2R)-2-Hydroxycylohexanecarbonitrile [(1R,2R)-**2b**]*

The same procedure as for (1*S*,2*S*)-**2b** was followed, but using 10 mg of 2-oxocyclohexanecarbonitrile and KRED-P1-B12 (13 mg). After 24 h of reaction, and following the procedure described for its stereoisomer, pure (1*R*,2*R*)-**2b** was isolated. Yield, 99% (10 mg); $[\alpha]_D^{20}$ +28.1 (*c* 0.83, CH_2Cl_2), *ee* >99%.

*1.4.3.5. (1S,2S)-2-Hydroxycyloheptanecarbonitrile [(1S,2S)-**2c**]*

In a 100 mL Erlenmeyer flask, which contains 30 mL of 125 mM phosphate buffer pH 7.0 (also containing 1.25 mM $MgSO_4$ and 1.0 mM $NADP^+$), a solution of 2-oxocycloheptanecarbonitrile (30 mg) in isopropylic alcohol (3.8 mL), and KRED-P1-A04 (40 mg) were added. The solution was shaken at 250 rpm and 30°C for 24 h. Then the mixture was extracted with ethyl acetate (2 × 30 mL), the organic layers separated by centrifugation (3 min, 5000 rpm), combined and finally dried over Na_2SO_4. The crude material was purified by flash chromatography (hexane/ethyl acetate 6:1) to yield the pure compound as a colorless oil. Yield, 96% (30 mg); $[\alpha]_D^{20}$ –14.4 (*c* 1.8, CH_2Cl_2), *ee* >99%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.35-1.48 (m, 1H), 1.52-2.02 (m, 9H), 2.83 (s, 1H, OH), 3.06-3.11 (m, 1H, H-1), 3.90 [dt, 1H, ^3J 4.2 (d) and 8.1 (t) Hz, H-2]; ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 21.7 (CH_2), 24.4 (CH_2), 25.7 (CH_2), 26.3 (CH_2), 34.6 (CH_2), 39.0 (C-1), 71.8 (C-2), 120.8 (CN); HRMS (ESI^+): $[M+Na]^+$ found: 162.0895. $C_8H_{13}NNaO$ requires 162.0889.

*1.4.3.6. (1R,2R)-2-Hydroxycyloheptanecarbonitrile [(1R,2R)-**2c**]*

The same procedure as for (1*S*,2*S*)-**2c** was followed, but using 15 mg of 2-oxocycloheptanecarbonitrile and KRED-P1-B10 (19.5 mg). After 24 h of reaction, and following the procedure described for its stereoisomer, pure (1*R*,2*R*)-**2b** was isolated. Yield, 99% (15 mg); $[\alpha]_D^{20}$ +10.0 (*c* 1.3, CH_2Cl_2), *ee* >99%, *dr* 98:2.

*1.4.3.7. (1S,2S)-2-Hydroxycylopentanecarboxylic acid [(1S,2S)-**4a**]*

Starting from 2-oxocyclopentanecarbonitrile (20 mg) and, after the bioreduction with KRED-P2-D11 was completed (24 h), the pH was adjusted at 7.0 and a suspension of resting cells of *R.r.* IFO 15564 was added to get a final A_{650} = 4.0. The resulting reaction mixture was left shaking for another 24 hours. Then the mixture was centrifuged (3 min, 5000 rpm), the solution basified until pH 8.0 and continuously extracted with dichloromethane for 8 h. The water phase was collected, acidified until pH 2.0 with concentrated HCl and continuously extracted with dichloromethane for 12 h. Finally, the organic phase was dried over Na_2SO_4 and the solvent eliminated. Flash chromatography [neutral silica gel (200-425 mesh), hexane-ethyl acetate 4:1 as the eluent] of the crude material yielded the final product as a colorless oil. Yield, 52% (12 mg). $[\alpha]_D^{20}$ +31.8 (*c* 0.33, CH_2Cl_2), *ee* >99%; *dr* 96:4. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.58-2.17 (m, 6H), 2.67-2.75 (m, 1H, H-1), 4.38-4.44 (q, 1H, *J* 6.6 Hz, H-2), 3.5-6.0 (br s, 2H, OH + CO_2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 21.9 (CH_2), 26.9 (CH_2), 34.1 (CH_2), 52.3 (C-1), 76.3 (C-2), 180.1 (CO_2H). These spectroscopic data are in agreement with published data.[37]

*1.4.3.8. (1R,2S)-2-Hydroxycyclohexanecarboxylic acid [(1R,2S)-**4b**]*

2-Oxocyclohexanecarbonitrile (30 mg), KRED-P1-A04 (40 mg), and *i*-PrOH (1.5 mL) were added to a 100 mL Erlenmeyer flask containing 30 mL of a suspension of harvested cells of *R.r.* IFO 15564 (A_{650}= 4.0) in 125

[37] H. Baumann, N. C. Franklin, H. Möhrle, *Tetrahedron*, **1967**, *23*, 4331–4336.

mM phosphate buffer pH 7.0 (also containing 1.25 mM $MgSO_4$, 1.0 mM $NADP^+$). The mixture was shaken at 200 rpm and 28 ºC for 48 h. After this time, the mixture was centrifuged (3 min, 5000 rpm), the solution basified until pH 8.0 and continuously extracted with dichloromethane for 8 h. Then, the aqueous phase was collected, acidified until pH 2.0 with conc. HCl and continuously extracted with dichloromethane for 12 h. Finally, the organic phase was dried over Na_2SO_4 and the solvent eliminated under reduced pressure. The crude product **4b** was isolated as a colorless oil with a high level of purity. If it is required, **4b** can be purified by flash chromatography (neutral silica gel, hexane/ethyl acetate 4:1). Yield, 90% (32 mg). $[\alpha]_D^{20}$ +18,4 (*c* 1.4, CH_2Cl_2) *ee*>99%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.22-1.98 (m, 8H), 2.51-2.57 [dt, *J* 2.7 (t) and 6.0 (d) Hz, 1H, H-1], 4.18-4.20 (m, 1H, H-2), 6.2-8.0 (br s, 2H, OH + CO_2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 20.2 (CH_2), 23.7 (CH_2), 24.4 (CH_2), 31.7 (CH_2), 46.4 (C-1), 67.0 (C-2), 180.2 (CO_2H). These spectroscopic data are in agreement with published data.[37]

*1.4.3.9. (1S,2R)-2-Hydroxycylohexanecarboxylic acid [(1S,2R)-**4b**]*

The same procedure as for (1*R*,2*S*)-**4b** was followed, but using 10 mg of 2-oxocyclohexanecarbonitrile and KRED-P1-B12 (13 mg). After 24 h of reaction, and following the procedure described for its stereoisomer, pure (1*S*,2*R*)-**4b** was isolated. Yield, 83% (10 mg); $[\alpha]_D^{20}$ –16.4 (*c* 0.50, CH_2Cl_2), *ee* >99%.

*1.4.3.10. (1R,2S)-2-Hydroxycycloheptanecarboxylic acid [(1R,2S)-**4c**]*

The same procedure was followed, but starting from 2-oxocycloheptanecarbonitrile (30 mg) and KRED-P1-A04 (40 mg). After 24 h of reaction, compound (1*R*,2*S*)-**4c** was isolated. Yield, 97% (34 mg); $[\alpha]_D^{20}$ +12.0 (*c* 1.4, CH_2Cl_2), *ee*>99%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.34-2.14 (m, 11H), 2.65-2.70 [dt, *J* 5.1 (d) and 2.7 (t) Hz, 1H, H-1], 4.25-4.29 (m, 1H, H-2), 5.7-7.2 (br s, 1H, CO_2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 21.9 (CH_2), 24.0 (CH_2), 26.4 (CH_2), 27.7 (CH_2), 34.8 (CH_2), 49.6

(C-1), 70.3 (C-2), 181.1 (CO_2H). HRMS (ESI^+): $[M+Na]^+$ found: 181.0835. $C_8H_{14}NaO_3$ requires 181.0835.

*1.4.3.11. (1S,2R)-2-Hydroxycycloheptanecarboxylic acid [(1S,2R)-**4c**]*

The same procedure was followed, but using in this case 20 mg of 2-oxocycloheptanecarbonitrile and KRED-P1-B10 (26 mg). After 24 h of reaction and following the same procedure described for its stereoisomer, pure (1*R*,2*S*)-**4c** was isolated. Yield, 91% (20mg); $[\alpha]_D^{20}$ –10.7 (*c* 1.0, CH_2Cl_2), *ee* >99%

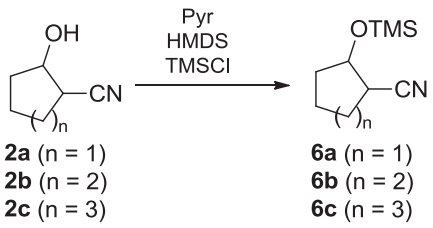
*1.4.3.12. (1R,2S)-2-Hydroxycyclohexanecarboxamide [(1R,2S)-**3b**]*

2-Oxocyclohexanecarbonitrile (30 mg), KRED-P1-B12 (40 mg), and *i*-PrOH (1.5 mL) were added to a 100 mL Erlenmeyer flask containing 30 mL of a suspension of harvested cells of *R.r.* IFO 15564, which amidase activity was inhibited during its growth phase with DEPA[28] (A_{650}= 4.0) in 125 mM phosphate buffer pH 7.0 (also containing 1.25 mM $MgSO_4$, 1.0 mM $NADP^+$). The mixture was shaken at 200 rpm and 28 ºC for 6 h. After this time, the mixture was centrifuged (3 min, 5000 rpm), the solution basified until pH 7.0 and continuously extracted with ethyl acetate for 6 h. Finally, the organic phase was dried over Na_2SO_4 and the solvent eliminated. The crude material was purified by flash chromatography (10% methanol:$CHCl_3$) to yield the pure compound as a colourless oil. Yield, 62% (21 mg); $[\alpha]_D^{20}$ +20.7 (*c* 1.05, EtOH), *ee*>99%. ^{1}H NMR (300 MHz, CD_3OD) δ (ppm): 1.24-1.96 (m, 9H), 2.30-2.36 [dt, *J* 6.0 (d) and 3.0 (t) Hz, 1H, H-1], 4.11-4.15 (m, 1H, H-2); ^{13}C NMR (300 MHz, CD_3OD) δ (ppm): 19.51 (CH_2), 23.80 (CH_2), 24.62 (CH_2), 31.85 (CH_2), 47.2 (C-1), 66.8 (C-2), 179.6 ($CONH_2$). These spectroscopic data are in agreement with published data.[19]

1.4.4. Synthesis of racemic cis/trans *mixtures of* ***2a-c****,* ***4a-c****,* ***cis-3b****, and their derivatives for ee and dr determinations*

1.4.4.1. 2-Hydroxycycloalkanecarbonitriles ***2a-c*** *and their derivatives* ***6a-c****.*

Racemic *cis*/*trans* mixtures of 2-hydroxycyloalkanecarbonitriles **2a-c** were obtained by reduction of the corresponding 2-oxocycloalkanecarbonitrile **1a-c** with $NaBH_4$ (4.0 equiv) in methanol (0.10 M). In all the cases, a mixture of *cis*/*trans* diastereomers was obtained such it was proven by ^{1}H-NMR analysis of the reaction crude. These mixtures were converted into the corresponding trimethylsilyl derivatives **6a-c** such as it is shown in the following Scheme 1.4.4.1. Finally, the GC-analysis of derivatives **6a-c**, using an optically active column, showed four well-defined peaks for each derivative.



Scheme 1.4.4.1

On the other hand, in order to differentiate which peaks corresponds to each *cis*- and *trans*-diastereomer, *trans*-(±)-**2a** and *trans*-(±)-**2b** were prepared by opening of the corresponding commercially available cyclopentene or cyclohexene oxide with sodium cyanide in the presence of

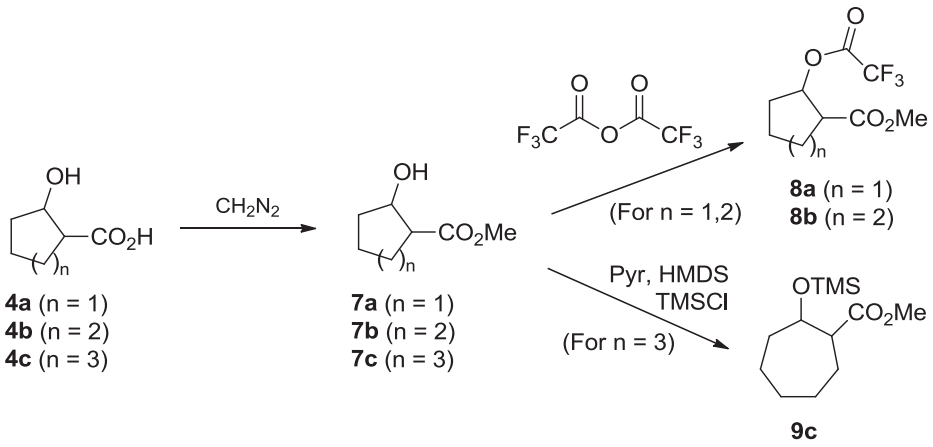
$LiClO_4$.[38] Then, they were also converted into the corresponding *trans*-(±)-**6a** and *trans*-(±)-**6b** and analyzed by chiral-GC.

In the case of **2c**, the analysis of the ^1H NMR spectrum and the comparison with published data for *trans*-(±)-**2c** [39] allowed us to differentiate the signals corresponding to both diastereomers. In addition, a sample of *cis*-(±)-**2c** was isolated with a high diastereomeric ratio (95:5) after a careful separation by flash column chromatography (hexane-ethyl acetate 5:1 as eluent). Then, the conversion of this diastereomerically enriched sample into the derivative *cis*-(±)-**5c** and further GC-analysis allowed us to assign the peaks corresponding to *cis*- and *trans*-diastereomers.

*1.4.4.2. 2-Hydroxycycloalkanecarboxylic acids **4a-c** and their derivatives **8a,b** and **9c**.*

Analyses of all the 2-hydroxycycloalkanecarboxylic acids **4a-c** required their previous transformation into the methyl ester derivatives **7a-c** by treatment with a solution of diazomethane in a mixture of diethyl ether/methanol (40 mM). Then, **7a,b** was transformed into the diester **8a,b** with trifluoroacetic anhydride meanwhile **7c** was converted into the silyl derivative **9c**, as shown in the Scheme 1.4.4.2.

[38] M. Chini, P. Crotti, L. Favero, F. Macchia, *Tetrahedron Lett.*, **1991**, *32*, 4775–4778.
[39] S. Yamasaki, M. Kanai, M. Shibasaki, *J. Am. Chem. Soc*., **2001**, *123*, 1256–1257.

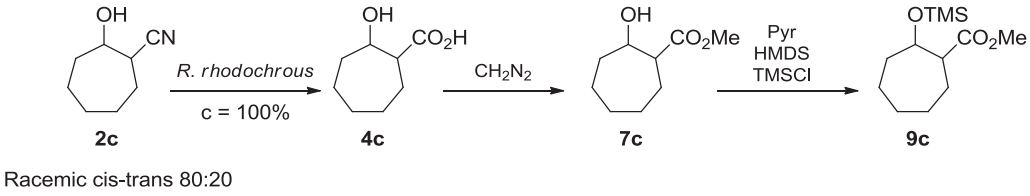


Scheme 1.4.4.2

Racemic *cis*/*trans* mixtures of **8a,b** were prepared from the corresponding mixture of *cis*-(±)- and *trans*-(±)-**7a,b**, which in turn were obtained by $NaBH_4$ (4 equiv.) reduction of the corresponding methyl 2-oxocycloalkanecarboxylate. Moreover, a little amount of diastereomerically pure *cis*-(±)-**7a,b** and *trans*-(±)-**7a,b** were achieved by flash chromatography of the mixture (hexane-dichloromethane-diethyl ether 2:4:1 as eluent). The chiral-GC analysis of **8a** revealed the presence of four well-defined peaks, but the GC analysis of **8b** showed a slight overlapping between two peaks corresponding to a *trans* enantiomer and the *cis*-(1*R*,2*S*) stereoisomer. Nevertheless, this was not an inconvenient for the GC-analysis of the preparative samples of optically active *cis*-**4b** (from the biocascade reactions) since only a *cis*-stereoisomer was obtained.

On the other hand, the racemic *cis*/*trans* mixture of **6c** was prepared starting from an 80:20 mixture of *cis*-(±)-/*trans*-(±)-2-hydroxycycloheptanecarbonitrile **2c** and carrying out its hydrolysis with *R.r.* IFO 15564 until completion. The diastereomeric ratio 80:20 was maintained after the hydrolysis such it was proven by ^{1}H NMR analysis of the resulting **4c**. The GC-analysis of the trimethylsilyl derivative **9c** revealed the

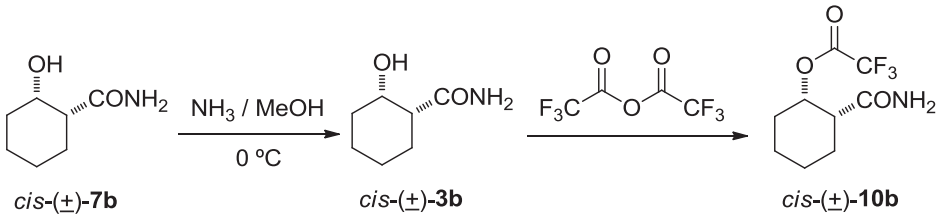
presence of only three peaks because of the overlapping of one enantiomer of the *trans* diastereomer and the *cis*-(1*R*,2*S*) stereoisomer. For this reason, for samples obtained in the biocascade of **1c**, a combined analysis by ^{1}H-NMR of **4c** and the chiral-GC of its derivative **9c** allowed us to determine the *ee* and *dr*. A complete sequence is shown in Scheme 1.4.4.3.



Scheme 1.4.4.3

*1.4.4.3. 2-Hydroxycyclohexanecarboxamide **3b** and its derivative **10b.***

Analysis of the racemic *cis*-2-hydroxycylohexanecarboxamide required the previous transformation into the ester derivative **10b** with trifuoroacetic anhydride (Scheme 1.4.4.4).



Scheme 1.4.4.4

Racemic *cis* mixture of **3b** was prepared from the corresponding mixture of *cis*-(±)-**7b**, which was obtained by $NaBH_4$ (4equiv.) reduction of the corresponding methyl 2-oxocyclohexanecarboxylate and further purification by flash chromatography. Then, *cis*-(±)-**7b** was treated with a saturated methanol solution of ammonia at 0 ºC during 24 h to give the desired compound after evaporation of the solvent.

1.4.5. GC analytical data for ee and dr determinations

In the following Tables 1.4.5.1-1.4.5.3, all data corresponding to the GC analyses using a chiral column are shown. Assignment of the configuration for every peak is included in most cases. Temperature program is given as follows, taking the program 90/10/1/115/20/180 as a model: to start with an initial temperature of 90 ºC / to maintain 90 ºC during 10 min / to increase the temperature with a slope of 1 ºC/min until to reach 115 ºC / to increase the temperature with a slope of 20 ºC/min until to reach the final 180 ºC. ChiraSil column was used in all cases, except for derivative **6b** when RT-β-DEXse column was used.

Table 1.4.5.1. GC analyses data for the KRED-catalyzed reduction of 2-oxocycloalkanecarbonitriles 1a-1c

Product	Derivative	T programme[a]	Retention time for derivative (min)	Retention time for substrate (min)
2a	**6a**	90/10/1/115/20/180	19.02 (1*S*,2*R*) and 20.38 (1*R*,2*S*) 24.30 (1*S*,2*S*) and 26.06 (1*R*,2*R*)	32.21 (ketone **1a**)
2b	**6b**	90/10/1/130/20/180	33.59 (1*S*,2*R*) and 34.32 (1*R*,2S) 35.60 (1*S*,2*S*) and 36.36 (1*R*,2*R*)	43.31 (ketone **1b**)
2c	**6c**	85/10/1/130/20/180	45.80 (*trans*)[b] and 47.63 (*trans*)[b] 46.56 (1*S*,2*S*) and 49.90 (1*R*,2*R*)	51.75 (ketone **1c**)

[a] GC programme: initial temperature (ºC) / time (min) / slope (ºC/min) / temp. (ºC) / slope (ºC/min) / final temp (ºC). [b] Absolute configuration of the *trans* derivative has not been determined.

Table 1.4.5.2. GC analyses data for the biotransformation of 2-oxocycloalkanecarbonitriles 1a-1c into 2-hydroxycycloalkanecarboxylic acids 4a-4c

Product	Derivative	T programme[a]	Retention time final compound (min)	Retention time substrate and hydroxynitriles (min)
4a	**8a**	80/10/1/115/20/180	16.43 (1*R*,2*R*) 17.16 (1*S*,2*S*) 23.80 (1*R*,2*S*) 24.79 (1*S*,2*R*)	34.41 (ketone **1a**) 15.14,16.68, 26.65,29.10
4b	**8b**	85/10/1/120/20/180	25.14 (*trans*)[b] 26.72 (*trans*)[b] 27.02 (1*R*,2*S*) 28.26 (1*S*,2*R*)	34.51 (ketone **1b**) 29.83,33.69
4c	**8c**	90/10/1/135/20/180	39.05 (*trans*)[b] 39.66 (*trans*)[b] 39.66 (1*R*,2*S*) 40.66 (1*S*,2*R*)	46.35 (ketone **1c**) 40.44,42.19

[a] GC programme: initial temperature (ºC) / time (min) / slope (ºC/min) / temp. (ºC) / slope (ºC/min) / final temp (ºC). [b] Absolute configuration of the *trans* derivative has not been determined.

Table 1.4.5.3. GC analysis data for the biotransformation of 1b into 2-hydroxycyclohexanecarboxamide 3b

Product	Derivative	T programme[a]	Retention time final compound (min)	Retention time substrate and intermediate (min)
3b	**10b**	85/10/1/115/20/1180	30.53 (1*R*,2*S*) and 33.84 (1*S*,2*R*)	34.51 (ketone **1b**) 29.83, 33.69

[a] GC programme: initial temperature (ºC) / time (min) / slope (ºC/min) / temp. (ºC) / slope (ºC/min) / final temp (ºC).

1.4.6. CD Support

In the CD support, detailed information about the experimental of this Chapter is included. A complete section of enzymatic screening, copy of GC chromatograms and NMR spectra for all the synthesized optically pure compounds will be found.

Chapter 2

Cooperation between metals and enzymes in aqueous medium: concurrent ruthenium-catalyzed nitrile hydration and asymmetric ketone bioreduction to obtain optically pure β-hydroxyamides

2.1. Bibliographic Background

Combination of biocatalysis with metal catalysis in cascade processes has fast emerged in recent years as a challenging and promising field for both academic and industrial sectors. Historically, chemists have combined reactions belonging to a particular field, as chemocatalysis (in organic solvents) or biocatalysis (mainly in water).[40]

Notably, the first proof of concept combining catalysts from different fields was reported by van Bekkum *et al*.[41] in 1980, demonstrating a successful combination of an enzymatic isomerisation with a heterogeneous catalytic hydrogenation. In detail, a D-glucose isomerase catalyzed the isomerization of D-glucose into D-fructose, while a preferential *in situ* platinum-catalyzed hydrogenation of D-fructose gave an enhanced amount of the corresponding sugar D-mannitol.

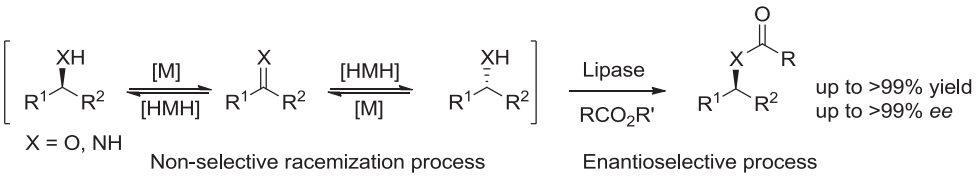
After this pioneering work, further breakthroughs were achieved by several groups in the 1990s. Thus, interesting dynamic kinetic resolution

[40] a) A. Bruggink, R. Schoevaart, T. Kieboom, *Org. Process Res. Dev*., **2003**, *7*, 622−640; b) O. Pamies, J. E. Bäckvall, *Chem. Rev*., **2003**, *103*, 3247−3262.

[41] M. Makkee, A. P. G. Kieboom, H. van Bekkum, J. A. Roels, *J. Chem. Soc., Chem. Commun.*, **1980**, 930–931.

(DKR) protocols were developed combining a chemocatalytic racemization and a hydrolase-catalysed process.[42]

Regarding this, metal-catalyzed racemization conducted in organic media has attracted attention as a valuable strategy for *in situ* racemization of chiral organic molecules such as alcohols and amines. Most of these racemizations are based on the reversible hydrogen transfer between the chiral secondary alcohol or its structurally analogue primary amine and the metal (Scheme 2.1.1). In addition, lipases, a kind of hydrolases catalytically active in organic media, have shown a great efficiency in the kinetic resolution of alcohols (through esterification or transesterification) and amines (through aminolysis reactions). Thanks to the compatibility between both catalytic systems and reaction conditions, the combination of both reactions has allowed to carry out a great number of highly effective DKR processes.



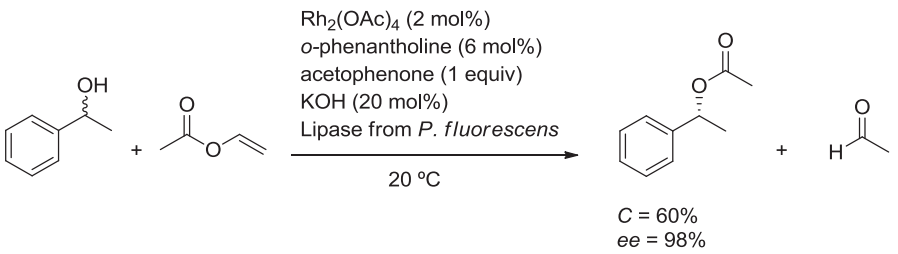
Scheme 2.1.1

Pioneering work on this field had been done by the group of Williams and Harris and, specially, by Bäckvall group. Williams, Harris *et al.*[43] demonstrated a proof of concept for the combination of a rhodium-catalyzed

[42] For some reviews see: a) O. Verho, J. E. Bäckvall, *J. Am. Chem. Soc*., **2015**, *137*, 3996−4009; b) F. Rebolledo, J. González-Sabín, V. Gotor, 'Enzymatic Dynamic Kinetic Resolution in Stereoselective Synthesis', In Stereoselective Synthesis of Drugs and Natural Products, 2V Set, 1st Ed. Edited by Vasyl Andrushko and Natalia Andrushko, JohnWiley & Sons, Inc., **2013**; Chapter 57, pp 1683–1711.

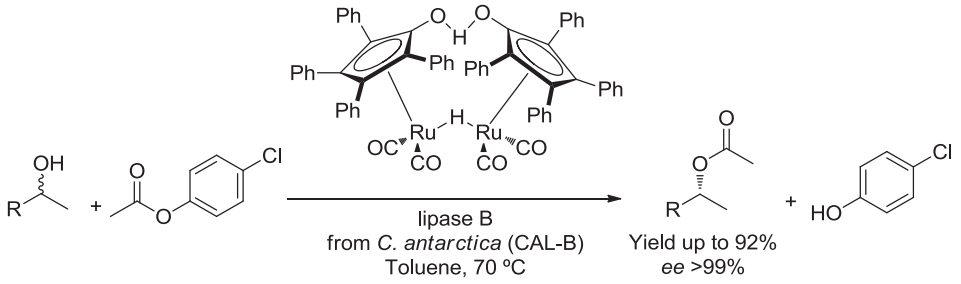
[43] P. M. Dinh, J. A. Howarth, A. R. Hudnott, J. M. J. Williams, W. Harris, *Tetrahedron Lett.*, **1996**, *37*, 7623–7626.

racemization of 1-phenylethanol with a lipase-catalyzed transesterification using vinyl acetate as the acyl donor (Scheme 2.1.2). In this first attempt, a moderate degree of conversion was registered and some additives had to be added in the reaction medium.



Scheme 2.1.2

More impressing results were obtained by Bäckvall *et al*.[44] by using the Shvó ruthenium complex as an efficient catalyst for the racemization of alcohols. This racemization reaction did not require any ketone or base as additives and it was elegantly combined with a lipase-catalyzed transesterification using *p*-chlorophenyl acetate as the acyl donor.



Scheme 2.1.3

[44] a) A. L. E. Larsson, B. A. Persson, J. E. Bäckvall, *Angew. Chem. Int. Ed.*, **1997**, *36*, 1211–1212; b) B. A. Persson, A. L. E. Larsson, M. Le Ray, J. E. Bäckvall, *J. Am. Chem. Soc.*, **1999**, *121*, 1645–1650.

The protocol was applied to a wide range of secondary alcohols, the corresponding esters being obtained in high yields (up to 92%) and excellent enantiomeric excesses (>99%) when reactions were performed at 70 ºC (Scheme 2.1.3). Later developments by the Bäckvall group led to the discovery of several ruthenium catalysts which enabled efficient DKRs of secondary alcohols at room temperature.[45]

Without any doubt, these contributions have been a solid starting point for the multi-catalytic methodologies that are being developed nowadays. However, examples of metal catalysts working concurrently with enzymes in aqueous media are still very scarce. Despite the elegance of many multi-catalytic reactions, there are several challenges that have to be met on the way to a wide application of this concept. In addition to overlapping substrate spectra, compatibility of the different catalysts and their respective reaction conditions is a key requirement for a successful cascade. This includes cross-inhibitions of metal cofactors, substrates and intermediates as well as demands for different solvents, reaction temperatures and pH-values.[46] Although the number of industrially used cascade reactions is steadily increasing, the intrinsic complexity of cascades has so far prevented a wide application of the concept.

In this section, we have selected those examples where multi-catalytic cascades imply the use of metal catalysts and ketoreductases. For a more general background, Kroutil *et al.*[16a] recently have published an extensive review on "Artificial Biocatalytic Linear Cascades for Preparation of Organic Molecules".

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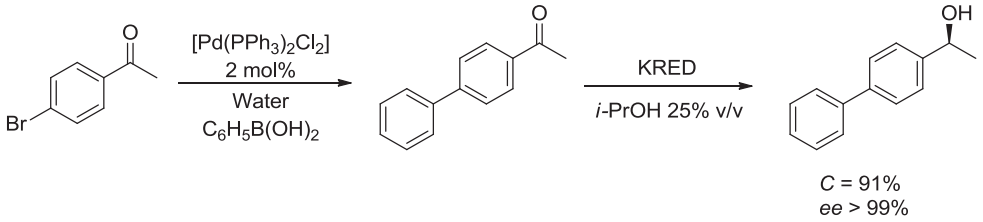
[45] B. Martin-Matute, M. Edin, K. Bogár, J. E. Bäckvall, *Angew. Chem. Int. Ed.*, **2004**, *43*, 6535–6539.

[46] S. Schmidt, K. Castiglione, R. Kourist, *Chem. Eur. J.*, **2018**, *24*, 1755–1768.

One of the first examples of this kind of processes was reported by the Gröger and Hummel group,[47] combining the Suzuki reaction with an asymmetric KRED-catalyzed ketone reduction in an aqueous reaction medium. In this process, a one-pot two-step strategy was developed based on the use of 1 equiv of boronic acid in the Suzuki reaction and addition of the enzyme directly to the reaction mixture once the first step was finished (Scheme 2.1.4). A detailed analysis of the inhibitory factors revealed that, quite surprisingly, the palladium complex did not exert a detrimental effect on the enzyme. This methodology can also be applied for the synthesis of chiral C_2-symmetric diols as monomers for chiral polymer synthesis.[48]

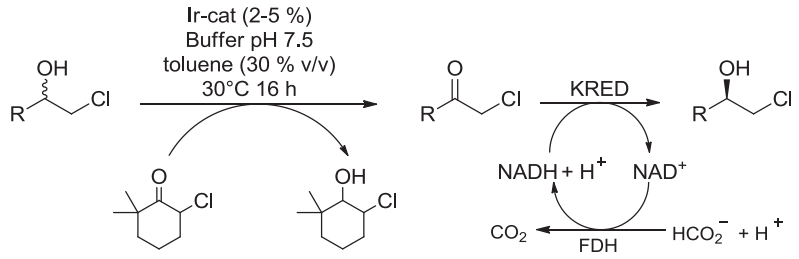


Scheme 2.1.4

In another contribution, an iridacycle was used in an oxidation-reduction deracemization sequence of chlorohydrins (Scheme 2.1.5).[49] This was achieved by coupling of a not stereoselective iridium-catalyzed oxidation to a stereoselective reduction catalyzed by an alcohol dehydrogenase. The metal and the catalyst were shown to be compatible thanks to the identification of orthogonal reagents for each step: a specific hydrogen acceptor for the oxidation in the form of 6-chloro-2,2-dimethylcyclohexanone and a specific hydride donor for the reduction in the

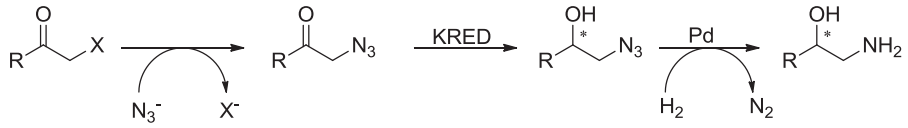
[47] E. Burda, W. Hummel, H. Gröger, *Angew. Chem. Int. Ed.,* **2008**, *47*, 9551–9554.
[48] E. Burda, W. Bauer, W. Hummel, H. Gröger, *ChemCatChem*, **2010**, *2*, 67–72.
[49] F. G. Mutti, A. Orthaber, J. H. Schrittwieser, J. G. de Vries, R. Pietschnig, W. Kroutil, *Chem. Commun.,* **2010**, *46*, 8046–8048.

form of formate. Since the iridacycle catalyst partially racemized the secondary enantioenriched alcohol, *ee* values were only moderate.



Scheme 2.1.5

Hollmann *et al.*[50] demonstrated that in a step-wise fashion, the metal catalyzed step may also follow the enzymatic reaction. They combined the azidolysis of aliphatic α-haloketones with an enzymatic ketoreduction and the subsequent hydrogenation of the azide group by lignin-stabilized palladium nanoparticles (Scheme 2.1.6). The application of this methodology led to the synthesis of the pharmacologically active (*S*)-tembamide.

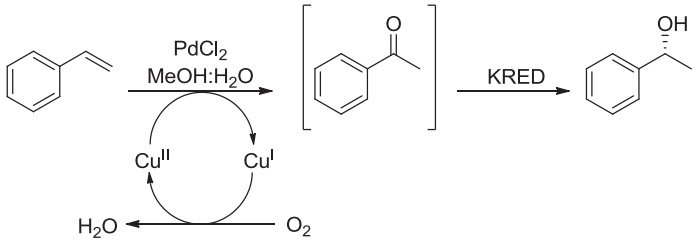


Scheme 2.1.6

While concurrent or sequential reactions appear as an elegant concept, sometimes they are not really feasible, when the component of the second

[50] J. H. Schrittwieser, F. Coccia, S. Kara, B. Grieschek, W. Kroutil, N. D'Alessandro, F. Hollmann, *Green Chem.*, **2013**, *15*, 3318–3331.

step is not compatible with the first reaction. A typical example is the inactivation or inhibition of enzymes by metal ions. Gröger *et al*.[51] elegantly demonstrated that this limitation can be solved by spatial separation of both reactions. They combined the Wacker oxidation of styrenes by a $CuCl/PdCl_2$ catalyst with a stereoselective reduction of the intermediate ketone (Scheme 2.1.7). A strong inhibition of the alcohol dehydrogenase by the copper salts was solved by the use of polidimethylsiloxane thimbles. In this way, being physically separated, both reactions could undergo in a sequential fashion, without inhibition of the enzymatic step.

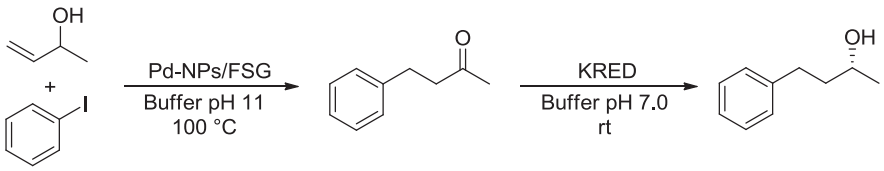


Scheme 2.1.7

Another successful example includes the assembly of the Heck cross-coupling with bioreduction.[52] The Pd-catalyzed reaction of aryl iodides with allylic alcohols was successfully linked to the enzymatic reduction of the intermediate ketones in aqueous medium in a one-pot sequential fashion and applied to the synthesis of (*R*)-rhododendrol (Scheme 2.1.8). The Heck reaction was catalyzed by Pd nanoparticles immobilized in fluorous silica gel under aerobic conditions in water at pH 11. Once the coupling was fisnished, the pH was adjusted to 7.0 and the alcohol dehydrogenase added. The methodology was applied to five substrates and the chiral alcohols were

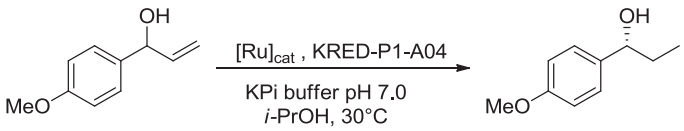
[51] H. Sato, W. Hummel, H. Gröger, *Angew. Chem. Int. Ed*., **2015**, *54*, 4488−4492.
[52] A. Boffi, S. Cacchi, P. Ceci, R. Cirilli, G. Fabrizi, A. Prastaro, S. Niembro, A. Shafir, A. Vallribera, *ChemCatChem*, **2011**, *3*, 347−353.

obtained in high yields (77-92%) and excellent enantiomeric excesses (>99%) in all the cases.



Scheme 2.1.8

Recently, our research group has reported a ruthenium-catalysed isomerization of allylic alcohols combined with an asymmetric bioreduction catalysed by ketoreductases in a one-pot concurrent process.[53] Both the metal catalyst and enzyme were able to coexist and work simultaneously from the beginning at 200 mM concentration, with no need of isolation of the intermediate ketone or compartmentalization of the metal catalyst. The final products were isolated with yields near to 85% and perfect optical purity (Scheme 2.1.9).

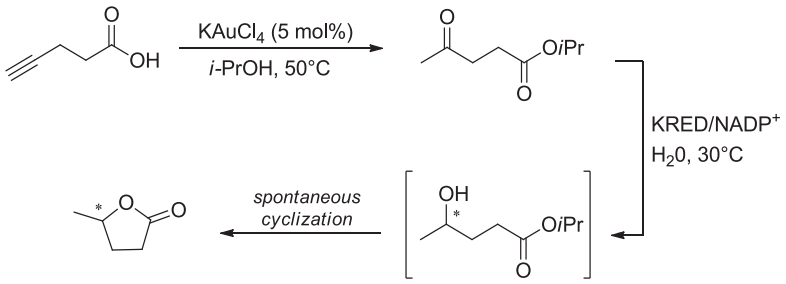


Scheme 2.1.9

In another contribution, a metal-catalyzed cycloisomerization of alkynols, γ-alkynoic acids, or alkynyl amides, was coupled with the enantioselective bioreduction of the transiently formed prochiral carbonyl

[53] N. Ríos-Lombardía, C. Vidal, E. Liardo, F. Morís, J. García-Álvarez, J. González-Sabín *Angew. Chem. Int. Ed.,* **2016**, *55*, 8691−8695.

compounds.[54] In an example, enantiopure γ-valerolactone was obtained starting from 4-pentynoic acid. Thus, the golden catalyst quantitatively converted the starting material into the corresponding isopropyl levulinate, which was reduced by the enzyme to give the transitorily γ-hydroxy ester. Spontaneous cyclization gave rise to enantiopure γ-valerolactone (Scheme 2.1.10).

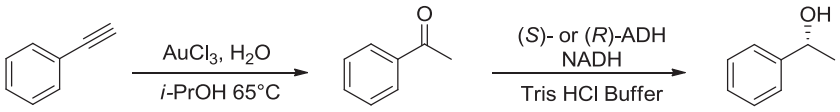


Scheme 2.1.10

Lastly, a gold catalyzed hydration reaction was combined by an enzymatic reduction for the production of (*S*)- and (*R*)-aryl alkyl alcohols.[55] The complete reaction mixture from the metal-catalyzed step could be used in the subsequent enzymatic reduction without any spatial separation. This was realized by applying *i*-PrOH as a solvent for the Au-catalyzed hydration; it served as auxiliary substrate for cofactor regeneration in the subsequent enzymatic reduction (Scheme 2.1.11).

[54] M. J. Rodríguez-Álvarez, N. Ríos-Lombardía, S. Schumacher, D. Pérez-Iglesias, F. Morís, V. Cadierno, J. García-Álvarez, J. González-Sabín, *ACS Catal*., **2017**, *7*, 7753−7759.

[55] P. Schaaf, V. Gojic, T. Bayer, F. Rudroff, M. Schnürch, M. D. Mihovilovic, *ChemCatChem*, **2018**, *10*, 920−924.

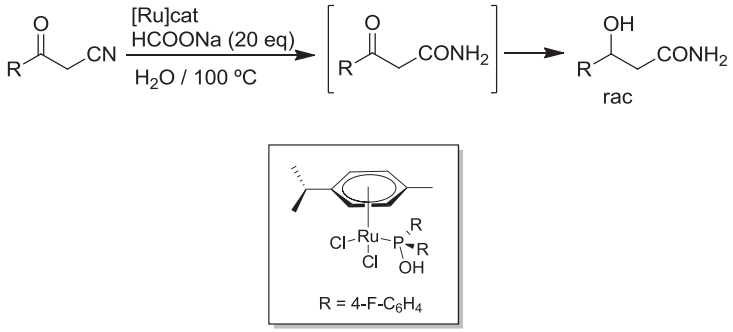


Scheme 2.1.11

On the basis of these findings, and taking into account the limitations we found in the biocatalytic route towards optically active β-hydroxyamides from β-ketonitriles (see Chapter 1, section 2), we have designed a new hybrid process for this transformation in order to overcome those drawbacks. In addition, it must be considered that the development of new approaches to obtain enantiopure β-hydroxyamides is an important area of synthetic organic chemistry since they are interesting building blocks and precursors of other highly valuable compounds.

The aforementioned hybrid process has consisted of the combination of a Ru-catalyst to achieve the hydration of the cyano group and a ketoreductase. The selection of the Ru-catalysts was based on the previous results reported by Cadierno *et al*.[56] These authors have carried out the hydration of a wide variety of nitriles in water at high temperatures using different ruthenium(II) complexes (Scheme 2.1.12). When the reaction was applied to β-ketonitriles and the hydration was performed in the presence of sodium formate, the reduction of the carbonyl group also took place and the corresponding β-hydroxyamides were obtained. This method was performed exclusively in a non-asymmetric version and required harsh reaction conditions (100 °C and excess of reactant).

[56] R. González-Fernández, P. Crochet, V. Cadierno, *Org. Lett*., **2016**, *18*, 6164−6167.



Scheme 2.1.12

The mechanism of this Ru-catalysed nitrile hydration has been fully investigated and confirmed by Cadierno *et al*.[57] It starts with the attack of the hydroxyl group of the P-donor ligand (P-OH) on $C_{nitrile}$ with simultaneous proton transfer to the chain of assisting water molecules. At the metallacyclic intermediate, the $O_{hydroxyl}$-$C_{nitrile}$ bond is formed and a proton ends up bonded to $N_{nitrile}$. Then, the five-membered metallacycle is opened through attack on the phosphorous atom of a solvent water molecule in an S_N2-type reaction. Finally, the formation of the amide ligand takes place through proton migration to the nitrile nitrogen atom (Figure 2.1.1).

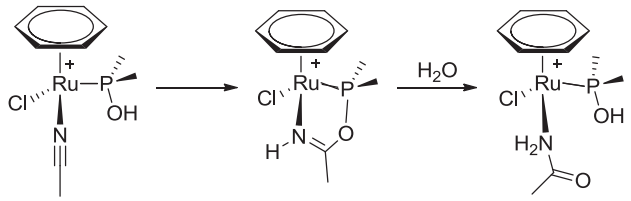


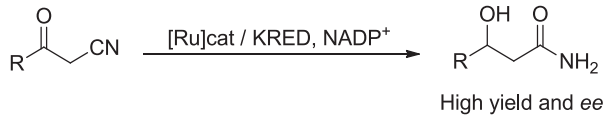
Figure 2.1.1

[57] E. Tomás-Mendivil, V. Cadierno, M. I. Menéndez, R. López, *Chem. Eur. J*., **2015**, *21*, 16874−16886.

On the light of this background, our purpose of using the dual catalytic system combining a ruthenium catalyzed nitrile hydration with an enzymatic ketone reduction should exploit the benefits of each catalysis area and bypass the drawbacks pointed out above.

2.2. Results and Discussion

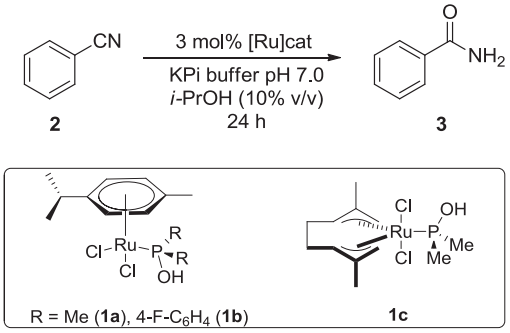
For this study, a wide range of β-ketonitriles has been selected: benzoylacetonitrile with different substituents in the aromatic moiety, 2-furoylacetonitrile, 2-thenoylacetonitrile and other more complex cyclic and acyclic β-ketonitriles bearing a chiral centre. The idea was the development of a multi-catalytic methodology in which we could take advantage of both the high enantioselectivity of the KRED in the reduction of the ketonic group and the activity of the Ru-based catalyst to hydrate the nitrile moiety (Scheme 2.2.1).



Scheme 2.2.1. Proposal of concurrent [Ru]cat and KRED catalyzed process

In the preliminary stage of the project, both reactions have been deeply studied in order to find the optimal conditions where a concurrent process could take place. In the previous contributions concerning the two independent processes, the ruthenium-catalyzed hydration was performed in pure water at 100 °C, while the enzymatic step required a more complex medium with phosphate buffer pH 7.0 (1.25 mM $MgSO_4$, 1.0 mM $NADP^+$), IPA (10% v/v), and lower temperatures. For this reason, firstly, two different Ru(II) complexes (**1a** and **1b**) and a Ru(IV) complex (**1c**) were tested for the hydration of benzonitrile –a typical substrate– under the reaction conditions required for the bioreduction. Results are collected in the following Table 2.2.1.

Table 2.2.1. Checking the hydration activity of [Ru] catalysts

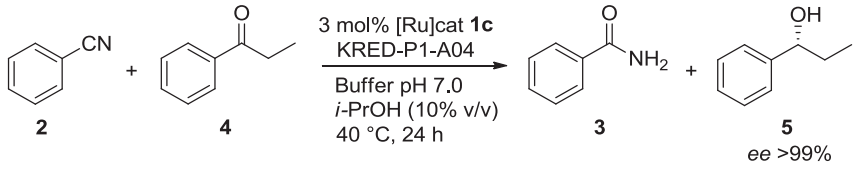


Substrate conc. (mM)	[Ru]cat	T (ºC)	*C*[a] (%)
330	**1a**	40	58
330	**1b**	40	10
330	**1c**	40	>95
200	**1c**	40	>95
100	**1c**	60	>95

[a] Degree of conversion was determined by ^{1}H-NMR analysis of the reaction crude.

The initial reactions were performed at 330 mM substrate concentration, as in the general procedure previously described for nitrile hydration. Both Ru(II) catalysts **1a** and **1b** gave low or moderate conversions, while **1c** appeared as a good candidate for this project, catalyzing the formation of the benzamide (**3**) with a conversion degree higher than 95%. In order to efficiently combine this process with the enzymatic step, other lower substrate concentrations were checked. Thus, carrying out the reaction at 200 mM and 40 ºC, or at 100 mM and 60 ºC, the Ru catalyst **1c** continued being active and *C* >95% were achieved. It is of note the efficiency of the catalyst at 100 mM, an appropriate concentration for enzymatic reactions but away from the commonly used with these Ru catalysts.

At this point, another crucial issue had to be considered: if both catalysts are compatible and do not experiment cross-inhibition or poisoning. To investigate these aspects, a reaction was performed in which the ruthenium complex and the KRED could simultaneously react with a mixture of two different substrates, as benzonitrile and propiophenone (each one typical of only one catalyst), in the previous buffer at 200 mM concentration (Scheme 2.2.2). Gratifyingly, after 24 h at 40 °C, both processes occurred with complete conversion and the reduction step did not show any loss of enantioselectivity, such as it was demonstrated by the high *ee* (>99%) measured for the resulting (*R*)-1-phenylpropan-1-ol (**5**).



Scheme 2.2.2. Compatibility test of [Ru]cat and KRED

This test demonstrated that a perfect coexistence of both catalysts was possible and that a tandem catalytic process could be envisaged. At this moment another important issue had to be analyzed: the level of orthogonality between the two processes that will take place with the β-ketonitriles under study (Figure 2.2.1). If the nitrile hydration and ketone reduction occur at the same time, two different intermediates could be present in the reaction medium, as β-ketoamide and β-hydroxynitrile. With this scenario in mind, the KRED could reduce not only the starting β-ketonitrile, but also the intermediate β-ketoamide. Thus, the optical purity of the final product β-hydroxyamide would depend on the stereoselectivity exhibited by KRED towards both species. In the same way, the ruthenium catalyst could hydrate the starting material β-ketonitrile, but also the intermediate β-hydroxynitrile.

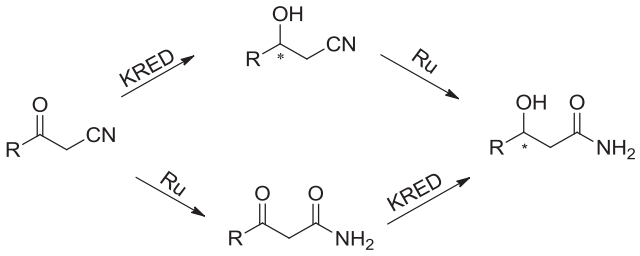
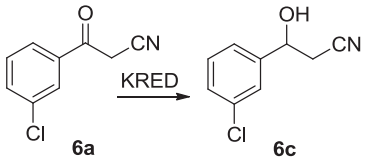


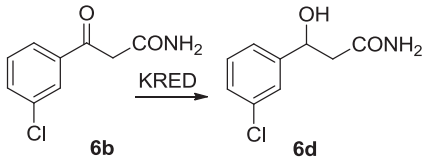
Figure 2.2.1. Routes towards β-hydroxyamides

With these premises in consideration, the bioreduction of both the selected β-ketonitriles and their corresponding β-ketoamides was studied. Reactions were carried out at 20 mM concentration and 40 °C. As result, KREDs exhibited high activity towards the β-ketonitriles, giving access to both enantiomers, while only in few cases good results were registered for the β-ketoamides. To exemplify this common trend observed for all substrates here investigated, the most representative results of the screening of *m*-chlorobenzoylacetonitrile (**6a**) and *m*-chlorobenzoylacetamide (**6b**) are collected in Table 2.2.2.

Table 2.2.2. Screening of the KRED-catalyzed reduction of 6a and 6b



6a **6c**

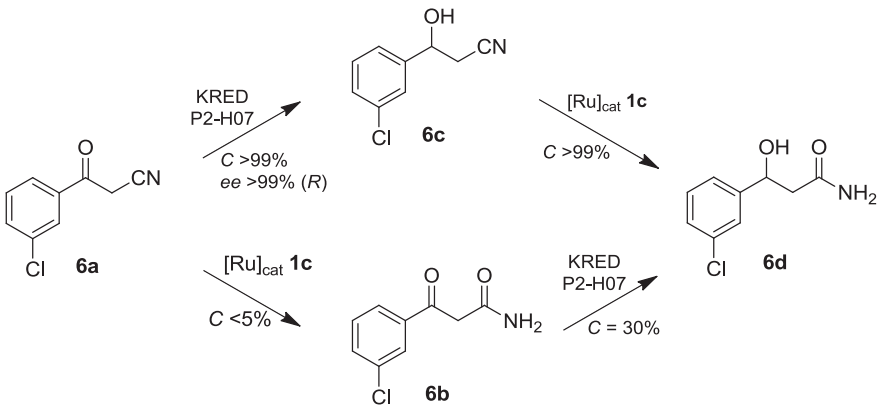


6b **6d**

KRED	***C* (%)**	***ee* of 6c (%)**	***C* (%)**	***ee* of 6d (%)**
P1-A04	48	>99 (*R*)	3	-
P1-B02	>99	78 (*S*)	>99	20 (*R*)
P1-B10	>99	28 (*R*)	>99	40 (*S*)
P1-B12	>99	90 (*R*)	>99	50 (*R*)
P1-H08	>99	92 (*S*)	8	-
P2-B02	>99	74 (*S*)	>99	92 (*R*)
P2-C02	>99	28 (*S*)	>99	>99 (*R*)
P2-C11	>99	>99 (*R*)	91	>99 (*R*)
P2-G03	>99	>99 (*R*)	>99	86 (*R*)
P2-H07	>99	>99 (*R*)	30	-
P3-H12	>99	>99 (*S*)	15	-

Simultaneously, the hydration reaction was also studied for the β-ketonitriles and β-hydroxynitriles, and at 40 °C many of the β-ketonitriles were recovered almost unalterated, while the β-hydroxynitriles were completely converted into the amide.

The following Scheme 2.2.3 collects a summary of the best results achieved when the corresponding *m*-chlorophenyl derivative was subjected to each individual process.



Scheme 2.2.3. Testing pathways for the tandem bioreduction-nitrile hydration of β-ketonitrile 6a

In view of the results before mentioned, one thinks that the conditions to pose a concurrent process are appropriate. Ru catalyst is highly chemoselective towards β-hydroxynitriles and KRED shows higher activity towards β-ketonitriles than β-ketoamides. Thus, in the concurrent process, KRED should act first and then, Ru catalyst should hydrate the resulting optically active β-hydroxynitrile.

Concurrent KRED-[Ru]cat process

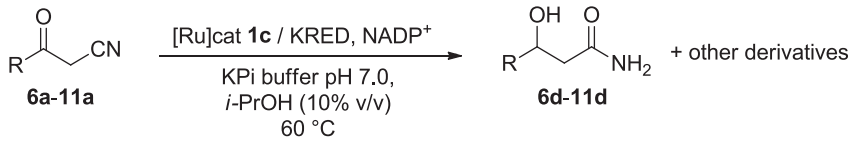
At this point, a concurrent reaction was essayed on β-ketonitrile **6a** (200 mM) by the combined action of KRED-P2-H07 and Ru catalyst **1c** (3 mol%) at 40 ºC, working together from the beginning. After 24 h, all the starting **6a** disappeared but a 79:21 mixture of enantiomerically pure (*R*)-**6c** and (*R*)-**6d** was obtained, thus showing a high efficiency of the enzyme and a loss of activity of the Ru catalyst during the process. Because the hydration Ru activity is improved at higher temperatures (60-100 ºC),[56] we decided to test the process at 60 °C. Under these conditions, the bioreduction step was studied and no significant changes were detected. Regarding the hydration step, both β-ketonitrile **6a** and β-hydroxynitrile **6c**

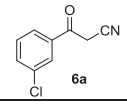
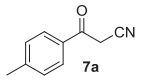
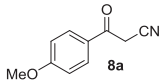
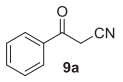
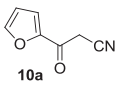
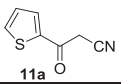
were fully converted. So the previous cascade reaction was carried out with substrate **6a**, KRED-P2-H07, and Ru (3 mol%) at 60 °C. However, although all the starting material disappeared, a mixture consisting of ketoamide (29%), hydroxyamide (45%), and hydroxynitrile (26%) was detected. The presence of ketonic and nitrile species in the mixture continue being a consequence of the low activity of the enzyme towards the intermediate ketoamide as well as of a decreasing of activity of the Ru catalyst. To confirm this hypothesis, an incubation test of the Ru catalyst was carried out. The catalyst was allowed to stay in the reaction mixture and after 4 h the hydroxynitrile **6c** was added. After 24 h at 60 ºC, only 22% of hydroxyamide was obtained. This test confirmed that the activity of the catalyst is very high during the first hours of the reaction and a dramatic decrease in its efficiency is registered after extended reaction times. For this reason, the choice of the right ketoreductase was crucial. The enzyme should be very active towards both keto-species and, obviously, exhibit the same enantiopreference.

The concurrent approach was then designed from **6a** by using a KRED (P2-C11) that displayed matched stereopreference towards the β-ketonitrile/β-ketoamide **6a**/**6b** pair, 6 mol% of Ru complex, 60 °C and 100 mM substrate concentration. Gratifyingly, under these reaction conditions the tandem hydration-reduction process took place and the β-hydroxyamide **6d** was obtained with >99% conversion and >99% *ee*. Encouraged by the success of this dual catalytic system, we extended the methodology to β-ketonitriles **7a**-**11a** (Table 2.2.3). Thus, by using the appropriate KRED and a higher catalyst loading (6 mol%), it was possible to reach the (*R*)-enantiomer of most β-hydroxyamides (**6d**-**10d**) as well as (*S*)-**11d** in enantiopure form and with high degrees of conversion. Although it was challenging to find KREDs meeting the requirements to prepare the hydroxyamide antipodes, these examples are a valuable proof of concept of the successful simultaneous action of enzymes and metal catalysts. Moreover, it is worth highlighting the excellent outcome of the process in

several cases, which allowed us to isolate the resulting β-hydroxyamides with very high yields (92-94% for compounds **6d**, **7d**, and **9d**).

Table 2.2.3. Orthogonal tandem protocol

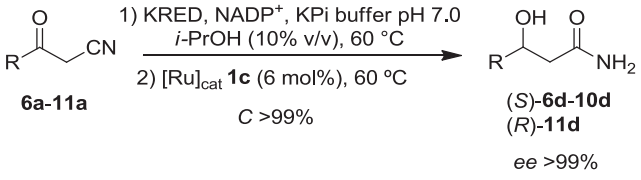


Substrate	KRED	t (h)	Keto-		Hydroxy-		6d-11d
			nitrile[a]	amide[a]	nitrile[a]	amide[a]	*ee* (%)
 6a	P2-C11	16	-	-	-	>99	>99 (*R*)
 7a	P2-C11	16	3	-	-	97	>99 (*R*)
 8a	P2-C11	18	-	9	17	74	>99 (*R*)
 9a	P2-G03	18	-	-	-	>99	>99 (*R*)
	L.kefir	24	-	11	2	87	>99 (*R*)
 10a	P2-G03	18	-	-	5	95	>99 (*R*)
 11a	P1-B10	18	-	7	10	83	>99 (*S*)

[a] Percentage measured in the ^{1}H-NMR spectrum of the reaction crude.

Sequential KRED-[Ru]cat process

For those cases with either mismatched KRED stereoselectivity towards the β-ketonitrile/β-ketoamide pair or incomplete conversion, a practical solution would be a sequential one-pot two-step process. Although less elegant than the concurrent one with both steps running simultaneously, the stepwise approach offers essentially the same advantages and low ecological footprint. Actually, the only setting change would be the addition of the ruthenium complex **1c** once the bioreduction was completed. Concerning the order of the steps, this was established in view of the superior activity displayed by KREDs towards β-ketonitriles respect to β-ketoamides. Accordingly, the reduction of β-ketonitriles **6a-11a** was accomplished in the conditions of the previous screening by choosing stereoselective KREDs (that is, P3-H12 or *R. ruber* for **6a**, P3-B03 for **7a**, P3-H12 for **8a**, P1-H08 for **9a**, P1-B10 or *R. ruber* for **10a**, and P2-G03 or *L. kefir* for **11a**). Thus, once the enzymatic step was complete at 60 ºC (18-24 h), complex **1c** (6 mol%) was added and the reaction mixture stirred overnight at the same temperature. As a result, the (*S*)-enantiomer of **6d-10d** and (*R*)-**11d** were isolated in essentially pure form (*C* >99%) and *ee* >99% (Scheme 2.2.4).



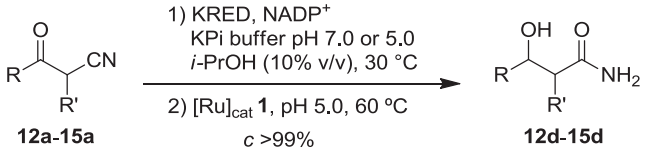
Scheme 2.2.4. Chemoenzymatic one-pot synthesis of optically active 6d-11d in a sequential mode

Additionally, some cyclic and acyclic β-ketonitriles bearing a stereogenic carbon (**12a-15a**) were also considered (see Table 2.2.4). The main feature of these compounds relies on the lability of their chiral center

which is prone to racemize in the reaction medium thus triggering a dynamic reductive kinetic resolution (DYRKR). This fact along with the proven poor activity of the KREDs towards the corresponding α-substituted β-ketoamides **12b-15b**, precluded the option of a concurrent process and turned our attention towards the sequential protocol. The first step –KRED reduction– was carried out at 30 ºC by applying the conditions already reported for **13a-15a** (see Chapter 1). Moreover, it was also shown that the intermediate 3-hydroxy-2-phenylbutanenitrile (**12c**) undergoes epimerization of C-2 under heating at 60 ºC[58], so the bioreduction step of **12a** should also be run at lower temperature. With these premises, the reduction of **12a**, **14a**, and **15a** were carried out with the selected KRED at pH 7.0 and 30 ºC and quantitatively afforded the *syn*-**12c** and *cis*-**14c**-**15c** stereoisomers in >99:<1 *dr* and >99% *ee*. To obtain the analogous **13c** with high diastereoselectivity, the bioreduction of **13a** was conducted at pH 5.0. Finally, after proving that the Ru complex **1c** catalyzed the hydration of **13c** more efficiently at pH 5.0 than 7.0, the Ru-catalyzed second step was carried out at this pH value in all cases. No epimerization of any chiral center was observed in the second step, being the optically active α-substituted β-hydroxyamides **12d-15d** isolated with the same *dr* and *ee* than the corresponding β-hydroxynitrile intermediate. Table 2.2.4 collects some representative examples.

[58] When a sample of diastereomerically pure *syn*-**12c** was dissolved in the aqueous medium and heated at 60°C during 10 h, the isolated compound consited of a diastereomeric *syn*:*anti* 95:5 mixture.

Table 2.2.4. A sequential one-pot dynamic reductive kinetic resolution – hydration of α-substituted β-ketonitriles 12a-15a



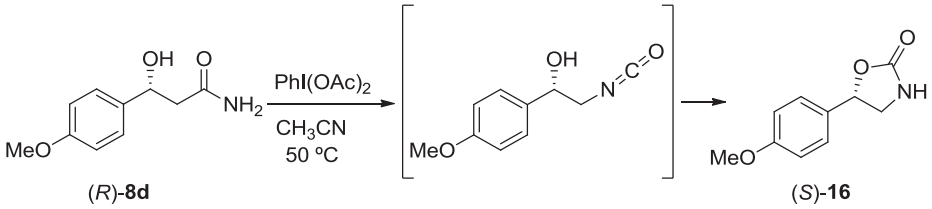
Ent.	Subst	R	R'	KRED	Product	*dr*	*ee* (%)
1	**12a**	Me	Ph	P3-H12	(2*R*,3*S*)-**12d**	>99:<1	>99
2	**13a**	-$(CH_2)_3$-		P2-D11	(1*S*,2*S*)-**13d**	15:85	>99
3	**13a**	-$(CH_2)_3$-		P1-B12	(1*S*,2*R*)-**13d**	80:20	>99
4	**14a**	-$(CH_2)_4$-		P1-B12	(1*S*,2*R*)-**14d**	>99:<1	>99
5	**14a**	-$(CH_2)_4$-		P1-A04	(1*R*,2*S*)-**14d**	>99:<1	>99
6	**15a**	-$(CH_2)_5$-		P1-A04	(1*R*,2*S*)-**15d**	>99:<1	>99

As a result, this multi-catalytic methodology gave access to optically pure β-hydroxyamides with excellent yields, thus improving the results of the previous contributions. In the concurrent process, the enzyme and the metal worked together from the beginning and for this reason a KRED which exhibited the same enantioselectivity towards the pair ketonitrile/ketoamide was required. When this case did not subsist, a sequential process was applied. Here the bioreduction was followed by the metal step by a simple addition of the Ru catalyst in the reaction medium. The final compounds were isolated with excellent yields and, in many cases, without any need of further purification.

Chemoenzymatic syntheses of (*S*)-tembamide and (*R*)-aegeline

Finally, to demonstrate the utility of the final compounds as building blocks for pharmaceuticals, the synthesis of two naturally occurring alkaloids as (*S*)-tembamide (active against HIV) [59] and (*R*)-aegeline (hypoglycemic activity) [60] was carried out. Starting from 3-(4-methoxyphenyl)-3-oxopropanenitrile (**8a**), the sequential one-pot reaction using KRED-P2-C11 or KRED-P3-H12 and complex **1c** (6 mol%) gave access to the key enantiopure precursors (*R*)-**8d** and (*S*)-**8d**, respectively, in very high yields (93%).

The Hofmann rearrangement of (*R*)-**8d** with iodobenzene diacetate in acetonitrile at 50 °C yielded the oxazolidinone (*S*)-**16**, which was formed as a consequence of the intramolecular attack of the hydroxyl group to the isocyanate intemediate (Scheme 2.2.5).

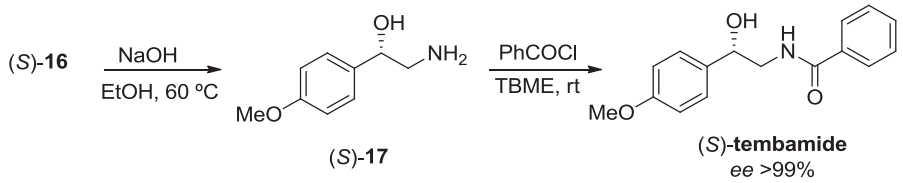


(*R*)-**8d** (*S*)-**16**

Scheme 2.2.5. Hofmann rearrangement of (*R*)-8d

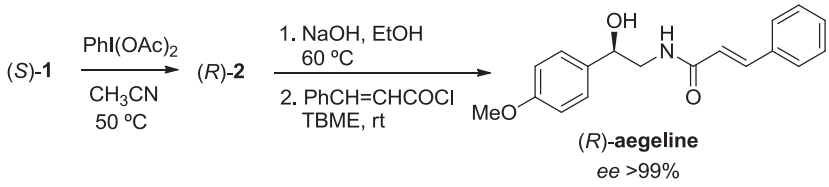
Then a basic hydrolysis of (*S*)-**16** yielded the corresponding β-amino alcohol (*S*)-**17**, which was treated with benzyl chloride to finally obtain the (*S*)-tembamide with >99% *ee* and 77% overall yield, as shown in Scheme 2.2.6.

[59] M. J. Cheng, K. H. Lee, I. L. Tsai, I. S. Chen, *Bioorg. Med. Chem*., **2005**, *13*, 5915−5920.
[60] A. Shoeb, R. S. Kapil, S. P. Popli, *Phytochemistry,* **1973**, *12*, 2071−2072.



Scheme 2.2.6. Synthesis of (*S*)-tembamide

In the same way, by applying the same protocol to (*S*)-**8d** and using *trans*-cinnamoyl chloride in the last step, (*R*)-aegeline was obtained with >99% *ee* and 73 % overall yield (Scheme 2.2.7).



Scheme 2.2.7. Synthesis of (*R*)-aegeline

The *ee* of both compounds tembamide and aegeline and, thus, the absence of racemization in the processes, was determined by chiral-HPLC analysis. In addition, the absolute configuration for each compound was corroborated by comparison of the sign of the optical rotation with published data (for tembamide[50] and for aegeline[61]).

[61] N. A. Cortez, G. Aguirre, M. Parra-Hake, R. Somanathan, *Tetrahedron: Asymmetry*, **2013**, 24, 1297−1302.

2.3. Conclusions

In summary, a new multi-catalytic methodology for the synthesis of optically pure β-hydroxyamides was developed. The process consisted in a concurrent-tandem cascade in aqueous medium, where a Ru(IV) catalyst and a ketoreductase were able to coexist from the beginning of the reaction. For more complex β-ketonitriles bearing a chiral centre a sequential approach was applied. In this case, all the β-hydroxyamides were isolated with perfect diastereomeric ratios, thanks to the efficient dynamic kinetic resolution displayed in the bioreduction step.

This work represents another contribution of the compatibility between biocatalysis and metal-catalysis without any need of compartmentalization, immobilization or dilution.

Furthermore, to underline the importance of β-hydroxyamides as building blocks in medicinal chemistry, two interesting natural occurring alkaloids as (*S*)-tembamide (active against HIV) and (*R*)-aegeline (hypoglycemic activity) have been synthesized in very high yields and excellent enantiomeric excesses.

2.4. Experimental Section

2.4.1. General Information

Enzymes

Codex® KRED Screening Kit was purchased from Codexis.

Substrates

β-Ketonitriles **6a-12a** are commercially available. Racemic 2-oxocycloalkanecarbonitriles **13a-15a** were prepared as previously described.[19,31] β-Ketoamides **6b-15b** were obtained by Ru-catalysed hydration. β-Hydroxynitriles **6c-15c** and β-hydroxyamides **6d-15d** were prepared by conventional reduction of the corresponding ketones with $NaBH_4$ (4.0 equiv) in MeOH (0.10 M).

2.4.2. General procedures

2.4.2.1. General procedure for the Ru-catalyzed hydration of benzonitrile

In a 2.0 mL eppendorf tube, benzonitrile (100 mM), *i*-PrOH (10% v/v), and 125 mM phosphate buffer (also containing 1.25 mM in $MgSO_4$) pH 7.0 were added. Complex **1c** (3.0-6.0 mol%) was then added at room temperature. The resulting reaction mixture was shaken at 250 rpm and 60 °C overnight. After this time, the mixture 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 identity of the resulted compound was confirmed by ^{1}H-NMR.

2.4.2.2. General procedure for the enzymatic reduction of β-ketonitriles and β-ketoamides

In a 2.0 mL eppendorf tube, KRED (2.0 mg), ketone (20 mM), *i*-PrOH (190 μL) were added to 900 μL of 125 mM phosphate buffer, pH 7.0 or pH 5.0. This buffer also contains $MgSO_4$ (1.25 mM) and the cofactor $NADP^+$

(1.0 mM). The resulting reaction mixture was shaken at 250 rpm and 30 °C or 60 °C for 24 h. After this time, a 10 μL aliquot was removed for the determination of the degree of conversion by HPLC or GC analysis.Then, the mixture 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 diastereomeric ratio (if applicable) and enantiomeric excess of the corresponding product was determined by chiral HPLC or GC.

2.4.3. Hydration-bioreduction concurrent process: general procedure and characterisation of optically active compounds

In a 2.0 mL eppendorf tube, the corresponding nitrile (100 mM), KRED (the same weight as nitrile), complex **1** (3.0 mol% for **6a**, 6.0 mol% for **7a-11a**), *i*-PrOH (10% v/v) and 125 mM phosphate buffer (also containing 1.25 mM $MgSO_4$ and 1.0 mM $NADP^+$) pH 7.0 were added. The resulting reaction mixture was shaken at 250 rpm and 60 °C for 16-24 h. After this time, the mixture was extracted with ethyl acetate (2 × 500 μL), centrifugated (90 s, 13000 rpm), and the combined organic layers were finally dried over Na_2SO_4. The degree of conversion was measured by ^{1}H-NMR. The enantiomeric excess of the corresponding product was determined by chiral HPLC or GC.

For a better overview, a selection of compounds included in this section is reported in the following Figure 2.4.3.1.

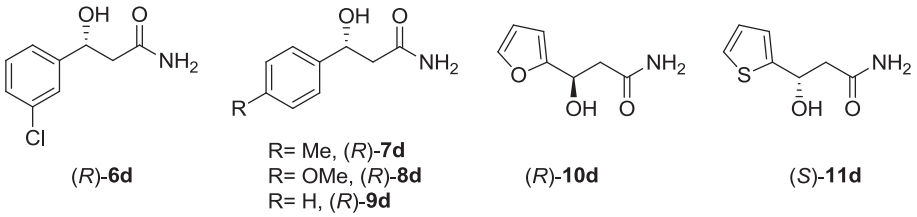


Fig. 2.4.3.1. A selection of synthesized compounds

2.4.3.1. (R)-3-(3-Chlorophenyl)-3-hydroxypropanamide **6d**

The typical procedure was applied to 10.0 mg (56 μmol) of the starting material **6a** using KRED-P2-C11 and 0.6 mg (3.0 mol%) of complex **1**. After 24 h conversion was complete but 3% of intermediate β-hydroxynitrile was detected. The crude β-hydroxyamide was purified by flash column chromatography (AcOEt) to give (*R*)-**6d** with 90% yield (9.9 mg); $[\alpha]_D^{19}$ +16.9 (*c* 0.52, EtOH), *ee* >99%, mp 99-100 °C. ^{1}H NMR (300 MHz, CD_3OD) δ (ppm): 2.52 (dd, *J* 14.4 Hz, *J* 5.1 Hz, 1H), 2.62 (dd, *J* 14.4 Hz, *J* 8.4 Hz, 1H), 5.06 (dd, *J* 8.4 Hz, *J* 5.1 Hz, 1H), 7.23-7.43 (m, 4H); ^{13}C-NMR (75.5 MHz, CD_3OD) δ (ppm): 45.98 (CH_2), 71.22 (CH), 125.32 (CH), 126.99 (CH), 128.47 (CH), 130.92 (CH), 135.27 (C), 147.94 (C), 176.04 (C=O). The spectroscopic data are in good agreement with the reported literature.[56]

2.4.3.2. (R)-3-Hydroxy-3-(4-methylphenyl)propanamide **7d**

The typical procedure was applied to 10.0 mg (63 μmol) of the starting material **7a** using KRED-P2-C11 and 1.5 mg (6.0 mol%) of complex **1**. Reaction was allowed to react during 16 h (97% of conversion). Then, the crude β-hydroxyamide was purified by flash column chromatography (Hexane:AcOEt 3:1, then AcOEt) to give (*R*)-**7d** with 86% yield (9.5 mg); $[\alpha]_D^{20}$ +34.5 (*c* 0.45, EtOH), *ee* >99%, mp 109-110 °C. ^{1}H NMR (300 MHz, CD_3OD) δ (ppm): 2.31 (s, 3H), 2.51 (dd, *J* 14.4 Hz, *J* 5.0 Hz,1H), 2.63 (dd, *J* 14.4 Hz, *J* 8.7 Hz, 1H), 5.03 (dd, *J* 8.7 Hz, *J* 5.0 Hz, 1H), 7.15 (d, *J* 8.1 Hz, 2H), 7.27 (d, *J* 8.1 Hz, 2H); ^{13}C-NMR (75.5 MHz, CD_3OD) δ (ppm): 21.14 (CH_3), 46.03 (CH_2), 71.80 (CH), 126.87 (CH), 129.96 (CH), 138.25 (C), 142.27 (C), 176.51 (C=O). The spectroscopic data are in good agreement with the reported literature.[56]

2.4.3.3. (R)-3-Hydroxy-3-(4-methoxyphenyl)propanamide **8d**

The typical procedure was applied to 15.0 mg (83 μmol) of the starting material **8a** using KRED-P2-C11 and 1.9 mg (6.0 mol%) of

complex **1**. After 18 h the degree of conversion was measured by ^{1}H-NMR and the conversion into the desired product was 74%, with 17% and 9% of hydroxynitrile and ketoamide, respectively. The crude β-hydroxyamide was purified by flash column chromatography (Hexane:AcOEt 3:1, then AcOEt) to give (*R*)-**8d** with 69% yield (11.8 mg); $[\alpha]_D^{19}$ +27.7 (*c* 0.55, EtOH), *ee* >99%, mp 162-163 °C. ^{1}H NMR (300 MHz, CD_3OD) δ (ppm): 2.50 (dd, *J* 14.2 Hz, *J* 5.0 Hz, 1H), 2.64 (dd, *J* 14.2 Hz, *J* 8.6 Hz, 1H), 3.77 (s, 3H), 5.01 (dd, *J* 8.6 Hz, *J* 5.0 Hz, 1H), 6.89 (d, *J* 8.7 Hz, 2H), 7.31 (d, *J* 8.7 Hz, 2H); ^{13}C-NMR (75.5 MHz, CD_3OD) δ (ppm): 46.01 (CH_2), 55.67 (OCH_3), 71.59 (CH), 114.72 (CH), 128.16 (CH), 137.28 (C), 160.60 (C), 176.52 (C=O). The spectroscopic data are in good agreement with the reported literature.[56]

2.4.3.4. (R)-3-Hydroxy-3-phenylpropanamide **9d**

The typical procedure was applied to 10.0 mg (69 μmol) of the starting material **9a** using KRED-P2-G03 and 1.6 mg (6.0 mol%) of complex **1**. After 18 h conversion was complete. The crude β-hydroxyamide was purified by flash column chromatography (AcOEt) to give (*R*)-**9d** with 91% yield (10 mg); $[\alpha]_D^{20}$ +32.7 (*c* 0.40, EtOH), *ee* >99%, mp 106-107 °C. ^{1}H NMR (300 MHz, CD_3OD) δ (ppm): 2.53 (dd, *J* 14.4 Hz, *J* 4.8 Hz, 1H), 2.64 (dd, *J* 14.4 Hz, *J* 8.7 Hz, 1H), 5.07 (dd, *J* 8.7 Hz, *J* 4.8 Hz, 1H), 7.22-7.41 (m, 5H); ^{13}C-NMR (75.5 MHz, CD_3OD) δ (ppm): 46.08 (CH_2), 71.93 (CH), 126.90 (CH), 128.51 (CH), 129.37 (CH), 145.35 (C), 176.43 (C=O). The spectroscopic data are in good agreement with the reported literature.[56]

2.4.3.5. (R)-3-(2-Furyl)-3-hydroxypropanamide **10d**

The typical procedure was applied to 10.0 mg (74 μmol) of the starting material **10a** using KRED-P2-G03 and 1.7 mg (6.0 mol%) of complex **1**. After 18 h the degree of conversion was measured by ^{1}H-NMR and the desired product was converted in 95%, with 5% of β-hydroxynitrile left. The crude β-hydroxyamide was purified by flash column chromatography (Hexane:AcOEt 3:1, then AcOEt) to give (*R*)-**10d** with

82% yield (9.0 mg); $[\alpha]_D^{20}$ +30.1 (*c* 0.40, EtOH), *ee* >99%, mp 102-103 °C. ^{1}H NMR (300 MHz, CD_3OD) δ (ppm): 2.64-2.77 (m, 2H), 5.08 (dd, *J* 7.8 Hz, *J* 6.0 Hz, 1H), 6.30 (dt, *J* 3.3 Hz (d), *J* 0.8 Hz (t), 1H), 6.35 (dd, *J* 3.3 Hz, *J* 1.8 Hz, 1H), 7.44 (dd, *J* 1.8 Hz, *J* 0.8 Hz, 1H); ^{13}C-NMR (75.5 MHz, CD_3OD) δ (ppm): 42.50 (CH_2), 65.30 (CH), 106.94 (CH), 111.15 (CH), 143.23 (CH), 157.39 (C), 175.86 (C=O). The spectroscopic data are in good agreement with the reported literature.[56]

2.4.3.6. (S)-3-Hydroxy-3-(2-thienyl)propanamide **11d**

The typical procedure was applied to 10.0 mg (66 μmol) of the starting material **11a** using KRED-P1-B10 and 1.5 mg (6.0 mol%) of complex **1**. After 18 h the degree of conversion was measured by ^{1}H-NMR and the desired product was converted in 83%, with 10% and 7% of β-hydroxynitrile and ketoamide, respectively. The crude β-hydroxyamide was purified by flash column chromatography (Hexane:AcOEt 3:1, then AcOEt) to give (*S*)-**11d** with 77% yield (8.5 mg); $[\alpha]_D^{20}$ –11.7 (*c* 0.50, EtOH), *ee* >99%, mp 83-84 °C. ^{1}H NMR (300 MHz, CD_3OD) δ (ppm): 2.66 (dd, *J* 14.4 Hz, *J* 5.4 Hz, 1H), 2.74 (dd, *J* 14.4 Hz, *J* 8.3 Hz, 1H), 5.32 (dd, *J* 8.3 Hz, *J* 5.4 Hz, 1H), 6.96 (dd, *J* 3.6 Hz, *J* 5.1 Hz, 1H) 7.00-7.02 (m, 1H), 7.30 (dd, *J* 5.4 Hz, *J* 1.5 Hz, 1H); ^{13}C-NMR (75.5 MHz, CD_3OD) δ (ppm): 46.19 (CH_2), 67.83 (CH), 124.56 (CH), 125.38 (CH), 127.53 (CH), 149.32 (C), 175.85 (C=O). The spectroscopic data are in good agreement with the reported literature.[56]

2.4.4. Bioreduction-hydration sequential process: general procedure and characterization of optically active compounds

2.4.4.1.General procedure for β-ketonitriles ***6a-11a***

In a 2.0 mL eppendorf tube, the corresponding ketonitrile (100 mM), KRED (the same weight as nitrile), *i*-PrOH (10% v/v) and 125 mM phosphate buffer pH 7.0 (also containing 1.25 mM $MgSO_4$, 1.0 mM $NADP^+$) were added. The resulting reaction mixture was shaken at 250 rpm

and 60 °C for 24 h. After this time, complex **1** (6.0 mol %) was added and the reaction was left stirring overnight at 60°C. Then, the mixture was extracted with ethyl acetate (2 × 500 μL), the organic layers were separated by centrifugation (90 s, 13000 rpm), combined, and finally dried over Na2SO4. The degree of conversion was measured by ^{1}H-NMR and it was complete in all cases. The enantiomeric excess of the corresponding product was determined by chiral HPLC or GC.

2.4.4.1.1. (S)-3-(3-Chlorophenyl)-3-hydroxypropanamide **6d**

The typical procedure was followed using 15.0 mg (84 μmol) of the starting material and KRED-P3-H12 as the catalyst in the reduction step. The crude product was submitted to flash column chromatography (AcOEt) to give pure (*S*)-**6d** with 94% yield (15.0 mg); $[\alpha]_D^{19}$ –17.8 (*c* 0.50, EtOH), *ee* >99%, mp 99-100 °C.

*2.4.4.1.2. (S)-3-Hydroxy-3-(4-methylphenyl)propanamide **7d***

The typical procedure was followed using 20.0 mg (126 μmol) of the starting material and KRED-P3-B03 as the catalyst in the reduction step. The crude product was purified by flash column chromatography (AcOEt) to give (*S*)-**7d** with 90% yield (19 mg); $[\alpha]_D^{18}$ –34.3 (*c* 0.70, EtOH), *ee* >99%, mp 109-110 °C.

2.4.4.1.3. (S)-3-Hydroxy-3-(4-methoxyphenyl)propanamide **8d**

The typical procedure was followed using 50.0 mg (285 μmol) of the starting material and KRED-P3-H12 as the catalyst in the reduction step. The crude product was purified by flash column chromatography (AcOEt) to give (*S*)-**8d** with 93% yield (52.0 mg); $[\alpha]_D^{19}$ –28.3 (*c* 0.70, EtOH), *ee* >99%, mp 162-163 °C.

2.4.4.1.4. (S)-3-Hydroxy-3-phenylpropanamide **9d**

The typical procedure was followed using 15.0 mg (103 μmol) of the starting material and KRED-P1-H08 as the catalyst in the reduction step. The product (*S*)-**9d** was isolated in a pure state with 94% yield (16.0 mg); $[\alpha]_D^{19}$ –33.9 (*c* 0.58, EtOH), *ee* >99%, mp 106-107 °C.

2.4.4.1.5. (S)-3-(2-Furyl)-3-hydroxypropanamide **10d**

The typical procedure was followed using 15.0 mg (111 μmol) of the starting material and KRED-P1-B10 as the catalyst in the reduction step. After the typical work-up, ^{1}H-NMR showed complete conversion and no need of further purification. The product (*S*)-**10d** was isolated with 94% yield (16.0 mg); $[\alpha]_D^{20}$ –29.3 (*c* 0.45, EtOH), *ee* >99%, mp 102-103 °C.

2.4.4.1.6. (R)-3-Hydroxy-3-(2-thienyl)propanamide **11d**

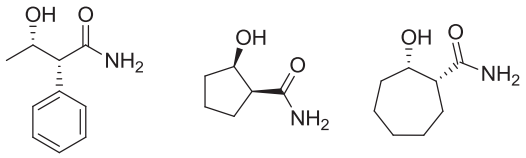
The typical procedure was followed using 15.0 mg (99 μmol) of the starting material and KRED-P2-G03 as the catalyst in the reduction step. The crude product was purified by flash column chromatography (AcOEt) to give (*S*)-**11d** with 93% yield (15.9 mg); $[\alpha]_D^{19}$ +12.3 (*c* 0.63, EtOH), *ee* >99%, mp 83-84 °C.

*2.4.4.2. General procedure for β-ketonitriles **12a-15a***

In a 2.0 mL eppendorf tube, the corresponding ketonitrile (100 mM), KRED (the same weight as nitrile), *i*-PrOH (10% v/v) and 125 mM phosphate buffer (also containing 1.25 mM $MgSO_4$, 1.0 mM $NADP^+$) pH 7.0 (**12a**, **14a** and **15a**) or pH 5.0 (**13a**) were added. The resulting reaction mixture was shaken at 250 rpm and 30 °C for 24 h. After this time, the pH was adjusted to 5.0 by adding conc. H_3PO_4 before the hydration step, complex **1** (6.0 mol %) was added and the reaction was left stirring overnight at 60°C. Then, the mixture was extracted with ethyl acetate (2 × 500 μL), the organic layers were separated by centrifugation (90 s, 13000 rpm), combined, and finally dried over Na2SO4. The degree of conversion

was measured by ^{1}H-NMR and it was complete in all cases. The diastereomeric ratio and enantiomeric excess of the corresponding product was determined by chiral HPLC or GC.

For a better overview, a selection of compounds included in this section is reported in the following Figure 2.4.4.1.



(2*R*,3*S*)-**12d** (1*S*,2*R*)-**13d** (1*R*,2*S*)-**15d**

Fig. 2.4.4.1. Examples of synthesized compounds

2.4.4.2.1. (2R,3S)-3-Hydroxy-2-phenylbutanamide **12d**

The typical procedure was followed using 20.0 mg (126 μmol) of the starting material and KRED-P3-H12 as the catalyst in the reduction step. The crude product was purified by flash column chromatography (AcOEt) to give (3*S,*2*R*)-**12d** with 89% yield (18.8 mg); $[\alpha]_D^{20}$ +72.4 (*c* 0.90, EtOH), *ee* >99%, mp 111-112 °C. ^{1}H-NMR (300 MHz, CD_3OD) δ (ppm): 1.25 (d, *J* 6.3 Hz, 3H), 3.40 (d, *J* 8.7 Hz, 1H), 4.33 [dq, *J* 8.4 Hz (d), *J* 6.3 Hz (q), 1H], 7.21-7.45 (m, 5H); ^{13}C-NMR (75.5 MHz, CD_3OD) δ (ppm): 21.96 (CH_3), 61.16 (CH), 69.43 (CH), 128.18 (CH), 129.23 (CH), 130.04 (CH), 139.10 (C), 178.01 (C=O). HRMS (ESI^+): $[M+Na]^+$ found: 202.0839. $C_{10}H_{13}NNaO_2$ requires 202.0838.

2.4.4.2.2. (1S,2S)-2-Hydroxycyclopentanecarboxamide **13d**

The starting material **13a** (137 μmol) was added in an eppendof tube and KRED-P2-D11was used. After the typical work-up, ^{1}H-NMR showed complete conversion and *dr* 15:85 (*cis:trans*). The crude product was purified by flash column chromatography (AcOEt) to give (1*S,*2*S*)-**13d** with

77% yield (13.5 mg). $[\alpha]_D^{20}$ +59.7 (*c* 0.35, EtOH), *ee* >99%, mp 112-113 °C. ^{1}H NMR (300 MHz, CD_3OD) δ (ppm): 1.53-1.80 (m, 4H), 1.90-2.04 (m, 2H), 2.51-2.58 (m, 1H), 4.27 (dd, *J* 12.9 Hz, *J* 6.6 Hz, 1H); ^{13}C-NMR (75.5 MHz, CD_3OD) δ (ppm): 23.48 (CH_2), 29.37 (CH_2), 35.82 (CH_2), 54.51 (CH), 77.51 (CH), 180.34 (C=O). The spectroscopic data are in good agreement with the reported literature.[62]

2.4.4.2.3. (1S,2R)-2-Hydroxycyclopentanecaboxamide **13d**

The previous procedure was followed, but in this case KRED-P1-B12 was used in the bioreduction step. After the typical work-up, ^{1}H-NMR showed complete conversion and *dr* 80:20 (*cis:trans*). The crude product was purified by flash column chromatography (AcOEt) to give (1*S*,2*R*)-**13d** with 74% yield (13.0 mg). $[\alpha]_D^{22}$ –26.5 (*c* 0.55, EtOH), *ee* >99%, mp 95-97 °C. ^{1}H-NMR (300 MHz, CD_3OD) δ (ppm): 1.61-2.00 (m, 6H), 2.53-2.61 (m, 1H), 4.35-4.38 (m, 1H); ^{13}C-NMR (75.5 MHz, CD_3OD) δ (ppm): 22.75 (CH_2), 27.57 (CH_2), 35.36 (CH_2), 51.12 (CH), 75.24 (CH), 179.42 (C=O). The spectroscopic data are in good agreement with the reported literature.[19]

2.4.4.2.4. (1S,2R)-2-Hydroxycyclohexanecarboxamide **14d**

The typical procedure was followed using 15.0 mg (122 μmol) of the starting material and KRED-P1-B12 as the catalyst in the reduction step. The crude product was purified by flash column chromatography (AcOEt) to give (1*S*,2*R*)-**14d** with 89% yield (15.0 mg). $[\alpha]_D^{20}$ –27.4 (*c* 0.55, EtOH), *ee* >99%, mp 126-128 °C. ^{1}H-NMR (300 MHz, CD_3OD) δ (ppm): 1.24-1.96 (m, 8H), 2.33 (ddd, *J* 2.4 Hz, *J* 3.9 Hz, *J* 11.7 Hz, 1H), 4.11-4.12 (m, 1H); ^{13}C-NMR (75.5 MHz, CD_3OD) δ (ppm): 20.93 (CH_2), 25.22 (CH_2), 26.04 (CH_2), 33.27 (CH_2), 48.43 (CH), 68.23 (CH), 181.07 (C=O). The spectroscopic data are in good agreement with the reported literature.[19]

[62] M. Quirós, F. Rebolledo, V. Gotor, *Tetrahedron: Asymmetry*, **1999**, *10*, 473–486.

2.4.4.2.5. (1R,2S)-2-Hydroxycyclohexanecarboxamide **14d**

The typical procedure was followed using 15.0 mg (122 μmol) of the starting material and KRED-P1-A04 as the catalyst in the reduction step. The crude product was purified by flash column chromatography (AcOEt) to give (1*R*,2*S*)-**14d** with 89% yield (15.2 mg). $[\alpha]_D^{21}$ +29.1 (*c* 0.47, EtOH), *ee* >99%, mp 126-128 °C.

2.4.4.2.6. (1R,2S)-2-Hydroxycycloheptanecarboxamide **15d**

The typical procedure was followed using 15.0 mg (109 μmol) of the starting material and KRED-P1-A04 as the catalyst in the reduction step. The crude product was purified by flash column chromatography (AcOEt) to give (1*R*,2*S*)-**15d** with 92% yield (15.6 mg). $[\alpha]_D^{20}$ +18.3 (*c* 0.75, EtOH), *ee* >99%, mp 106-107 °C. ^{1}H-NMR (300 MHz, CD_3OD) δ (ppm): 1.39-1.90 (m, 9H), 1.95-2.08 (m, 1H), 2.47 (dt, *J* 10.5 Hz (d), *J* 2.4 Hz (t), 1H), 4.14-4.18 (m, 1H); ^{13}C-NMR (75.5 MHz, CD_3OD) δ (ppm): 23.09 (CH_2), 25.85 (CH_2), 27.78 (CH_2), 28.86 (CH_2), 36.52 (CH_2), 51.34 (CH), 71.61 (CH), 181.85 (C=O). HRMS (ESI^+): $[M+Na]^+$ found: 180.0993. $C_8H_{15}NNaO_2$ requires 180.0995.

2.4.5. Synthesis of (S)-tembamide and (R)-aegeline

2.4.5.1. Hofmann rearrangement of the optically pure hydroxyamide **8d**

Iodobenzene diacetate (1.2 eq) was added to a solution of the corresponding amide **8d** (50.0 mg, 266 μmol) in MeCN (1.33 mL) and the mixture was left stirring at 50 °C for 2.5 h. When reaction was complete, the solvent was removed and the crude product was submitted to flash chromatography (Hexane:AcOEt 2:1) to get pure oxazolidinone **16**.

2.4.5.1.1. (R)-5-(4-Methoxyphenyl)-1,3-oxazolidin-2-one **16**

Starting from 50.0 mg of (*S*)-**8d**, the desired product (*R*)-**16** was obtained with 90% yield (44.0 mg). $[\alpha]_D^{21}$ –18.7 (*c* 0.50, $CHCl_3$), *ee* >99%,

mp 155-156 °C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 3.54 (t, *J* 8.4 Hz, 1H), 3.81 (s, 3H), 3.92 (t, *J* 8.7 Hz, 1H), 5.56 (t, *J* 8.1 Hz, 1H), 6.34 (bs, 1H), 6.92 (d, *J* 8.7 Hz, 2H), 7.31 (d, *J* 8.7 Hz, 2H); ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ (ppm): 48.44 (CH_2), 55.46 (OCH_3), 78.12 (CH), 114.36 (CH), 127.58 (CH), 130.35 (C), 160.21 (C and C=O). The spectroscopic data are in good agreement with the reported literature.[63]

2.4.5.1.2. (S)-5-(4-Methoxyphenyl)-1,3-oxazolidin-2-one **16**

The general procedure was applied to 52.0 mg of (*R*)-**8d** and the final oxazolidinone (*S*)-**16** was isolated with 90% yield (46.0 mg). $[\alpha]_D^{21}$ +18.3 (*c* 0.58, $CHCl_3$), *ee* >99%, mp 155-156 °C.

2.4.5.2. Synthesis of (S)-tembamide

To a solution of the oxazolidinone (*S*)-**16** (233 μmol) in EtOH (582 μL) was added NaOH (1.17 mmol) in 310 μL of H_2O at 50 °C and the reaction was left stirring for 4 h. Then the solvent was evaporated and the crude (*S*)-2-amino-1-(4-methoxyphenyl)ethanol was used for the next reaction without need of further purification. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.94 (bs, 3H), 2.80 (dd, *J* 7.8 Hz, *J* 12.6 Hz, 1H), 2.97 (dd, *J* 4.2 Hz, *J* 12.9 Hz, 1H), 3.80 (s, 3H), 4.59 (dd, *J* 4.2 Hz, *J* 7.8 Hz), 6.89 (d, *J* 8.7 Hz, 2H), 7.28 (d, *J* 8.1 Hz, 1H). The spectroscopic data are in good agreement with the reported literature.[50]

The crude aminoalcohol was dissolved in TBME (2.0 mL) and 1.0 mL of H_2O was added. Then benzoyl chloride (1.2 eq) was added and the reaction was left stirring for 2 h. When TLC revealed no presence of the starting material, 5.0 mL of AcOEt were added and the organic phase was washed with brine, dried over Na_2SO_4 and finally evaporated to get the crude product that was purified by flash chromatography (Hexane:AcOEt 1:1). Yield 85% (54.3 mg). $[\alpha]_D^{21}$ +53.8 (*c* 0.50, $CHCl_3$), *ee* >99%, mp 149-150 °C. [Lit.[50] (*S*)-tembamide: $[\alpha]_D^{20}$ +54.9 (*c* 0.52, $CHCl_3$), *ee* >99%, mp

[63] A. Slassi, B. Joseph, F. Ma, I. Egle, J. Clayton, M. Isaac, K. Swierczek, *PCT Int. Appl*., 2007078523, 12 Jul **2007**.

148-149°C]. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 2.18 (bs, 1H), 3.52 (ddd, ^{3}J 4.8 and 8.0 Hz, $|^{2}J|$ 14.0 Hz, 1H), 3.81 (s, 3H), 3.88 (ddd, ^{3}J 3.4 Hz and 6.9 Hz, $|^{2}J|$ 14.0 Hz, 1H), 4.91 (dd, *J* 3.4 Hz, *J* 8.0 Hz, 1H), 6.60 (bs, 1H), 6.90 (d, *J* 8.7 Hz, 2H), 7.33 (d, *J* 8.7 Hz, 2H), 7.40-7.54 (m, 3H), 7.76 (d, *J* 7.2 Hz, 2H). ^{13}C-NMR (75.5 MHz, CD_3OD) δ (ppm): 47.71 (CH_2), 55.68 (OCH_3), 73.14 (CH), 114.75 (CH), 128.27 (CH), 128.42 (CH), 129.52 (CH), 132.65 (CH), 135.68 (C), 135.94 (C), 160.73 (C), 170.54 (C=O). The spectroscopic data are in good agreement with the reported literature.[50]

2.4.5.3. Synthesis of (R)-aegeline

After hydrolysis of oxazolidinone (*R*)-**16** (233 μmol) as previously described, the resulting crude (*R*)-2-amino-1-(4-methoxyphenyl)ethanol was dissolved in TBME (2.0 mL) and 1.0 mL of H_2O was added. Then cinnamoyl chloride (1.2 eq) was added and the reaction was left stirring for 2 h. When TLC revealed no presence of the starting material, 5.0 mL of AcOEt were added and the organic phase was washed with brine, dried over Na_2SO_4 and finally evaporated to get the crude product that was purified by flash chromatography (Hexane:AcOEt 1:1). Yield, 81% (53.5 mg). $[\alpha]_D^{21}$ –40.1 (*c* 0.35, $CHCl_3$), *ee* >99%, mp 196-197 °C. [Lit.[61] (*R*)-aegeline: $[\alpha]_D^{20}$ –39.3 (*c* 0.40, $CHCl_3$), *ee* >99%, mp 193-195 °C]. ^{1}H NMR (300 MHz, CD_3OD) δ (ppm): 3.44 (dd, *J* 7.8 Hz, *J* 13.6 Hz, 1H), 3.56 (dd, *J* 5.0 Hz, *J* 13.6 Hz, 1H), 3.78 (s, 3H), 4.77 (dd, *J* 5.0 Hz, *J* 7.8 Hz, 1H), 6.64 (d, *J* 15.9 Hz, 1H), 6.91 (d, *J* 8.7 Hz, 2H), 7.33 (d, *J* 8.7 Hz, 2H), 7.35-7.42 (m, 3H), 7.50-7.57 (m, 3H). ^{13}C-NMR (75.5 MHz, CD_3OD) δ (ppm): 47.71 (CH_2), 55.68 (OCH_3), 77.24 (CH), 114.77 (CH), 121.79 (C=C), 128.41 (CH), 128.84 (CH), 129.94 (CH), 130.82 (CH), 135.93 (C), 136.31 (C), 141.84 (C=C), 160.75 (C), 168.91 (C=O).

These spectroscopic data are in good agreement with the reported literature.[61]

2.4.6. HPLC and CG analytical data for C, dr, and ee determinations

2.4.6.1 HPLC analyses for determination of C in the KRED-catalysed reduction of β-ketonitriles and β-ketoamides

HPLC Method:

HPLC analyses were carried out using a reversed phase column (Zorbax Eclipse XDB-C18, RR, 18μm, 4.6 x 50 mm, Agilent) with acetonitrile and water (0.1% trifluoroacetic acid) as solvent. 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 2.0 ml/min. Detection of peaks (UV absorption) were performed at 220 and 324 nm.

Determination of *C, dr,* and *ee* for reactions starting from compounds **13a-15a** was done as previously described in Chapter 1.

Table 2.4.6.1.1. Analytical data for determination of *C* in the bioreduction of β-ketonitriles 6a-12a

Method	Retention time (t_R, min)			
	β-ketonitrile	t_R	β-hydroxynitrile	t_R
HPLC	**6a**	3.82	**6c**	3.11
HPLC	**7a**	3.64	**7c**	2.88
HPLC	**8a**	2.70	**7c**	2.01
HPLC	**9a**	3.36	**9c**	2.46
HPLC	**10a**	1.10	**10c**	1.01
HPLC	**11a**	2.03	**11c**	1.63
HPLC	**12a**	3.60	**12c**	2.90 (*syn*) 2.70 (*anti*)

Table 2.4.6.1.2. Analytical data for determination of *C* in the bioreduction of β-ketoamides

Method	Retention time (t_R, min)			
	β-ketoamide	t_R	β-hydroxyamide	t_R
HPLC	**6b**	2.24	**6d**	1.88
HPLC	**7b**	2.06	**7d**	1.65
HPLC	**8b**	1.31	**8d**	0.95
HPLC	**9b**	1.96	**9d**	1.42
HPLC	**10b**	0.44	**10d**	0.28
HPLC	**11b**	0.80	**11d**	0.60

2.4.6.2. HPLC and GC analytical data for C, dr (if applicable) and ee determination

Table 2.4.6.2.1. Analytical data for determination of *dr* (if applicable) and *ee* in the bioreduction of β-ketonitriles

β-Hydroxynitrile	Conditions[a]	Retention time (min)
6c	Chiralpak AD-H, Hexane:IPA	17.57 (*R*), 19.14 (*S*)
	95:5, 40 °C, 0.8 mL/min	R_S = 1.7
7c	Chiralcel OJ-H, Hexane:IPA	21.99 (*S*), 24.56 (*R*)
	90:10, 40 °C, 0.7 mL/min	R_S = 11.7
8c	Chiralpak AD-H, Hexane:IPA	19.29 (*R*), 20.90 (*S*)
	95:5, 40 °C, 0.8 mL/min	R_S = 2.2
9c	Chiralcel OJ-H, Hexane:IPA	22.20 (*S*) , 25.80 (*R*)
	90:10, 40 °C, 0.8 mL/min	R_S = 5.1
10c	Chiralcel OJ-H, Hexane:IPA	23.24 (*S*) , 25.81 (*R*)
	90:10, 40 °C, 0.8 mL/min	R_S = 10.7
11c	Chiralcel OJ-H, Hexane:IPA	27.21 (*S*), 30.46 (*R*)
	90:10, 40 °C, 0.8 mL/min	R_S = 7.6
12c	Chiralpak IC, Hexane:IPA	12.32 (2*R*,3*R*), 13.61 (2*S*,3*S*)
	90:10, 40 °C, 0.8 mL/min	R_S = 4.6
		16.45 (*anti*)[b], 17.60 (*anti*)[b]

[a] Detection of peaks (UV absorption) was performed at λ = 216 nm.

[b] The absolute configuration has not been determined.

Table 2.4.6.2.2. Analytical data for determination of *dr* (if applicable) and *ee* in the bioreduction of β-ketoamides

β-Hydroxyamide	Conditions[a]	Retention time (min)
6d	Chiralpak AD-H, Hexane:IPA	8.56 (*R*), 9.24 (*S*)
	85:15, 40 °C, 0.8 mL/min	R_S = 0.7
7d	Chiralcel OJ-H, Hexane:IPA	17.02 (*R*), 18.07 (*S*)
	90:10, 40 °C, 0.7 mL/min	R_S = 2.4
8d	Chiralpak IC, Hexane:IPA	40.62 (*R*), 46.32 (*S*)
	85:15, 40 °C, 0.8 mL/min	R_S = 4.5
9d	Chiralcel OJ-H, Hexane:IPA	9.74 (*R*), 10.64 (*S*)
	85:15, 40 °C, 0.8 mL/min	R_S = 1.1
10d	Chiralpak IC, Hexane:IPA	14.91 (*S*), 16.99 (*R*)
	75:25, 40 °C, 0.8 mL/min	R_S = 3.0
11d	Chiralcel OJ-H, Hexane:IPA	17.95 (*R*), 19.36 (*S*)
	90:10, 40 °C, 0.8 mL/min	R_S = 9.7
12d	Chiralpak IC, Hexane:IPA	9.05 (2*R*,3*S*) , 10.54 (2*S*,3*R*)
	73:27, 40 °C, 0.8 mL/min	9.76 (*anti*), 14.89 (*anti*)
13d[b]	ChiraSil	14.31 (1*R*,2*R*), 15.85 (1*S*,2*S*)
	80/10/1/102/20/180[c]	26.45 (1*R*,2*S*), 29.11 (1*S*,2*R*)
14d[b]	ChiraSil	30.53 (1*R*,2*S*), 33.84 (1*S*,2*R*)
	85/10/1/115/20/180[c]	

[a] Detection of peaks (UV absorption) was performed at λ = 216 nm. [b] Previously derivatised with trifluoroacetic anhydride. [c] GC program: initial temperature (°C) / time (min) / slope (°C/min) / temp (°C) / slope (°C/min) / final temp (°C).

For compound **15d**, no analytical method was found for the determination of the *ee*. Keeping in mind that the hydration step does not cause any loss of *ee,* we assume that also in this case the *ee* of the intermediate hydroxynitrile is maintained. However, the *dr* was confirmed by ^{1}H-NMR.

Table 2.4.6.2.2. Analytical data for *ee* determination of tembamide and aegeline

Compound	Conditions[a]	Retention time (min)
Tembamide	Chiralcel OJ-H, Hexane:IPA 85:15, 40 °C, 0.8 mL/min	12.61 (*R*), 13.45 (*S*) R_S = 1.7
Aegeline	Chiralcel OJ-H, Hexane:IPA 85:15, 40 °C, 0.8 mL/min	20.59 (*R*), 22.26 (*S*) R_S = 3.7

[a] Detection of peaks (UV absorption) was performed at λ = 216 nm.

2.4.7. CD Support

In the CD support, detailed additional information about the experimental of this Chapter is included. A complete section of enzymatic screening, copy of GC-HPLC chromatograms and NMR spectra for all the synthesized optically pure compounds will be found.

Chapter 3

Hybrid organo- and bio-catalytic process combining AZADO and ω-transaminase or ketoreductase. Synthesis of optically pure β-aminoalcohol derivatives, amines, alcohols, and diols.

3.1. Bibliographic Background

In the preceding chapters it has been shown that prochiral or racemic ketones are a potential source of interesting optically active compounds. Taking advantage of the high stereoselectivity of enzymes as ketoreductases, they can be used to easily convert ketones into enantiomerically pure or highly enantioenriched alcohols.[64] Moreover, prochiral ketones are also substrates of another kind of enzymes as ω-transaminases (omega-TA) that catalyze their reductive amination giving optically active amines,[65] which also constitute a valuable class of compounds.

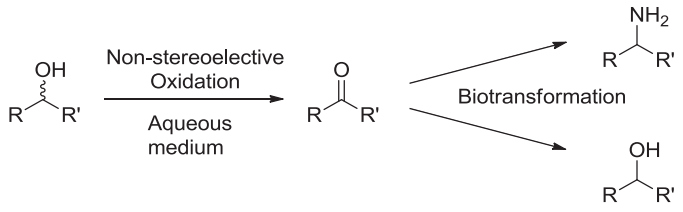
However, the easier availability of some racemic alcohols in comparison with their oxidized carbonyl derivatives makes them more convenient starting substrates and this has encouraged the chemical community to develop alternative and *greener* approaches for the alcohol

[64] For some recent examples, see: a) Y. Nie, S. Wang, Y. Xu, S. Luo, Y. L. Zhao, R. Xiao, G. Montelione, J. F. Hunt, T. Szyperski, *ACS Catal.*, **2018**, DOI: 10.1021/acscatal.8b00364; b) T. Nagai, S Sakurai, N. Natori, M. Hataoka, T. Kinoshita, H. Inoue, K. Hanaya, M. Shoji, T. Sugai, *Bioorg. Med. Chem*., **2018**, *26*, 1304−1313; c) A. I. Benítez-Mateos, E. San Sebastian, N. Ríos-Lombardía, F. Morís, J. González-Sabín, F. López-Gallego, *Chem. Eur. J*., **2017**, *23*, 16843−16852; d) D. Alsafadi, S. Alsalman, F. Paradisi, *Org. Biomol. Chem*., **2017**, *15*, 9169−9175.

[65] For recent reviews using a) transaminases: A. Gomm, E. O´Reilly, *Curr. Opin. Chem. Biol*., **2018**, *43*, 106−112; b) other redox biocatalysts: G. Grogan, *Curr. Opin. Chem. Biol*., **2018**, *43*, 15−22.

oxidation reaction. In these methods water appears as the selected reaction medium, not only to respond to the growing environmental requirements, but also to facilitate its assembly with other reactions working in the same aqueous *milieu* as the before mentioned biotransformations.

The following Scheme 3.1.1 summarizes the general objectives of this chapter, that is, the development of efficient methods to couple a non-stereoselective oxidation of racemic alcohols in aqueous medium with an asymmetric reductive amination catalyzed by omega-transaminase or with a ketoreductase-catalyzed reduction. As result, the synthesis of optically active amines or the deracemization of secondary alcohols, respectively, will be achieved.



Scheme 3.1.1

With these objectives in mind, the current section has been divided into the following three subsections.

3.1.1. Alcohol oxidation

Oxidation of alcohols is one of the most fundamental and important transformations in organic chemistry. Thus, although a variety of methods and reagents for the oxidation have been developed, until recently the traditional oxidation reactions had been performed with stoichiometric amounts of heavy metal-based reagents,[66] hypervalent iodine reagents,[67] or activated DMSO. [68] These methods often happen in environmentally undesirable media like chlorinated solvents which render them impractical in the pharmaceutical industry.[69]

Considerable efforts have been made to develop more efficient protocols in which the promoter oxidant reagent is used in catalytic amount along with a greener co-oxidant, as molecular oxygen, which has the function of recycling the reagent.[70]

As an example, the redox catalytic cycle proposed for the aerobic oxidation of alcohols using the hypervalent iodine $PhIO_2$ (1 mol%) and catalytic amount of Br_2 (2 mol%) and $NaNO_2$ (1 mol%) is shown in Scheme 3.1.1.1.[71]

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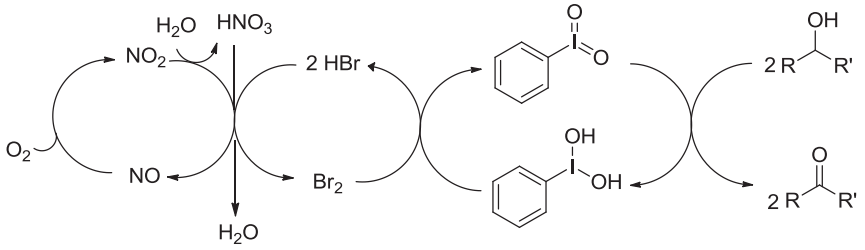
[66] For a review on metal-mediated oxidations, see: Organic Syntheses by Oxidation with Metal Compounds (Eds.: W. J. Mijs, C. R. H. de Jonge), Plenum, New York, **1986**.
[67] H. Tohma, Y. Kita, *Adv. Synth. Catal*., **2004**, *346*, 111−124.
[68] T. T. Tidwell, *Synthesis*, **1990**, 857−870.
[69] S. Caron, R. W. Dugger, S. G. Ruggeri, J. A. Ragan, D. H. Brown Ripin, *Chem. Rev*., **2006**, *106*, 2943−2989.
[70] a) T. Mallat, A. Baiker, *Chem. Rev*., **2004**, *104*, 3037−3058; b) M. Uyanik, K. Ishihara, *Chem. Commun*., **2009**, 2086−2099.
[71] R. Mu, Z. Liu, Z. Yang, Z. Liu, L. Wu, Z. L. Liu, *Adv. Synth. Catal*., **2005**, *347*, 1333−1336. It is proposed that NO_2^- experiments disproportination in the acidic aqueous solution to give NO and NO_3^-.

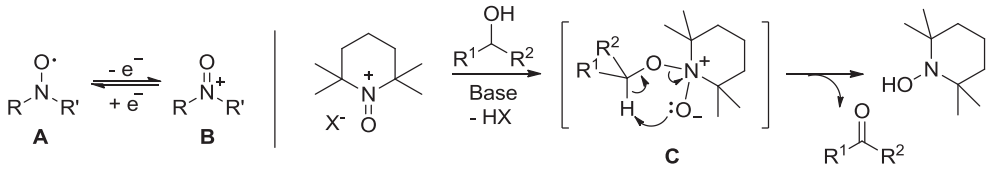


Scheme 3.1.1.1

In addition, performing the alcohol oxidation in water would represent a much cheaper, safer, and more environmentally benign oxidation protocol.[72]

Alcohol oxidations mediated by nitroxyl radicals, also called nitroxides, deserve special mention because of the close relationship with our work. Many nitroxides have been successfully synthesized and their properties studied,[73] being TEMPO (2,2,6,6-tetramethylpiperidine *N*-oxyl) the most widely investigated and applied in large-scale oxidations.[74] These nitroxides (**A**) undergo reversible oxidation to the corresponding oxoammonium salt (**B**), this last being the active oxidant in either acid or basic conditions. The Scheme 3.1.1.2 shows the suggested mechanism for the oxidation of alcohols under basic conditions with TEMPO.[75]

[72] a) Y. Uozumi, R. Nakao, *Angew. Chem. Int. Ed*., **2003**, *42*, 194−197; b) R. A. Sheldon, *Catal. Today*, **2015**, *247*, 4−13.
[73] a) L. Tebben, A. Studer, *Angew. Chem. Int. Ed*., **2011**, *50*, 5034−5068; b) Q. Cao, L. M. Dornan, L. Rogan, N. L. Hughes, M. J. Muldoon, *Chem. Commun*., **2014**, *50*, 4524−4543.
[74] R. Ciriminna, M. Pagliaro, *Org. Process Res. Dev*., **2010**, *14*, 245−251.
[75] W. F. Bailey, J. M. Bobbitt, K. B. Wiberg, *J. Org. Chem*., **2007**, *72*, 4504−4509.



Scheme 3.1.1.2

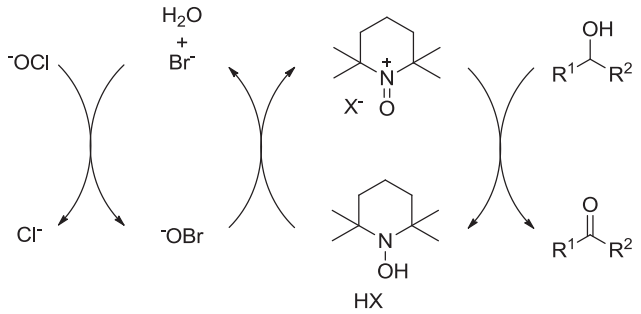
Although some stable oxoammonium salts have been used as stoichiometric mild oxidants for the oxidation of alcohols,[76] more interestingly, and more importantly in the context of the guiding principles of green chemistry, are the nitroxide-mediated oxidations in which the nitroxide compound is used in catalytic amounts.[77] In these cases, a stoichiometric co-oxidant, such as sodium hypochlorite (NaOCl), bromine, or $PhI(OAc)_2$, is required to complete the catalytic cycle reoxidizing the hydroxylamine to the oxoammonium salt.

One common protocol (the Anelli oxidation) features catalytic TEMPO (or a derivative, 1 mol%) and bromide (10 mol%) in a buffered organic/aqueous biphasic mixture, with NaOCl as the stoichiometric oxidant[78] (Scheme 3.1.1.3).

[76] a) In acid media: J. Zakrzewski, J. Grodner, J. M. Bobbitt, M. Karpińska, *Synthesis,* **2007**, 2491−2494; In basic media: b) N. Merbouh, J. M. Bobbitt, C. Brückner, *J. Org. Chem*., **2004**, *69*, 5116−5119; c) T. Breton, G. Bashiardes, J. M. Léger, K. B. Kokoh, *Eur. J. Org. Chem*., **2007**, *10*, 1567−1570.

[77] R. A. Sheldon, I. W. C. E. Arends, G. J. T. Brink, A. Dijksman, *Acc. Chem. Res*., **2002**, *35*, 774−781.

[78] a) P. L. Anelli, C. Biffi, F. Montanari, S. Quici, *J. Org. Chem*., **1987**, *52*, 2559−2562; b) P. L. Anelli, F. Montanari, S. Quici, *Org. Synth*., **1990**, *69*, 212−219.



Scheme 3.1.1.3

One distinguishing feature of the TEMPO-based method is its capability for the selective oxidation of primary alcohols in the presence of secondary alcohols.[79] The reaction mechanism (see Scheme 3.1.1.2) and the catalyst structure with four methyl groups flanking the nearby catalytic center could prevent bulky substrates from forming the key intermediate **C**. Regarding this, Iwabuchi *et al.*,[80] among other authors,[81] have shown that other structurally less hindered nitroxyl radicals as the tricyclic AZADO (2-azaadamantane *N*-oxyl), its methyl derivative, 1-Me-AZADO, or the bicyclic 9-azabicyclo[3.3.1]nonane *N*-oxyl (ABNO) significantly enhance the efficiency (see Scheme 3.1.1.4) and scope of this oxidation methodology.

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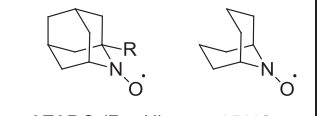
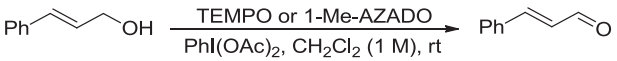
[79] For some selected examples, see: a) K. C. Nicolaou, H. Zhang, A. Ortiz, P. Dagneau, *Angew. Chem., Int. Ed*., **2008**, *47*, 8605−8610; b) D. W. Custar, T. P. Zabawa, J. Hines, C. M. Crews, K. A. Scheidt, *J. Am. Chem. Soc*., **2009**, *131*, 12406−12414; c) K. C. Nicolaou, H. Zhang, A. Ortiz, *Angew. Chem. Int. Ed*., **2009**, *48*, 5642−5647; d) R. Araoz, D. Servent, J. Molgo, B. I. Iorga, C. Fruchart-Gaillard, E. Benoit, Z. H. Gu, C. Stivala, A. Zakarian, *J. Am. Chem. Soc*., **2011**, *133*, 10499−10511.

[80] a) M. Shibuya, M. Tomizawa, I. Suzuki, Y. Iwabuchi, *J. Am. Chem. Soc*., **2006**, *128*, 8412–8413; b) M. Tomizawa, M. Shibuya, Y. Iwabuchi, *Org. Lett*., **2009**, *11*, 1829–1831; c) M. Shibuya, M. Tomizawa, Y. Sasano, Y. Iwabuchi, *J. Org. Chem*., **2009**, *74*, 4619–4622; d) M. Hayashi, M. Shibuya, Y. Iwabuchi, *J. Org. Chem*., **2012**, *77*, 3005–3009; e) M. Shibuya, R. Doi, T. Shibuta, S. I. Uesugi, Y. Iwabuchi, *Org. Lett*., **2012**, *14*, 5006–5009; f) R. Doi, M. Shibuya, T. Murayama, Y. Yamamoto, Y. Iwabuchi, *J. Org. Chem*., **2015**, *80*, 401–413.

[81] M. Rafiee, K. C. Miles, S. S. Stahl, *J. Am. Chem. Soc*., **2015**, *137*, 14751−14757.



AZADO (R = H) ABNO
1-Me-AZADO (R = Me)



Nitroxide (mol%)	yield (%) / time (h)	
	TEMPO	1-Me-AZADO
10	95 / 1.5	96 / 0.1
1.0	42 / 6.0	93 / 0.7

Scheme 3.1.1.4

Nitroxide-catalyzed alcohol oxidations in the presence of molecular oxygen as the terminal oxidant and a co-catalyst have also been profusely investigated. A variety of co-catalysts including $NaNO_2$, HNO_3, or other NO_x sources,[82] as well as transition-metal salts[83] have been used, the Cu/nitroxyl catalyst systems emerging as the most versatile and effective among those metal-based.[84]

On the other hand, a very interesting chemoenzymatic alternative for the aerobic alcohol oxidation is the combination of a nitroxyl radical with a laccase.[85] These enzymes (copper-dependant oxidases) contain four copper centres per protein molecule and catalyze the oxidation of electron rich aromatic substrates, usually phenols or aromatic amines via four single

[82] a) M. B. Lauber, S. S. Stahl, *ACS Catal*., **2013**, *3*, 2612−2616 and references cited herein; b) M. Shibuya, S. Nagasawa, Y. Osada, Y. Iwabuchi, *J. Org. Chem*., **2014**, *79*, 10256−10268.

[83] a) Y. Seki, K. Oisaki, M. Kanai, *Tetrahedron Lett*., **2014**, *55*, 3738−3746; b) R. A. Sheldon, I. W. C. E. Arends, *J. Mol. Catal. A*: *Chem.*, **2006**, *251*, 200−214.

[84] a) B. L. Ryland, S. S. Stahl, *Angew. Chem. Int. Ed*., **2014**, *53*, 8824−8838; b) K. C. Miles, S. S. Stahl, *Aldrichimica Acta*, **2015**, *48*, 8−10. Alternative mechanisms to the oxoammonium salts have been suggested by these processes. See as an example: c) R. A. Sheldon, I. W. C. E. Arends, *Adv. Synth. Catal*., **2004**, *346*, 1051−1071.

[85] For a recent review about enzymatic oxidation of alcohols, see: J. Liu, S. Wu, Z. Li, *Curr. Opin. Chem. Biol*., **2018**, *43*, 77−86.

electron oxidation steps concomitant with the four electron reduction of O_2 to H_2O.[86]

Additionally, laccases can also catalyze the oxidation of other compounds, as alcohols, but only under the presence of an electron mediator.[87] ABTS [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] was the first compound found to be capable of mediating the laccase from *Trametes versicolor* catalyzed oxidation of a series of benzylic alcohols to their corresponding benzaldehydes.[88] Later, some *N*-hydroxy compounds with propensity to give *N*-oxyl radicals, and TEMPO have been used for this purpose with laccases of different sources, being TEMPO the most effective.[89]

The active species in the laccase/TEMPO process, which is commonly carried out in buffer at pH 4.5, is also the oxoammonium salt, and the mechanism is similar to that shown in Scheme 3.1.1.2.[90] Once the oxidation takes place, laccase would then regenerate TEMPO from the hydroxylamine and the active species be restored either through an acid-induced disproportionation of TEMPO,[91] or through its oxidation by laccase (Scheme 3.1.1.5). A distinguish aspect of the non-enzymatic TEMPO-mediated oxidations is the high nitroxyl radical loadings required (typically 30 mol% on substrate).

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[86] a) E. I. Solomon, U. M. Sundaram, T. E. Machonik, *Chem. Rev*., **1996**, *96*, 2563−2605; b) R. Ten Have, P. J. M. Teunissen, *Chem. Rev*., **2001**, *101*, 3397−3413.

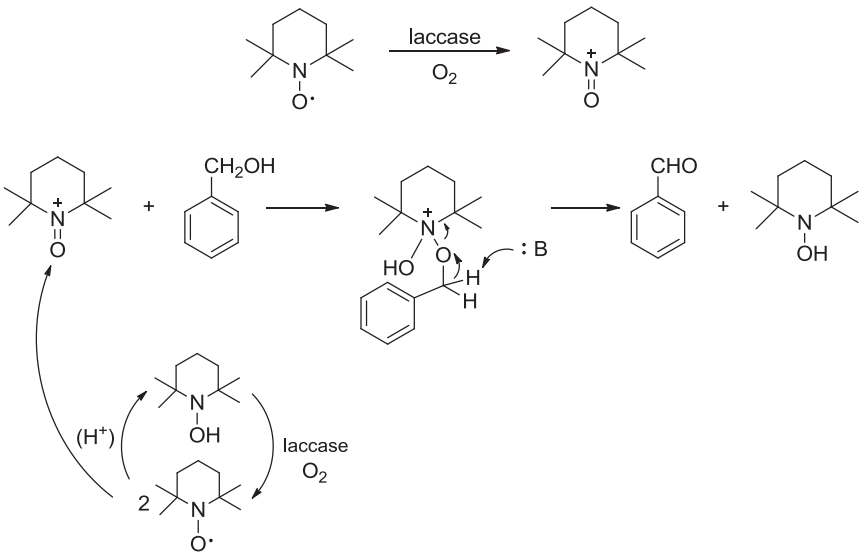
[87] S. Witayakrana, A. J. Ragauskas, *Adv. Synth. Catal*., **2014**, *356*, 897−927.

[88] A. Potthast, T. Rosenau, C. L. Chen, J. S. Gratzl, *J. Mol. Catal. A: Chem.,* **1996**, *108*, 5−9.

[89] a) M. Fabbrini, C. Galli, P. Gentili, *J. Mol. Catal. B: Enzym.*, **2002**, *16*, 231−240; b) M. Fabbrini, C. Galli, P. Gentili, D. Macchitella, *Tetrahedron Lett.,* **2001**, *42*, 7551−7553.

[90] I. W. C. E. Arends, Y. X. Li, R. Ausan, R. A. Sheldon, *Tetrahedron*, **2006**, *62*, 6659−6665.

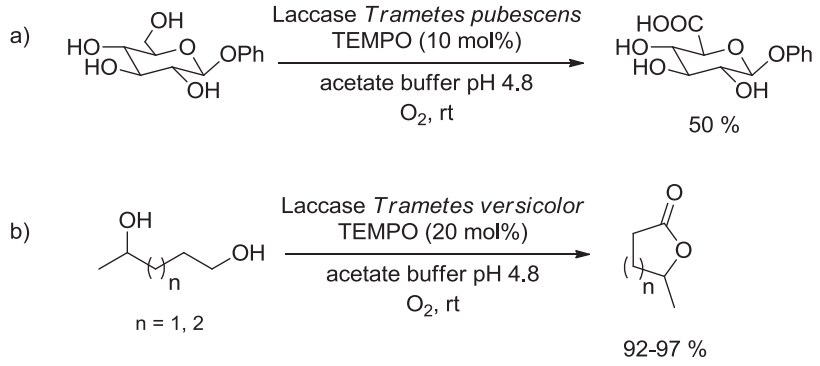
[91] a) Y. Ma, C. Loyns, P. Price, V. Chechik, *Org. Biomol. Chem*., **2011**, *9*, 5573−5578; b) V. D. Sen, V. A. Golubev, *J. Phys. Org. Chem*., **2009**, *22*, 138−143.



Scheme 3.1.1.5

The high selectivity of TEMPO towards primary alcohols over secondary ones has been exploited for the regioselective aerobic oxidation of different sugar derivatives in the presence of laccase from *Trametes pubescens* (Scheme 3.1.1.6a).[92] Another example is the regioselective oxidation of 1,4-pentanediol and 1,5-hexanediol to γ-valerolactone and δ-caprolactone, respectively (Scheme 3.1.1.6b).[93] Oxidations have occurred in a non-stereoselective fashion but with complete regio- and monoselectivity, obtaining lactones with excellent purity after a simple extraction.

[92] M. Marzorati, B. Danieli, D. Haltrich, S. Riva, *Green Chem*., **2005**, *7*, 310−315.
[93] A. Díaz-Rodríguez, I. Lavandera, S. Kanbak-Aksu, R. A. Sheldon. V. Gotor, V. Gotor-Fernández, *Adv. Synth. Catal*., **2012**, *354*, 3405−3408.

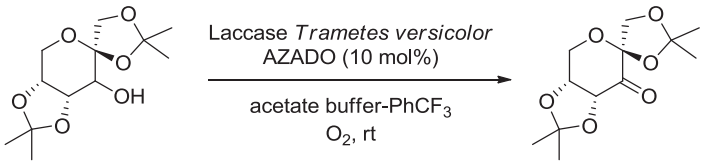


Scheme 3.1.1.6

Fabbrini *et al*.[89b] showed that the laccase-TEMPO system can also be applied to the oxidation of secondary alcohols as 1-(4-methoxyphenyl)ethanol, 1-(4-methoxyphenyl)propan-1-ol and cyclohexanol. The corresponding ketones were obtained with 85, 95, and 35% conversion, respectively, clearly indicating the preference towards benzylic substrates. Reactions were performed using the laccase from *Trametes villosa* and TEMPO (33 mol%) in citrate buffer at pH 4.5.

The oxidation of different complex and highly functionalized secondary alcohols was achieved by using laccase from *T. versicolor* in combination with the less-hindered nitroxyl radical AZADO.[94] With these substrates TEMPO was ineffective and very low conversions were attained (Scheme 3.1.1.7).

[94] C. Zhu, Z. Zhang, W. Ding, J. Xie, Y. Chen, J. Wu, X. Chen, H. Ying, *Green. Chem*., **2014**, *16*, 1131−1138.



Scheme 3.1.1.7

Other enzymes such as ADHs can be used for the oxidation of alcohols.[85] However, due to the excellent selectivity offered by ADHs, when a racemic alcohol is used, two enzymes with opposite stereopreference become compulsory. These enzymes require the cofactor $NAD(P)^+$ as the electron acceptor,[95] and a cofactor recycling system is then mandatory for practical applications.

[95] W. Kroutil, H. Mang, K. Eddeger, K. Faber, *Adv. Synth. Catal.*, **2004**, *346*, 125−142.

3.1.2. Enzymatic transamination reaction. Multi-catalytic methodologies combining alcohol oxidation and enzymatic transamination

Optically active amines are among the most valuable compounds in chemistry, not only for their presence in a variety of natural products but also as intermediates in the synthesis of pharmaceuticals and agrochemicals.[96] Currently, biocatalysis offers a variety of enzymes for amine synthesis,[97] among them transaminases being one of the most effective catalysts.[65a, 98]

Transaminases (TAs) are pyridoxal-5'-phosphate (PLP) dependent enzymes. The cofactor PLP, which is used in catalytic amount, serves thereby as molecular shuttle for ammonia and electrons, reversibly transferring an amino group between a suitable amine donor and an acceptor (ketone or aldehyde), pyridoxamine-5'-phosphate (PMP) being the intermediate (Scheme 3.1.2.1). α-TAs exclusively convert α-amino and α-keto acids whereas omega-TAs can accept substrates with a distal carboxylate group. A subgroup of omega-TAs, known as amine TAs (ATAs) are capable of accepting substrates that completely lack a carboxylate group.

ATAs can be used for the kinetic resolution of a racemic primary amine (the preferred enantiomer being transformed into the carbonyl

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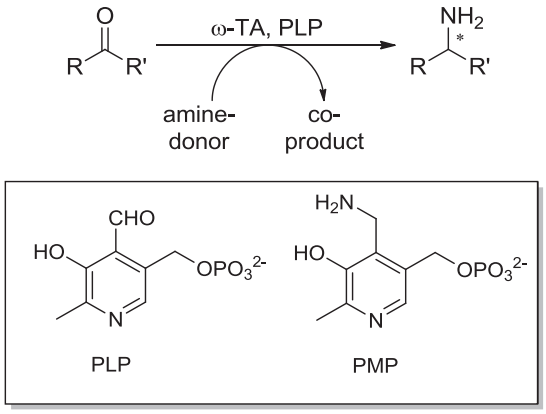
[96] a) D. J. Newman, G. M. Cragg, *J. Nat. Prod*., **2016**, *79*, 629−661. b) S. K. Talapatra, B. Talapatra, *Chemistry of Plant Natural Products*, Springer-Verlag: Berlin, Heidelberg, **2015**.

[97] a) W. Kroutil, E.-M. Fischereder, C. S. Fuchs, H. Lechner, F. G. Mutti, D. Pressnitz, A. Rajagopalan, J. H. Sattler, R. C. Simon, E. Siirola, *Org. Process Res. Dev*., **2013**, *17*, 751−759; b) H. Kohls, F. Steffen-Munsberg, M. Höhne, *Curr. Opin. Chem. Biol*., **2014**, *19*, 180−192.

[98] a) D. Koszelewski, K. Tauber, K. Faber, W. Kroutil, *Trends Biotechnol*., **2010**, *28*, 324−332; b) S. Mathew, H. Yun, *ACS Catal*., **2012**, *2*, 993−1001; c) J. Ward, R. Wohlgemuth, *Curr. Org. Chem*., **2010**, *14*, 1914−1927; c) D. Zhu, L. Hua, *Biotechnol. J*., **2009**, *4*, 1420−1431.

compound) or, more interesting, for the asymmetric reductive amination of a carbonyl compound which allows converting prochiral ketones and aldehydes in quantitative yield. The second process suffers mostly from unfavorable equilibrium which lies on the side of the starting materials. However, this issue can be circumvented either by utilizing a large excess of amine donor, a small excess of smart amine donors,[99] or removal of the coproduct, for example, by using of a multienzyme network.[100]



Scheme 3.1.2.1

There are a number of examples involving transaminases in asymmetric synthesis of pharmaceutical ingredients.[101]

One of the most successful examples is that of the antidiabetic drug sitagliptin.[102] In this case, a homologue of the (*R*)-selective omega-TA

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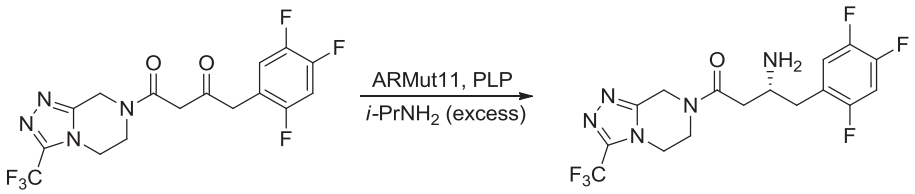
[99] a) B. Wang, H. Land, P. Berglund, *Chem. Commun.*, **2013**, *49*, 161−163; b) A. P. Green, N. J. Turner, E. O'Reilly, *Angew. Chem. Int. Ed*., **2014**, *53*, 10714−10717; c) D. Baud, N. Ladkau, T. S. Moody, J. M. Ward, H. C. Hailes, *Chem. Commun*., **2015**, *51*, 17225−17228; d) L. Martínez-Montero, V. Gotor, V. Gotor-Fernández, I. Lavandera, *Adv. Synth. Catal*., **2016**, *358*, 1618−1624; e) A. Gromm, W. Lewis, A. P. Green, E. O'Reilly, *Chem. Eur. J.*, **2016**, *22*, 12692−12695; f) S. E. Payer, J. H. Schrittwieser, W. Kroutil, *Eur. J. Org. Chem*., **2017**, *17*, 2553−2559.

[100] R. C. Simon, N. Richter, E. Busto, W. Kroutil, *ACS Catal*., **2014**, *4*, 129−143.

[101] For a complete review on the application of ATA in the pharmaceutical industry, see: S. A. Kelly, S. Pohle, S. Wharry, S. Mix, C. C. R. Allen, T. S. Moody, B. F. Gilmore, *Chem. Rev*., **2018**, *118*, 349−367.

ATA-117 from *Arthrobacter sp*. was used in the asymmetric synthesis of chiral amine sitagliptin from its corresponding prochiral ketone, prositagliptin (Scheme 3.1.2.2).



Scheme 3.1.2.2

Initially the enzyme was unable to bind to the substrate due to steric constraints caused by the bulky side groups of the prositagliptin ketone. Through use of in silico design and directed evolution, a substrate walking approach was employed to engineer the large binding pocket of the enzyme's active site, with further evolution of the enzyme directed at improving activity toward prositagliptin. The resultant effect was a reaction which proceeded with 92% yield (>99.95% *ee*), contributing to a process providing sitagliptin with a 10−13% increase in overall yield, 53% increase in productivity and 19% reduction in total waste.

The same evolved variant was used in the asymmetric synthesis of the dual orexin receptor antagonist, sovurexant.[103] Amination of the prochiral ketone catalyzed by transaminase with subsequent ring annulation, afforded the diazepane ring, a key feature of sovurexant (Scheme 3.1.2.3).

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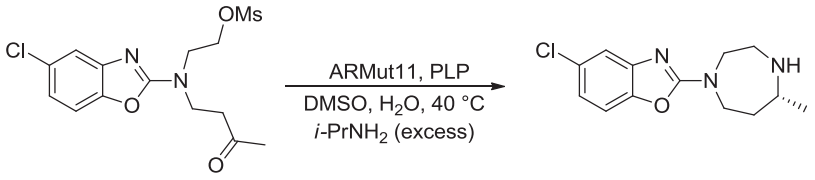
[102] C. K. Savile, J. M. Janey, E. C. Mundorff, J. C. Moore, S. Tam, W. R. Jarvis, J. C. Colbeck, A. Krebber, F. J. Fleitz, J. Brands, P. N. Devine, G. W. Huisman, G. J. Hughes, *Science*, **2010**, *329*, 305−309.

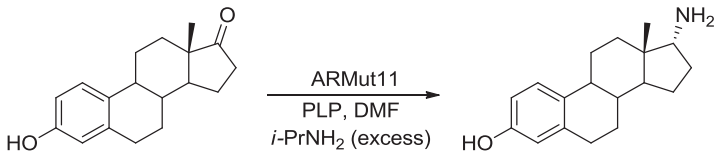
[103] I. K. Mangion, B. D. Sherry, J. Yin, F. J. Fleitz, *Org. Lett*., **2012**, *14*, 3458−3461.



Scheme 3.1.2.3

The starting ketone was added to the reaction medium via a syringe pump over 6 h in order to avoid the formation of other side cyclic compounds.

17-α-Amino steroids are interesting non-natural products used as intermediates in the preparation of pharmacologically active steroids.[104] The (*R*)-selective ARMut11 was used for the preparation of three analogous to the amine in Scheme 3.1.2.4. This enzymatic protocol enabled a 9-fold yield increase and a reduction in processing steps from three to one versus the existing conventional approach.[105]



Scheme 3.1.2.4

The selected examples are just a part of the contributions that have been reported during the last years about the synthesis of optically pure amines.[106] Different research groups are devoting a great effort for the

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[104] a) J. Geisler, H. Sasano, S. A. Chen, A. Purohit, *J. Steroid Biochem. Mol. Biol*., **2011**, *125*, 39–45; b) R. Maltais, D. Poirier, *Steroids*, **2011**, *76*, 929–948; c) Y. A. Mostafa, S. D. Taylor, *Bioorg. Med. Chem*., **2012**, *20*, 1535–1544.

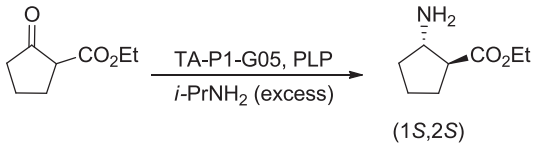
[105] N. Richter, R. C. Simon, W. Kroutil, J. M. Ward, H. C. Hailes, *Chem. Commun*., **2014**, *50*, 6098−6100.

[106] For recent examples of synthesis of precursors of pharmaceuticals, see: a) Y. Feng, Z. Luo, G. Sun, M. Chen, J. Lai, W. Lin, S. Goldmann, L. Zhang, Z. Wang**,** *Org. Process Res. Dev.*, **2017**, *21*, 648–654; b) N. Yasuda, E. Cleator, B. Kosjek, J. Yin, B. Xiang, F. Chen, S. C. Kuo, K. Belyk, P. R. Mullens, A. Goodyear, J. S. Edwards, B. Bishop, S.

development of innovative methods and the discovery of novel transaminases with unreported capabilities.[107]

On the other hand, in contrast to the case for dynamic reductive kinetic resolutions, reports of dynamic asymmetric transaminations involving two chiral centres are still very scarce.

Gotor *et al.* [108] studied the transamination reaction of ethyl 2-oxocyclopentanecarboxylate and the desired optically pure (1*S*,2*S*)-amino esther was obtained with 58% yield and 98% diastereomeric excess (Scheme 3.1.2.5).



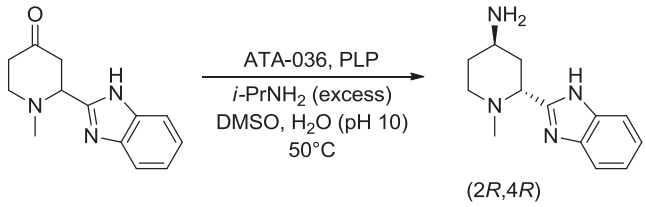
Scheme 3.1.2.5

Ceglia, J. Belardi, L. Tan, Z. J. Song, L. Di Michele, R. Reamer, F. L. Cabirol, W. L. Tang, G. Liu, *Org. Process Res. Dev.*, **2017**, *21*, 1851–1858; c) M. Burns, C. A. Martinez, B. Vanderplas, R. Wisdom, S. Yu, R. A. Singer, *Org. Process Res. Dev.*, **2017**, *21*, 871–877; d) D. L. Hughes, *Org. Process Res. Dev.*, **2017**, *21*, 1227–1244; e) C. S. Fuchs, J. E. Farnberger, G. Steinkellner, J. H. Sattler, M. Pickl, S. C. Simon, F. Zepeck, K. Gruber, W. Kroutil, *Adv. Synth. Catal*., **2018**, *360*, 768−778.

[107] a) S. P. France, G. A. Aleku, M. Sharma, J. Mangas-Sanchez, R. M. Howard, J. Steflik, R. Kumar, R. W. Adams, I. Slabu, R. Crook, G. Grogan, T. W. Wallace, N. J. Turner, *Angew. Chem. Int. Ed*., **2017**, *56*, 15589−15593; b) V. Erdmann, D. Rother, B. R. Lichman, J. Zhao, H. C. Hailes, R. C. Simon, W. Kroutil, J. M. Ward, *Angew. Chem. Int. Ed*., **2017**, *56*, 12503−12507; c) M. S. Weiss, I. V. Pavlidis, U. T. Bornscheuer, P. Spurr, S. P. Hanlon, B. Wirz, H. Iding, *ChemBioChem*, **2017**, *18*, 1022−1026; d) I. Slabu, J. L. Galman, R. C. Lloyd, N. J. Turner, *ACS Catal*., **2017**, *7*, 8263−8284; e) S. Calvelage, M. Dörr, M. Höhne, U. T. Bornscheuer, *Adv. Synth. Catal*., **2017**, *359*, 4235−4243; f) M. López-Iglesias, D. González-Martínez, M. Rodríguez-Mata, V. Gotor, E. Busto, W. Kroutil, V. Gotor-Fernandez, *Adv. Synth. Catal*., **2017**, *359*, 279−291; g) A. Mourelle-Insua, L. A. Zampieri, I. Lavandera, V. Gotor-Fernández, *Adv. Synth. Catal*., **2018**, *360*, 686−695; g) A. W. H. Dawood, R. O. M. A. de Souza, U. T. Bornscheuer, *ChemCatChem*, **2018**, *10*, 951−955; h) L. Marx, N. Ríos-Lombardía, J. F. Farnberger, W. Kroutil, A. I. Benitez-Mateos, F. Lopez-Gallego, F. Morís, J. González-Sabín, P. Berglund, *Adv. Synth. Catal*., **2018,** doi:10.1002/adsc.201701485.

[108] A. Cuetos, I. Lavandera, V. Gotor, *Chem. Commun*., **2013**, *49*, 10688−10690.

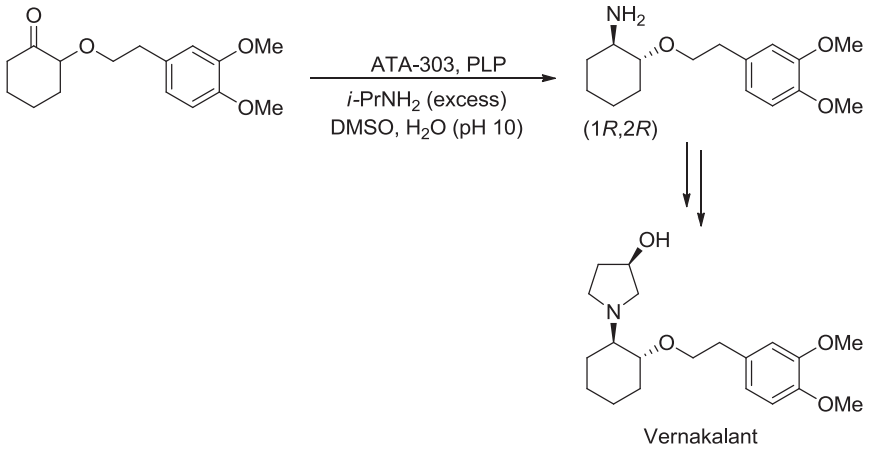
Another successful example was reported for the asymmetric synthesis of a smoothened receptor inhibitor, through enzymatic transamination and DKR of 4-piperidone.[109] ATA-036 afforded (2*R*,4*R*)-amine in 85% assay yield with an *anti*/*syn* ratio of >10:1 and >99% *ee* (Scheme 3.1.2.6).



Scheme 3.1.2.6

In an example reported by Merck,[110] the highly enantio- and diastereoselective bioamination of a 2-[2-(3,4-dimethoxyphenyl)ethyl]-cyclohexanone (Scheme 3.1.2.7) was the key asymmetric step toward vernakalant, an antiarrythmic drug for atrial fibrillation. The (1*R*,2*R*)-amine was obtained in 85% assay yield, 95:1 *dr*, and >99% *ee*.

[109] Z. Peng, J. W. Wong, E. C. Hansen, A. L. A. Puchlopek-Dermenci, H. J. Clarke, *Org. Lett.*, **2014**, *16*, 860−863.
[110] J. Limanto, E. R. Ashley, J. Yin, G. L. Beutner, B. T. Grau, A. M. Kassim, M. M. Kim, A. Kaplars, Z. Liu, H. R. Strotman, M. D. Truppo, *Org. Lett*., **2014**, *16*, 2716−2719.



Scheme 3.1.2.7

Transaminases have been implied in various types of cascade sequences.[16a] As alcohols are relatively common starting materials for chemical reactions,[111] the chemical community has focused its attention on the development of multistep approaches for the one-pot conversion of alcohols into amines.[112]

Kroutil *et al*.[113] had reported the amination of primary alcohols via a redox-self-sufficient hydrogen-borrowing cascade (Scheme 3.1.2.8). The oxidation of the primary alcohol by means of an ADH was coupled with a transaminase that catalyzed the reductive amination of the aldehyde. In this amination step, L-alanine was the amino source and, to shift the equilibrium towards the product, pyruvic acids was removed by the action of L-alanine dehydrogenase (AlaDH), which ensures reusability of the hydride abstracted in the oxidation step. Only alcohol dehydrogenases could be employed, since they can store the hydride at NAD(P)H.

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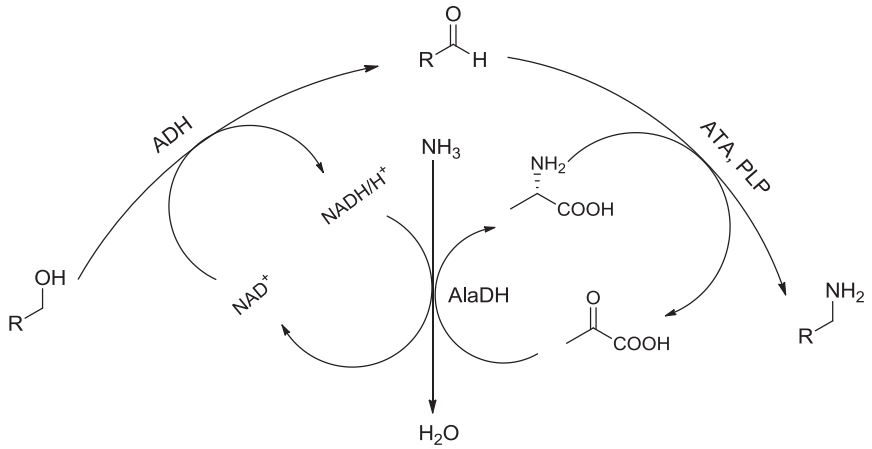
[111] A. J. A. Watson, J. M. J. Williams, *Science*, **2010**, *329*, 635−636.
[112] K. Barta, P. C. Ford, *Acc. Chem. Res*., **2014**, *47*, 1503−1512.
[113] J. H. Sattler, M. Fuchs, K. Tauber, F. G. Mutti, K. Faber, J. Pfeffer, T. Haas, W. Kroutil, *Angew. Chem. Int. Ed*., **2012**, *51*, 9156−9159.



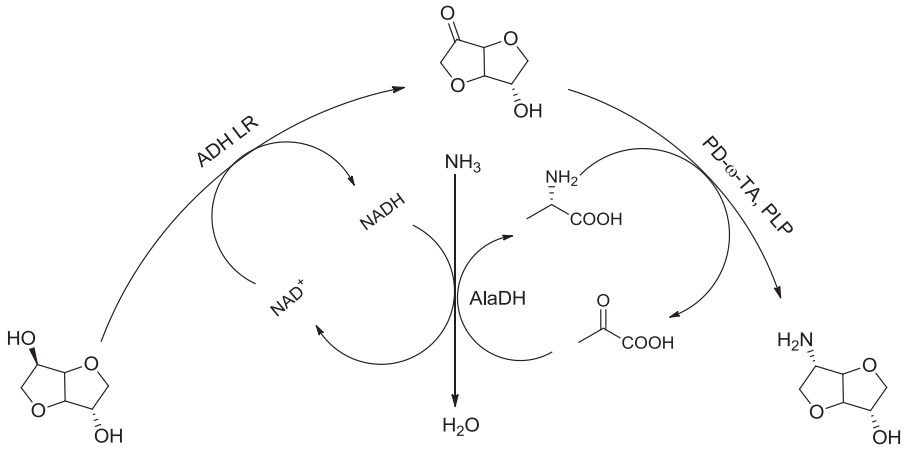
Scheme 3.1.2.8

For this reaction a thermostable ADH from *Bacillus stearothermophilus*, ATA from *Chromobacterium violaceum*, and AlaDH from *Bacillus subtilis* were used as biocatalysts and they were coexpressed to provide an *E. coli* single-cell catalyst.[114]

Studies aiming at extending the substrate scope to secondary alcohols revealed additional challenges. Racemic alcohol substrates, for instance, may require the use of two stereocomplementary alcohol dehydrogenases for their complete oxidation.[115] Furthermore, in contrast to primary alcohols, secondary alcohols were converted with maximum 64% conversion (cyclopentanol), indicating thermodynamic limitations. In a related study, the biocatalytic amination of isosorbide, a byciclic secondary diol obtainable from D-glucose, was investigated.[116] Choosing levodione reducase from *Leifsonia aquatica*, an engineered transaminase from *Paracoccus denitrificants* and AlaDH from *B. subtilis*, isosorbide was

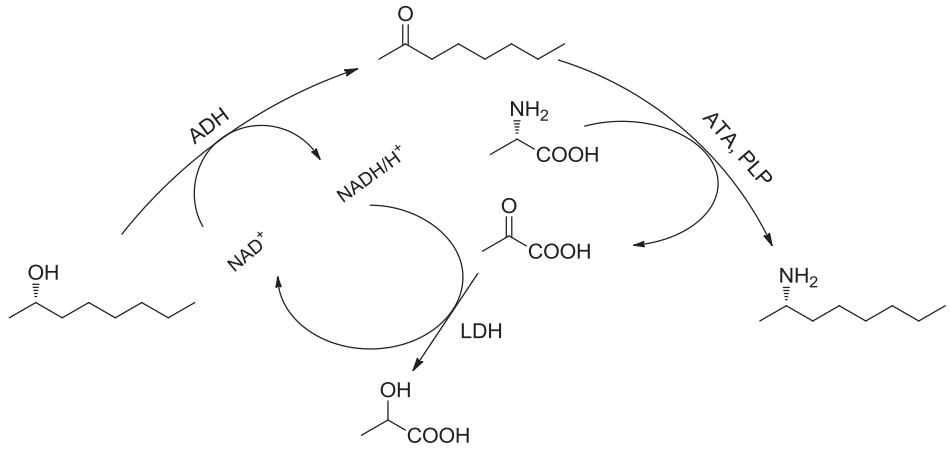
[114] S. Klatte, V. F. Wendisch, *Bioorg. Med. Chem*., **2014**, *22*, 5578−5585.
[115] K. Tauber, M. Fuchs, J. H. Sattler, J. Pitzer, D. Pressnitz, D. Koszelewski, K. Faber, J. Pfeffer, T. Haas, W. Kroutil, *Chem. Eur. J.*, **2013**, *19*, 4030−4035.
[116] A. Lerchner, S. Achatz, C. Rausch, T. Haas, A. Skerra, *ChemCatChem*, **2013**, *5*, 3374−3383.

transformed to the monoaminated (2*S*,5*S*)-amino alcohol with 7% conversion (Scheme 3.1.2.9).



Scheme 3.1.2.9

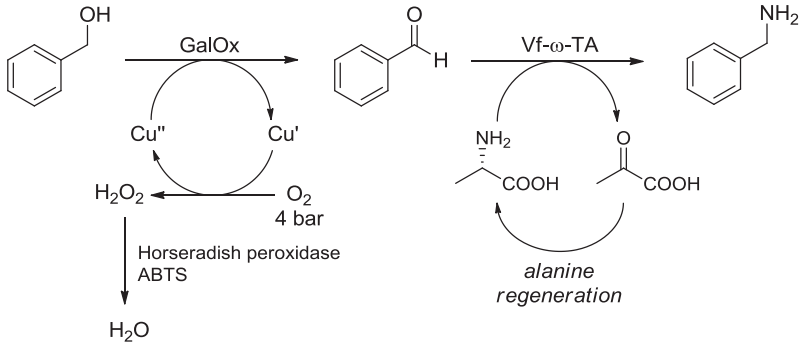
When using *E. coli* in hydrogen borrowing cascades with poorly reactive substrates, significant amounts of the intermediate ketone accumulated, which indicated an insufficient coupling of the oxidation and reductive amination reactions; this was attributed to the presence of NADH oxidase activity in *E. coli*, which consumed hydride equivalents.[115] To overcome these problems and to introduce a better thermodynamic driving force, a modified reaction system was designed using alanine as the only nitrogen source, which was transformed to pyruvate and further on to lactate as final coproduct; thus the AlaDH of the previous schemes got substituted by a lactate dehydrogenase (Scheme 3.1.2.10).[115] In this way, the levels of amine formation were improved, but the chiral centre of the *sec*-alcohol had to be considered, since a perfect enantioselective ADH could oxidize only 50% of the substrate. In order to improve the ketone formation, optically pure alcohols were also employed in combination with one enantioselective ADH, as in the case of (*S*)-2-octanol that was used as starting material in the cascade, to get the corresponding (*S*)-amine with 64% conversion and 96% *ee*. When non chiral *sec*-alcohols were used, as cyclopentanol and cyclohexanol, higher conversions were achieved (85 and 91% respectively).



Scheme 3.1.2.10

Alcohol oxidase can also be applied for oxidation of the alcohol consuming molecular oxygen and leading to hydrogen peroxide as coproduct. A variant of galactose oxidase (GalOx) from *Fusarium* sp. NRRL 2903 was used for alcohol oxidation and transaminases from *Vibro Fluvialis* or *P. denitrificants* for reductive amination (Scheme 3.1.2.11).[117] The formed hydrogen peroxide was removed in this case by horseradish peroxidase in combination with ABTS. Alanine, required for the amination step, was recycled employing AlaDH and GDH. For sufficient supply of molecular oxygen in the first step, the cascade was carried out in a pressurized chamber at 4 bar of oxygen. This set up represents an alternative methodology for the amination of primary benzylic alcohols and cinnamic alcohol, but it is not appropriate for the synthesis of optically pure amines.

[117] M. Fuchs, K. Tauber, J. Sattler, H. Lechner, J. Pfeffer, W. Kroutil, K. Faber, *RSC Adv*., **2012**, *2*, 6262−6265.



Scheme 3.1.2.11

For the amination of primary alcohols of medium chain length (C_4-C_{11}), the long chain alcohol oxidase from *Aspergillus fumigatus* was required. Quantitative conversion to the amine was observed for several substrates, but the reactions were run at a lower substrates concentration (10 mM).[118]

The previously described methodologies are highly efficient and the individual reaction steps of the cascades are interlinked not only by means of the transformed substrate, but also through the cofactors and cosubstrates. However, when chiral amines are needed, these strategies have the main drawback of requiring two enzymes with opposite stereopreference to fully convert the racemic alcohol into the intermediate ketone. For this reason, these examples find their main application for the synthesis of primary amines.

To overcome this limitation, a non-stereoselective oxidation of the racemic alcohol could represent a valid alternative to the fully enzymatic oxidation. More recently, the non-stereoselective oxidation catalyzed by laccase-TEMPO system has been coupled with omega-TAs for the transformation of racemic secondary benzylic alcohols into optically active amines (Scheme 3.1.2.12).[119]

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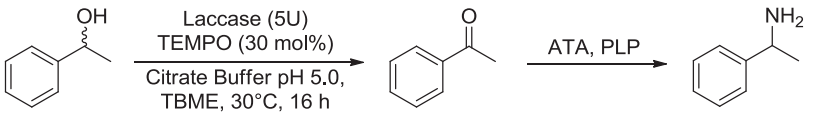
[118] M. Pickl, M. Fuchs, S. M. Glueck, K. Faber, *ChemCatChem*, **2015**, *7*, 3121−3124.
[119] L. Martínez-Montero, V. Gotor, V. Gotor-Fernández, I. Lavandera, *Green Chem*., **2017**, *19*, 474−480.



Scheme 3.1.2.12

In this case, the oxidation step was conducted in citrate buffer at pH 5.0 and TBME was used as co-solvent. In order to have a quantitative conversion into the corresponding ketone, TEMPO was used in 30 mol%. Once the first reaction was finished, all the components of the transamination reaction were added for a final concentration of 20 mM, pH 7.5 and the reaction was allowed to stir for 24 h at 30 °C. In this way, racemic *sec*-alcohols have been converted into enantioenriched amines with good to excellent selectivities (90-99% *ee*) and conversion values (67-99%).

On the basis of these findings, we envisaged to develop a new multi-catalytic method for the conversion of racemic alcohols into optically pure amines. The oxidation method should be efficient enough to completely oxidize the easy available racemic *trans*-2-(benzyloxy)cycloalkanols into the corresponding ketones, and the bioamination process should be deeply studied in order to find the conditions for a good dynamic kinetic resolution.

Results derived from this research will be exposed in the subsection 3.2.1. of Results and Discussion titled *Hybrid organo- and biocatalytic process for the asymmetric transformation of alcohols into amines*.

3.1.3. Deracemization of secondary alcohols: multi-enzymatic or chemoenzymatic methodologies

A variety of deracemization approaches have been successfully applied to the quantitative transformation of a racemic alcohol in an enantiopure compound.[120] These processes have attracted enormous interest because avoid the need to discard or recycle the unwanted enantiomer when a simple kinetic resolution is applied.[121]

As repeatedly mentioned along this thesis, ketoreductases are one of the most used enzymes in organic synthesis due to their availability and high stereoselectivity. As these enzymes promote either the selective oxidation of an alcohol or the reduction of the prochiral ketone, they have also been exploited to get the deracemization by stereoinversion of a range of secondary alcohols. In these cases, two enzymes with opposite stereopreference are required.

An option is to use microbial systems containing different redox enzymes, even two stereocomplementary ADHs within the cells which may be compartmentalized and have different cofactor requirements (NADH and NADPH), thus providing the thermodynamic driving force necessary for deracemization.[122]

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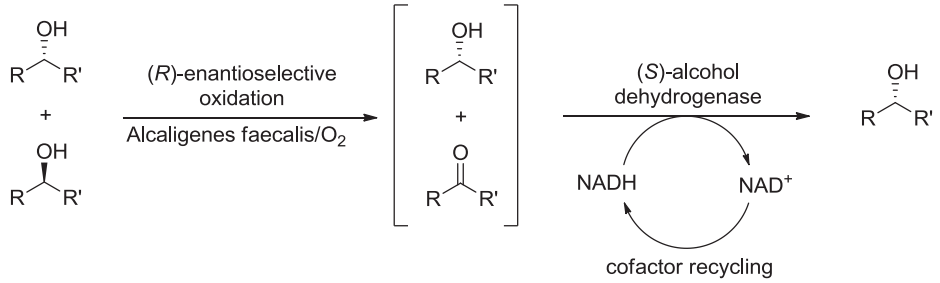
[120] For some reviews exemplifying different deracemisation processes, see: a) C. C. Gruber, I. Lavandera, K. Faber, W. Kroutil, *Adv. Synth. Catal.,* **2006**, *348*, 1789−1805; b) C. V. Voss, C. C. Gruber, W. Kroutil, *Synlett,* **2010**, 991−998; c) O. Verho, J. E. Bäckvall, *J. Am. Chem. Soc.,* **2015**, *137*, 3996−4009; d) A. Díaz-Rodríguez, I. Lavandera, V. Gotor, *Curr. Green Chem*., **2015**, *2*, 192−211; e) C. Moberg, *Acc. Chem. Res*., **2016**, *49*, 2736−2745.

[121] U. T. Bornscheuer, R. J. Kazlauskas in *Hydrolases in Organic Synthesis*, *2nd Ed.*, Wiley-VCH, Weinheim, **2006**, Chapters 5 and 6.

[122] For a recent review, see: a) A. Chadha, S. Venkataraman, R. Preetha, S. K. Padhi, *Bioorg. Chem*., **2016**, *68*, 187−213. For some examples, see: b) G. R. Allan, A. J. Carnell, *J. Org. Chem*., **2001**, *66*, 6495−6497; c) J. V. Comasseto, L. F. Assis, L. H. Andrade, I. H. Schoenlein-Crusius, A. L. M. Porto, *J. Mol. Catal. B: Enzym*., **2006**, *39*, 24−30; d) D. J. Palmeira, L. S. Araújo, J. C. Abreu, L. H. Andrade, *J. Mol. Catal. B: Enzym.,* **2014**, *110*, 117−125; e) Y. P. Xue, Y. G. Zheng, Y. Q. Zhang, J. L. Sun, Z. Q.

Kroutil *et al.* [123] reported a biocatalytic alcohol deracemization protocol involving the combination of freshly harvested cells from *Alcaligenes faecalis* DSM 13975 [for the enantioselective oxidation of the (*R*)-enantiomer] with a (*S*)-selective ADH and GDH/glucose as cofactor recycling system (Scheme 3.1.3.1). The use of the harvested cells with an intact cell membrane contributed to a sharp separation of the oxidation and reduction steps, and to the success of the method. The scalability of this protocol was also demonstrated on a preparative scale (up to 485 mg).



Scheme 3.1.3.1

Successively, Kroutil *et al.* [124] shown that NAD(P)H-dependent oxidation and reduction cycles could be run simultaneously in one pot without compartmental separation. In this case, enzymes with high cofactor specificity should be employed. Thereby, the (*S*)-enantiomer of the racemic alcohol was oxidized to the ketone, leaving the desired (*R*)-enantiomer untouched, or vice versa. By using a second stereocomplementary ADH, the intermediate ketone was reduced to the (*R*)-alcohol. In this system, oxidation and reduction worked concurrently with different cofactor pairs, and consequently, two specific cofactor recycling systems had to run simultaneously without interference: while $NADP^+$ was recycled by a

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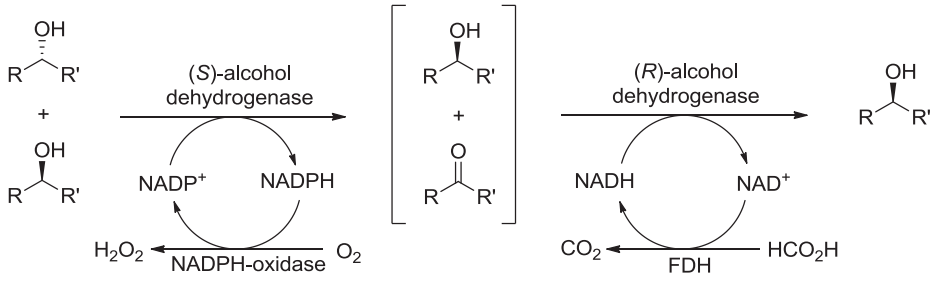
Liuab, Y. C. Shen, *Chem. Commun*., **2013**, *49*, 10706−10708; f) Y. P. Xue, H. Zeng, X. L. Jin, Z. Q. Liu, Y. G. Zheng, *Microb. Cell Fact.,* **2016**, *15*, 162−171.

[123] C. V. Voss, C. C. Gruber, W. Kroutil, *Angew. Chem. Int. Ed*., **2008**, *47*, 741–745.

[124] C. V. Voss, C. C. Gruber, K. Faber, T. Knaus, P. Macheroux, W. Kroutil, *J. Am. Chem. Soc*., **2008**, *130*, 13969−13972.

NADPH-dependent oxidase, NADH was recycled by a NAD^+ specific FDH (Scheme 3.1.3.2).



Scheme 3.1.3.2

In another contribution, Gotor *et al*. [125] reported a similar deracemization process based on the use of two stereocomplementary alcohol dehydrogenases from *R. ruber* and *L. brevis* with complementary cofactor preference ($NADH/NAD^+$ *vs* $NADPH/NADP^+$).

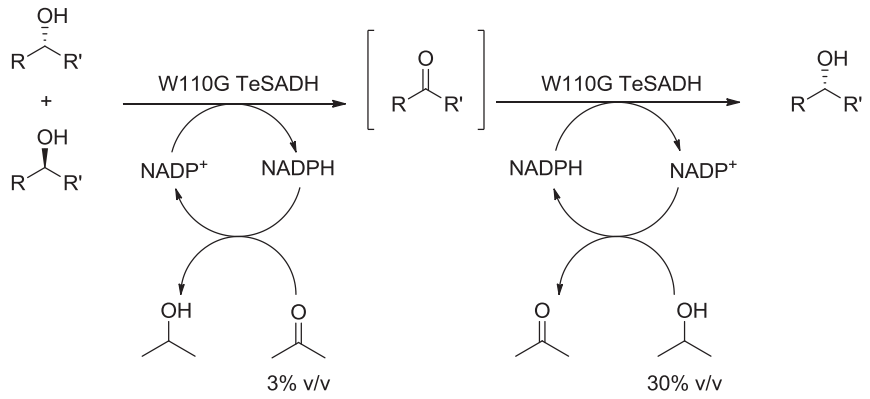
Recently, a single biocatalyst, a mutant of *Thermoanaerobacter ethanolicus* secondary alcohol dehydrogenase (TeSADH), enabled the non-stereoselective oxidation of racemic alcohols to ketones, followed by a stereoselective reduction process. By varying the amounts of acetone and 2-propanol cosubstrates, the authors controlled the stereoselectivities of the consecutive oxidation and reduction reactions, respectively (Scheme 3.1.3.3).[126]

The concentration of the cosubstrates was then crucial for the success of this protocol. In the oxidation step, a drastic drop of the enzymatic activity was registered at acetone concentrations above 3%, maybe attributed to the inhibitory effect of acetone on TeSADH at higher concentrations, as precipitation of enzyme was noticed. For the reduction

[125] C. E. Paul, I. Lavandera, V. Gotor-Fernández, W. Kroutil, V. Gotor, *ChemCatChem*, **2013**, *5*, 3875−3881.
[126] I. Karume, M. Takahashi, S. M. Hamdan, M. M. Musa, *ChemCatChem,* **2016**, *8*, 1459−1463.

step, various concentrations of 2-propanol were also tested, being 30% the optimal for a complete enantioselective reduction.

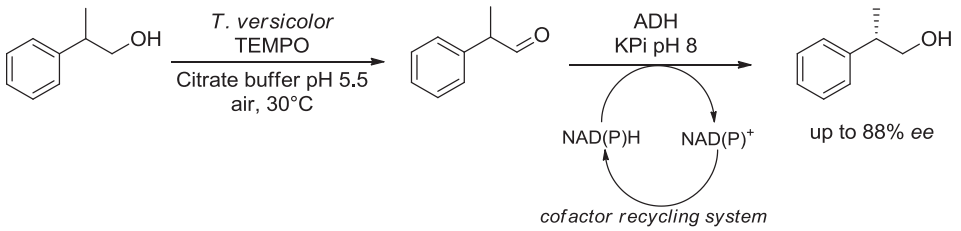
In this way, the intermediate ketones were fully converted into the optically active alcohols. However, the enantiomeric excess values obtained were, in general, moderate (20-87% *ee*) and only two of the five substrates investigated were isolated with very high *ee* (94 and >99%).



Scheme 3.1.3.3

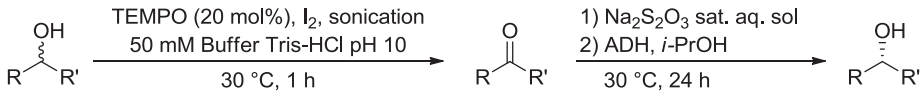
A mild one-pot methodology was also developed to deracemise *rac*-2-phenyl-1-propanol by combining the use of non-selective laccase/TEMPO-mediated oxidation with enantioselective bioreduction of the racemic aldehyde intermediate. [127] The cascade reaction consisted of aerobic oxidation mediated by laccase from *Trametes versicolor* and TEMPO, while the stereoselective reduction of the corresponding racemic aldehyde intermediate was mediated by an ADH under dynamic conditions. Both enantiomers were accessible by using two stereocomplementary ADHs, mainly HLADH and Evo-1.1.200 (Scheme 3.1.3.4).

[127] A. Díaz-Rodríguez, N. Ríos-Lombardía, J. H. Sattler, I. Lavandera, V. Gotor-Fernández, W. Kroutil, V. Gotor, *Catal. Sci. Technol.*, **2015**, *5*, 1443−1446.



Scheme 3.1.3.4

Another deracemization protocol was designed by combining different catalysts: the iodine/TEMPO system for the unselective oxidation of *sec*-alcohols followed by the bioreduction of the intermediate ketone mediated by ADH.[128] The oxidation reaction was carried out at 20 mM substrate concentration for 1 h in ultrasound, then an aqueous $Na_2S_2O_3$ saturated solution was added in order to destroy the iodine residues, followed by the addition of the components for the bioreduction reaction. In this way, after 24 h, the final optically pure alcohols were obtained with conversions over 90% (Scheme 3.1.3.5).



Scheme 3.1.3.5

[128] D. Méndez-Sánchez, J. Mangas-Sánchez, I. Lavandera, V. Gotor, V. Gotor-Fernández, *ChemCatChem,* **2015**, *7*, 4016−4020.

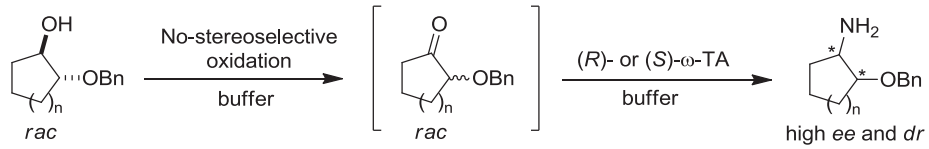
On the lights of these results, we decided to design a new multi-catalytic process for the deracemization of alcohols by employing an oxidation method in water and a subsequent bioreduction of the intermediate ketone mediated by ketoreductases. The protocol should be compatible with a large set of different alcohols and its efficiency would be also demonstrated for the conversion of *rac/meso*-diols into only one enantiomer.

Results derived from this research will be exposed in the subsection 3.2.2. of Results and Discussion titled *Deracemization of* sec-*alcohols*.

3.2. Results and discussion

3.2.1. Hybrid organo- and biocatalytic process for the asymmetric transformation of alcohols into amines

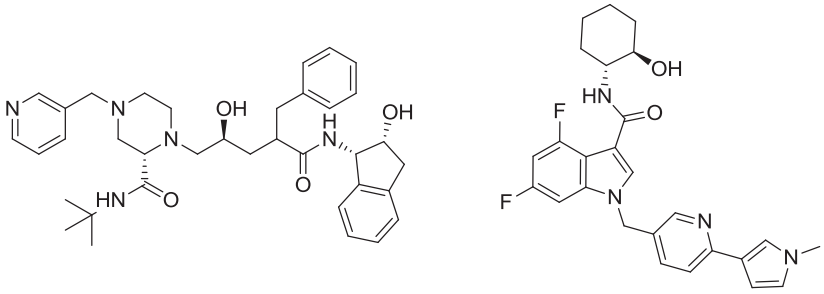
The aim of this project is the development of a water-compatible oxidation method to orchestrate a cascade process in aqueous medium. Ideally, the method should enable to convert the easily available racemic *trans*-2-(benzyloxy)cycloalkanols into optically active *cis*- or *trans*-2-(benxyloxy)cycloalkanamines (Scheme 3.2.1.1).



Scheme 3.2.1.1. Proposal of multi-catalytic oxidation-transamination cascade

These amine derivatives are highly valuable compounds since the β-amino alcohol moiety is ubiquitous in nature and the subfamily of β-aminocycloalkanols can be found in many natural products and synthetic bioactive molecules such as the antiarrhythmic agent vernakalant [129] (Scheme 3.1.2.7), the inhibitor of HIV indinavir[130a] or modulators of the M_1 muscarinic acetylcholine receptor[130b] (Figure 3.2.1.1).

[129] M. Finnin, *Am. J. Health-Syst. Pharm*., **2010**, *67*, 1157−1164.
[130] a) K. Izawa, T. Onishi, *Chem. Rev*., **2006**, *106*, 2811−2827; b) J. C. C. Dallagnol, E. Khajehali, E. T. van der Westhuizen, M. Jörg, C. Valant, A. G. Gonçalves, B. Capuano, A. Christopoulos, P. J. Scammells, *J. Med. Chem*., **2018**, *61*, 2875−2894.



Indinavir VU6004256

Fig. 3.2.1.1. Bioactive molecules

Starting racemic *trans*-2-(benzyloxy)cycloalkanols **1a** and **1b** were prepared by treatment of the corresponding cycloalkene oxide with benzyl alcohol. The selection of the benzylic ether in the structure of **1a,b** obeys to some practical reasons:

1. Benzyl group increases the synthetic value of the resulting protected cyclic β-aminoalcohol.

2. It is easily removed.

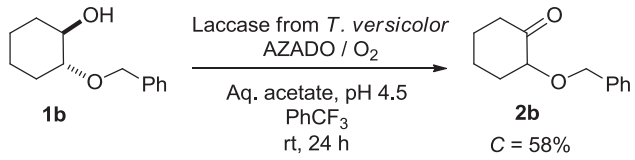
3. Benzyl group increases the hydrophobicity of the compound, thus facilitating the downstream processing based on extractions from aqueous media.

4. The inductive effect of the ether function exalts the acidity of the α-proton and it should facilitate the epimerization of the stereocenter, a prerequisite for a good DKR.

As a starting point, a highly efficient oxidation method of the 2-(benzyloxy)cycloalkanols had to be found. In order to check different reaction conditions, the oxidation of 2-(benzyloxy)cyclohexanol (**1b**) was selected as a model reaction.

Based on the promising results previously reported by applying the laccase-mediator system to the aerobic oxidation of benzylic alcohols, different reactions were planned with the use of various mediators and several laccases. Reaction conditions were analogous to the previously reported,[119] that is, laccase from *Trametes versicolor*, O_2, and TEMPO (33

mol%) in citrate buffer (50 mM, pH 5.0), at 30 ºC, and using TBME as a co-solvent. However, in these conditions, the starting **1b** was recovered unaltered. At that point, other nitroxyl-mediators (ABTS, AZADO, HAA, HOBT, NHPI, and VA) and co-solvents were tested and only in the reaction with AZADO and α,α,α-trifluorotoluene[131] as co-solvent, a little amount (12%) of 2-(benzyloxy)cyclohexanone (**2b**) was observed. After multiple attempts, the highest percentage (58%) of **2b** was achieved when *Trametes versicolor* laccase, 20 mol% of AZADO, O_2, acetate buffer pH 4.5, and $PhCF_3$ were used (Scheme 3.2.1.2).



Scheme 3.2.1.2. Oxidation of *trans*-(±)-1b promoted by laccase-AZADO system

From this preliminary study, AZADO had emerged as the best mediator, but the laccase/O_2 conditions resulted to be uneffective for the adequate acting of the mediator and thus, for the complete oxidation of this non-benzylic secondary alcohol.

Inspired by the pioneering research of Iwabuchi *et al*.,[80a] we tested other oxidant reagents in combination with the mediator (organocatalyst) AZADO. We selected the inexpensive aqueous NaOCl, which offers several benefits over traditional oxidants such as low material cost, mild reactions conditions and no metallic waste. Thus, a 0.40 M aqueous solution of NaOCl (pH 8.9)[132] was prepared from the commercially available aqueous solution of NaOCl (1.95 M, determined by reaction with potassium iodide in acetic acid and then, titration of the iodine liberated with sodium

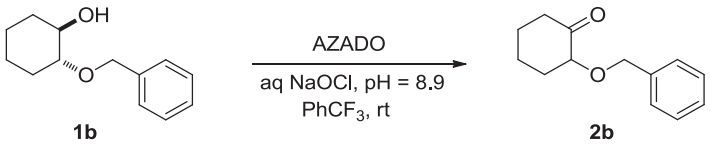
[131] A. Ogawa, D. P. Curran, *J. Org. Chem*., **1997**, *62*, 450−451.

thiosulfate[132]) by adding of solid $NaHCO_3$ and distilled water until the desired pH.

In a first attempt the oxidation of **1b** was essayed at room temperature in the freshly prepared aqueous solution of NaOCl (2.0 equiv) employing 5 mol% of AZADO and $PhCF_3$. Pleasantly, this oxidation system worked very efficiently, affording the ketone **2b** in quantitative yield in just 1 h at room temperature (Table 3.2.1.1, entry 1). Further parameterization included amount of NaOCl and organocatalyst loading. Thus, a decrease to equimolar amounts of NaOCl was detrimental and the conversion halted in 95% after 13 h (entry 2), being 1.2 equiv the minimum required (entry 3). On the other hand, AZADO retained its catalytic efficiency by using exclusively 1 mol% (entry 4). The conversion of alcohol into ketone was dependent on the catalyst loading, no ketone was formed in the absence of AZADO (entry 5) and lower amounts than 1 mol% of AZADO (0.1 and 0.5 mol%) lead to conversions of 76 and 94% (entries 6 and 7), even after longer reaction times. Besides, when AZADO was replaced by TEMPO under the optimal conditions, the conversion dropped to 51% after 24 h and did not evolve further (this result is not included in the Table). Lastly, other co-solvents of different nature were also checked (entries 8-11) but $PhCF_3$ remained as the optimal.

[132] T. Okada, T. Asawa, Y. Sugiyama, T. Iwai, M. Kirihara, Y. Kimura, *Tetrahedron*, **2016**, *72*, 2818−2827.

Table 3.2.1.1. AZADO-catalyzed oxidation of 1b with aqueous NaOCl[a]

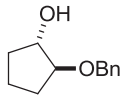
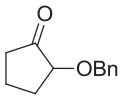
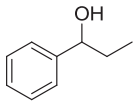
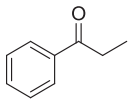
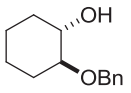
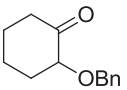
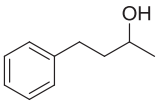
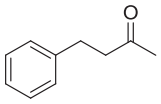
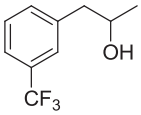
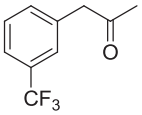
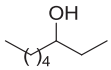
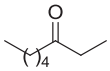
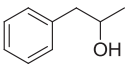
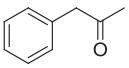
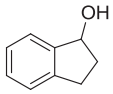
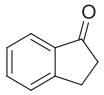
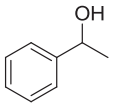
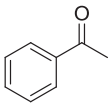
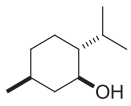
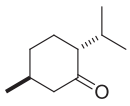


Entry	AZADO (equiv)	aq NaOCl (equiv)	Co-solvent (% v/v)	Reaction time (h)	*C* (%)
1	0.05	2.0	$PhCF_3$ (15)	1	>95
2	0.05	1.0	$PhCF_3$ (30)	13	95
3	0.05	1.2	$PhCF_3$ (25)	1	>95
4	**0.01**	**1.2**	**$PhCF_3$ (25)**	**1**	**>95**
5	-	1.2	$PhCF_3$ (25)	24	-
6	0.001	1.2	$PhCF_3$ (25)	14	76
7	0.005	1.2	$PhCF_3$ (25)	1.5	94
8	0.01	1.2	CH_2Cl_2 (40)	1	94
9	0.01	1.2	DMSO (25)	1	0
10	0.01	1.2	AcOEt (25)	1	91
11	0.01	1.2	CH_3CN (25)	1	92

[a] Reactions were carried out using 50 μmol of **1b**. Conversion degree (*C*) were determined by ^{1}H-NMR. Best conditions are highlighted in black.

In order to study the scope of this oxidation process, we applied this methodology to a variety of cyclic and acyclic alcohols (Table 3.2.1.2). All the substrates included in the study underwent complete oxidation to afford the corresponding ketones in excellent yields [>95% in all the cases except for **2h** and **2j** (92%)] after 1 h.

Table 3.2.1.2. Oxidation of alcohols 1a-1j with aq. NaOCl and AZADO[a]

Alcohol	NaOCl (equiv)	Ketone	Alcohol	NaOCl (equiv)	Ketone
 **1a**	1.2	 **2a**	 **1f**	1.2	 **2f**
 **1b**	1.2	 **2b**	 **1g**	1.2	 **2g**
 **1c**	1.2	 **2c**	 **1h**	1.4	 **2h**
 **1d**	1.4	 **2d**	 **1i**	1.0	 **2i**
 **1e**	1.2	 **2e**	 **1j**	1.4	 **2j**

[a] Alcohol (100 μmol), AZADO (1.0 μmol), $PhCF_3$ (100 μL), 0.40 M aq NaOCl (pH 8.9), rt (except reaction with **1d** at 5 ºC), 1 h.

Reaction conditions were only slightly modified in some cases to avoid over-oxidation products. Thus, when **1d** was allowed to react at room temperature with 1.2 equiv of NaOCl, the degree of conversion was only 79% and besides **2d**, a mixture of 1-chloro- and 1,1-dichloro-1-phenylpropan-2-ones was also observed. However, more than 95% of conversion was achieved lowering the temperature up to 5 ºC but using 1.4 equiv of oxidant. In contrast, the oxidation of 1-indanol (**1i**) was carried out

with equimolecular amount of NaOCl in order to avoid the formation of 3,3-dichloroindan-1-one as a side-product. In the other cases, no noticeable over-oxidation products were detected under the optimized conditions.

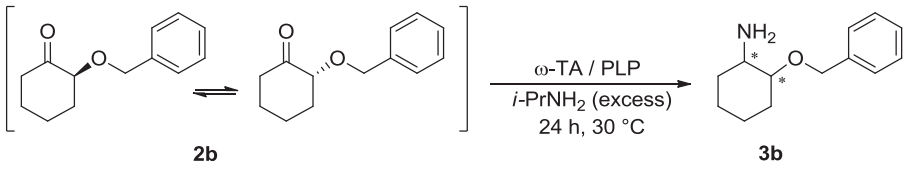
Next, the second step of the proposed cascade (Scheme 3.2.1.1), the reductive bioamination of the ketones **2**, was considered. Initially, we used both 2-(benzyloxy)cyclohexanone (**2b**) and its cyclopentylic homologue **2a** to check the reaction conditions. The main feature of these compounds relies on the liability of their chiral centre which is in α-position to both carbonyl and benzyloxy groups. Thus, they are potentially amenable to racemize at slightly basic pH and trigger a DKR which could allow to attain the amine product with 100% theoretical yield. Likewise, the bioamination process would deliver two contiguous stereocenters, with four possible stereoisomers.

Reductive amination of ketones **2a** and **2b** were tested with a series of 28 commercially available omega-TAs (from Codexis) and also with four omega-TAs overexpressed in *E.coli*: the *S*-selective TAs from *Chromobacterium violaceum* (Cv) and (*S*)-*Arthrobacter* (ArS) and the *R*-selective TAs from (*R*)-*Arthrobacter* (ArR) and its evolved variant ArRmut11. Reactions were carried out at 10 mM substrate concentration, with PLP (1.0 mM), and in the presence of a great excess of isopropylamine as amino donor.

A selection of the best results obtained in the reactions starting from ketone **2b** is shown in Table 3.2.1.3. Initially, under the standard conditions in phosphate buffer at pH 7.5 most of omega-TAs were very active and high conversions were reached after 24 h. More interestingly, all the omega-TAs displayed perfect asymmetry in the amination of the carbonyl group of **2b**, as it is deduced analyzing the C-1 configuration and the *ee* >99% obtained for both *cis*- and *trans*-2-(benzyloxy)cyclohexanamines **3b** in each reaction (entries 1-4). In addition, the diastereomeric ratios obtained for those reactions with $C > 90\%$ were low, meanwhile moderate or high *dr* (up to 88:12) was associated to reactions with lower conversion degree ($C \leq 69\%$).

With the aim of accelerating the racemization of **2b** and improving the diastereoselectivity of the process, the bioamination of **2b** was checked at higher pH values (8.5, 10, and 11.5). In general, a basic pH such as 10 or 11.5 was detrimental for the stability of omega-TAs, but a noteworthy increase in the *dr* was observed in some cases. Thus, changing pH from 8.5 to 10, the *dr* was increased until 6:1 and 7:1 in the reactions mediated by ATA-254 and ATA-P2-A07, respectively (entries 6 and 8). Similarly, ATA-412 displayed an enhanced selectivity at pH 11.5 despite a lower catalytic activity (entry 12). More interestingly, the ATA-303-catalyzed amination experienced the most significant improvement, with the enzyme retaining its catalytic activity and exhibiting the highest selectivity of the series to yield enantiopure (1*R*,2*R*)-**3b** with 24:1 *dr* (entry 10). Because of the enantiocomplementarity of ATA-254 and ATA-P2-A07, both enantiomers of *cis*-**3b** can be obtained from these processes at pH 10.

Table 3.2.1.3. A selection of omega-TA-catalyzed transamination of 2b[a]

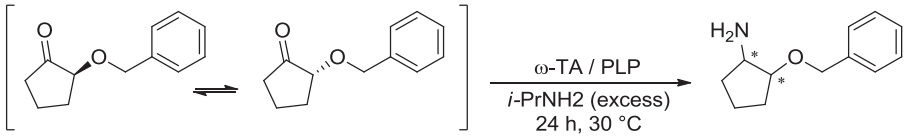


Entry	omega-TA	pH	*C* (%)	*dr* *cis:trans*	*ee* (%) *cis*	*ee* (%) *trans*
1	ATA-254	7.5	69	77:23	>99 (1*S*,2*R*)	>99 (1*S*,2*S*)
2	ATA-256	7.5	50	88:12	>99 (1*S*,2*R*)	-
3	ATA-303	7.5	>99	45:55	>99 (1*S*,2*R*)	>99 (1*S*,2*S*)
4	ATA-P1-G03	7.5	>99	67:33	>99 (1*S*,2*R*)	>99 (1*S*,2*S*)
5	ATA-254	8.5	>99	74:26	>99 (1*S*,2*R*)	>99 (1*S*,2*S*)
6	**ATA-254**	**10**	**90**	**85:15**	**>99 (1*S*,2*R*)**	>99 (1*S*,2*S*)
7	ATA-P2-A07	8.5	77	77:23	>99 (1*R*,2*S*)	>99 (1*R*,2*R*)
8	**ATA-P2-A07**	**10**	**90**	**87:13**	**>99 (1*R*,2*S*)**	>99 (1*R*,2*R*)
9	ATA-303	10	>99	32:68	>99 (1*R*,2*S*)	>99 (1*R*,2*R*)
10	**ATA-303**	**11.5**	**>99**	**4:96**	**-**	**>99 (1*R*,2*R*)**
11	ATA-412	10	>99	50:50	>99 (1*R*,2*S*)	>99 (1*R*,2*R*)
12	ATA-412	11.5	62	91:9	>99 (1*R*,2*S*)	-
13	ArS	10	>99	46:54	>99 (1*S*,2*R*)	>99 (1*S*,2*S*)
14	ArRmut11	10	>99	50:50	>99 (1*R*,2*S*)	>99 (1*R*,2*R*)
15	ArR	10	58	95:5	>99 (1*R*,2*S*)	-

[a] Reaction conditions: **2b** (10 mM), aq. KPi (100 mM, pH 7.5-11.5), PLP (1.0 mM), *i*-$PrNH_2$ (1.0 M), omega-TA [2.0 mg (ATA) or 5.0 mg for each mL of reaction mixture]. The most significant results taking into account *C*, *dr*, and *ee* values are highlighted in black.

Similarly, bioaminations of ketone **2a** were carried out at pH 7.5, 10 and 11. As shown in Table 3.2.1.4, we identified biocatalysts which preserved activity and gave rise to three of the four possible stereoisomers in high *dr* (up to 49:1) and >99% *ee*. ATA-303 was the most efficient catalyst towards the *trans*-diastereomer and (1*R*,2*R*)-**3a** was obtained with improved disatereoselectivity in the reaction at pH 11.5 (7:1 *dr*, and >99% *ee*, see entry 9). Regarding *cis*-diastereomer, significant enhances in the degree of conversion were achieved by raising the pH from 7.5 to 10 in the reactions with ATA-251 and ATA-P2-A01. Moreover, the *dr* continued being very high (19:1) with ATA-P2-A01 (entry 7). Also remarkable was the excellent *dr* (49:1) exhibited by ATA-117 at pH 10 (entry 5).

Table 3.2.1.4. A selection of omega-TA-catalyzed transamination of 2a[a]



2a **3a**

Entry	omega-TA	pH	*C* (%)	*dr* *cis:trans*	*ee* (%) *cis*	*ee* (%) *trans*
1	ATA-237	7.5	96	60:40	>99 (1*S*,2*R*)	>99 (1*S*,2*S*)
2	ATA-251	7.5	63	82:18	>99 (1*S*,2*R*)	>99 (1*S*,2*S*)
3	ATA-303	7.5	>99	48:52	>99 (1*R*,2*S*)	>99 (1*R*,2*R*)
4	ATA-P2-A01	7.5	59	>99:<1	>99 (1*R*,2*S*)	-
5	**ATA-117**	**10**	**90**	**98:2**	**>99 (1*R*,2*S*)**	-
6	**ATA-251**	**10**	**>99**	**86:14**	**>99 (1*S*,2*R*)**	>99 (1*S*,2*S*)
7	**ATA-P2-A01**	**10**	**>99**	**95:5**	**>99 (1*R*,2*S*)**	-
8	ATA-025	11.5	>99	81:19	>99 (1*R*,2*S*)	>99 (1*R*,2*R*)
9	**ATA-303**	**11.5**	**>99**	**12:88**	>99 (1*R*,2*S*)	**>99 (1*R*,2*R*)**
10	ArS	10	>99	27:73	>99 (1*S*,2*R*)	94 (1*S*,2*S*)
11	ArRmut11	10	>99	48:52	>99 (1*R*,2*S*)	>99 (1*R*,2*R*)
12	ArR	10	85	98:2	>99 (1*R*,2*S*)	-

[a] For reaction conditions, see Table 3.2.1.3. Highlighted in black are the most significant results.

Once both catalytic steps have been established, we took the challenge of performing a one-pot fully convergent approach furnishing optically active amines from the analogue racemic *trans*-cycloalkanols. At this point, a number of concerns should be addressed to implement such coupled process. A major challenge is the mutual compatibility of the involved catalysts as well as of their respective reaction conditions. In order to carry out some compatibility tests, we took 1-phenylpropan-1-ol (**1f**) as a model

substrate and investigated the impact of the biocatalyst (omega-TA) and its cofactor (PLP) on the AZADO/NaOCl system. Whereas omega-TA was innocuous, the presence of PLP caused a decrease of the conversion (78%) of the oxidation step. However, the most important observation was the deactivation of PLP in this medium.[133] In addition, it had been already found that AZADO is totally inhibited by DMSO, the co-solvent from the bioamination.

On the other hand, another issue to consider was the substrate concentration. Despite both steps are conducted under basic aqueous medium and mild temperature, the alcohol oxidation was optimized at higher than 200 mM substrate concentration, contrasting with the low concentration typically required by omega -TAs (10-20 mM). Thus, when the oxidation of **1f** was essayed under identical conditions as above but at lower concentration (10 mM), no ketone was produced even after addition of an excess of NaOCl (up to 8.4 equiv) and the use of a higher amount of AZADO (10 mol%).

At this point, the inhibitor effect of DMSO and the absence of oxidation at a low substrate concentration precluded the possibility of a one-pot concurrent reaction, while a stepwise process appeared more feasible.

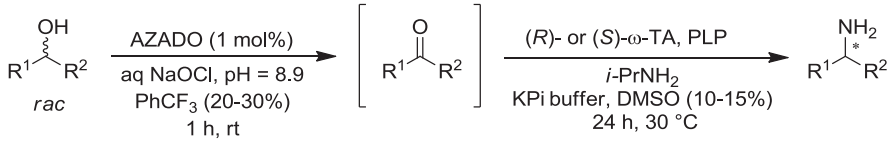
Accordingly, we firstly carried out the oxidation of *trans*-(±)-**1b** at 250 mM under the optimized conditions, namely 1 mol% of AZADO and 1.2 equiv of NaOCl. Once the oxidation was completed, the reaction mixture was diluted to 10 mM with a phosphate solution (KPi 100 mM, pH 11.5) containing *i*-$PrNH_2$ (1.0 M) and PLP (1.0 mM), and subsequently, DMSO (15% v/v) as a co-solvent and the omega -transaminase ATA-303 were added. Then, the reaction mixture was incubated during 24 h at 30 ºC and 250 rpm. After this time, HPLC analysis showed complete conversion

[133] To prove the deactivation of PLP, once the oxidation reaction was allowed to proceed during 1 h in the presence of PLP, all of the ingredients for the subsequent transamination step, except PLP, were added. In this case, no amine was formed and only a mixture of ketone and alcohol was observed. In addition, when pyridine-4-carbaldehyde, an analogue of PLP, was treated with 0.40 M aqueous NaOCl (pH 8.9), its oxidation to pyridine-4-carboxylic acid was totally produced after 12 h.

for the overall process, the corresponding *trans*-(1*R*,2*R*)-2-(benzyloxy)cyclohexanamine (**3b**) being isolated as the sole product with >99% *ee* and the same excellent *dr* (24:1) as that obtained starting from ketone **2b**. This result is collected in Table 3.2.1.5, entry 4. Interestingly, after a simple extraction-based work up, the optically active amine **3b** was recovered pure without need of further purification with very high yield (94%).

Once demonstrating the viability of this one-pot stepwise protocol, we extended the study to the cyclopentylic alcohol **1a** as well as the other alcohols used in the oxidation process (see Table 3.2.1.2). Previously, the activity of different omega-TAs was checked with substrates **1c-j** finding suitable biocatalysts for all alcohols except for **1i** and **1j**. Results obtained from the application of the oxidation-bioamination protocol are shown in Table 3.2.1.5.

Table 3.2.1.5. Chemoenzymatic transformation of racemic alcohols into optically active amines using AZADO/NaOCl and a omega-transaminase[a]



Entry	Substrate	omega-TA	Ratio (%)		*cis:trans*	*ee* (%)
			Ketone	Amine		
1	**1a**	ATA-303[d]	<1	>99	11:89	>99 (1*R*,2*R*)
2	**1a**	ArS[e]	<1	>99	27:73	94 (1*S*,2*S*)
3	**1a**	ATA-P2-A01[e]	<1	>99	95:5	>99 (1*R*,2*S*)
4	**1b**	ATA-303[d]	<1	>99	4:96	>99 (1*R*,2*R*)
5	**1c**	ATA-P2-B01	<1	>99		>99 (*R*)
6	**1c**	ArS	<1	>99		>99 (*S*)
7	**1d**	ATA-251	4	96		>99 (*S*)
8	**1d**	ArRmut11	<1	>99		>99 (*R*)
9	**1e**	ATA-P1-A06	20	80		>99 (*S*)
10	**1f**	ATA-033	13	87		>99 (*R*)
11	**1g**	ATA-P1-A06	<1	>99		>99 (*S*)
12	**1g**	ArR	<1	>99		>99 (*R*)
13	**1h**	ATA-033	15	85		>99 (*R*)

[a] Conditions analogous to that described in the text for **1b**. KPi buffer pH 7.5 was used for the second step except for some reactions (see footnotes d and e) [b] *cis:trans* ratio aplicable to the reactions starting from **1a** and **1b**. [c] *ee* and absolute configuration of the major stereoisomer. [d] pH 11.5. [e] pH 10.

All the starting alcohols were quantitatively oxidized in a concentration ranging from 200-300 mM.[134] The subsequent bioamination reaction was carried out with the omega-TA more adequate for each

[134] The differences between the concentration of each substrate are due to the different amount of NaOCl (see Table 3.2.1.2) used in each case. The oxidation step was carried at room temperature except for **1d** (5 ºC).

substrate,[135] at 10 mM substrate concentration. In these conditions, all omega-TAs worked exceptionally well leading to results (conversions, *ee*, and, for reactions with **1a**, also *dr* values) nearly identical to those measured in the corresponding single bioamination step.

As a result, fine-tuning the oxidation conditions and adequate biocatalyst selection provided a set of synthetically and pharmacologically interesting amines[136] with high overall yields (up to 97%) without need of chromatographic purification. Particularly remarkable is the simplicity of this chemoenzymatic setup, since the only required experimental setting, once the oxidation step is complete in 1 h, was a dilution of the reaction medium before the addition of the biocatalyst.

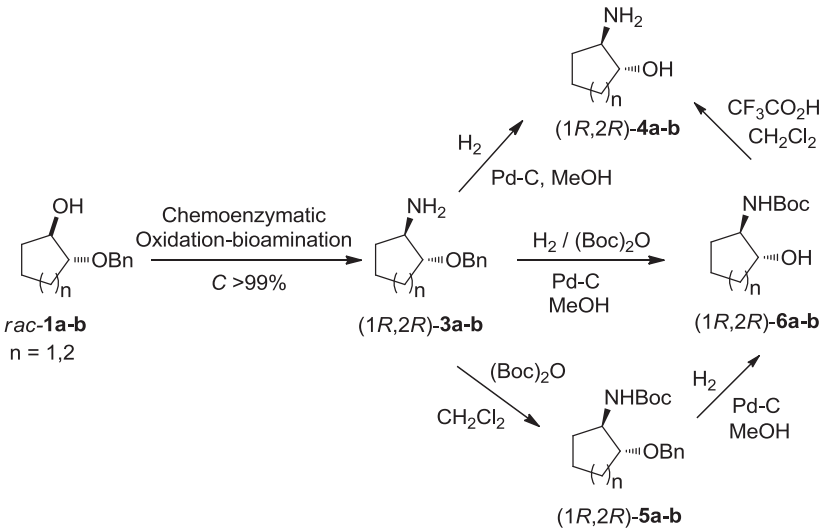
Finally, the synthetic utility of the chemoenzymatic platform developed herein was showcased by submitting the resulting optically active amino alcohol derivatives (1*R*,2*R*)-**3a** and (1*R*,2*R*)-**3b** to a panel of chemical transformations to provide a set of other valuable optically pure compounds (Scheme 3.2.1.3). First, a mild hydrogenolysis of **3a**,**b** furnished the free amino alcohols (1*R*,2*R*)-**4a**,**b** in quantitative yield. On the other hand, treatment of **3a**,**b** with di-*tert*-butyl dicarbonate led to the orthogonally protected derivatives (1*R*,2*R*)-**5a**,**b**. Actually, orthogonally protected amino alcohols are highly valuable molecules in both asymmetric catalysis and medicinal chemistry.[137] Debenzylation of **5a,b** gives entry to *N*-protected

[135] The results of the screenings of the different ω-TAs with all the ketone are available in electronic support.

[136] Optically active amine **3c** and its acetamide derivative are precursors of fenfluramine, an anorectic agent: a) J. Cerulli, B. M. Lomaestro, M. Malone, *Ann. Pharmacother*., **1998**, *32*, 88−102; b) G. Grignaschi, R. Samanin, *Eur. J. Pharmacol*., **1992**, *212*, 287−289. Amphetamine **3d** is a stimulant and anorectic agent: c) K. M. Taylor, S. H. Snyder, *Science*, **1970**, *168*, 1487−1489; d) G. T. Hajòs, S. Garattini, *J. Pharm. Pharmacol*., **1973**, *25*, 418−419. Optically active **3g** is a precursor of the antihypertensive agent labetalol: e) E. H. Gold, W. Chang, M. Cohen, T. Baum, S. Ehrreich, G. Johnson, N. Prioli, E. J. Sybertz, *J. Med. Chem*., **1982**, *25*, 1363−1370.

[137] a) S. Caron, R. W. Dugger, S. G. Ruggeri, J. A. Ragan, D. H. Brown Ripin, *Chem. Rev*., **2006**, *106*, 2943−2989; b) R. W. Dugger, J. A. Ragan, D. H. Brown Ripin, *Org. Process Res. Dev*., **2005**, *9*, 253−258.

amino alcohols (1*R*,2*R*)-**6a,b**, thus completing the synthesis of an interesting set of derivatives by means of quantitative and simple processes.



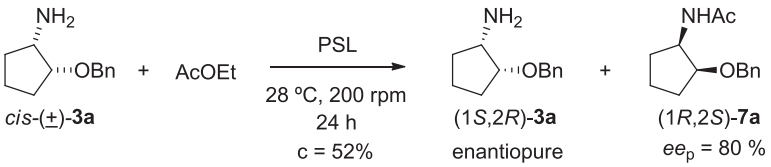
Scheme 3.2.1.3. Chemoenzymatic synthesis of optically active β-aminocycloalkanol derivatives

Assignment of the absolute configuration to the optically active amines

The absolute configuration of the isolated optically active amines *trans*-**3b**, **3c** and **3g** was assigned after comparison of the sign of the optical rotation of each sample with reported data (see experimental section). In addition, the absolute configuration of optically active amines *trans*-**3a** and **3d,e,f,h** was assigned by comparison of the chiral-HPLC chromatogram of the corresponding derivative with that obtained from a commercially available optically active sample (for *trans*-**3a**, **3e,** and **3f**) or by comparison with the previously published chiral-HPLC chromatogram (for **3d** and **3h**). In all cases these data were in good agreement with the stereopreference indicated in the specifications of the used omega-TAs.

The assignation of the absolute configuration of the optically active *cis*-**3a** required a more laborious procedure. This amine was always obtained partially impurified by its *trans*-diastereomer. The separation of both diastereomers was achieved by flash column chromatography, but required its previous transformation into the diastereomeric mixture of acetamides **7a**. As no data for optically active *cis*-**7a** were available, we decided to carry out its preparation by another route. For this, we planned the enzymatic resolution of a sample of diastereomerically pure *cis*-(±)-2-(benzyloxy)cyclopentanamine [*cis*-(±)-**3a**][138] by means of a lipase-catalyzed aminolysis reaction with ethyl acetate. Thus, the incubation of a solution of *cis*-(±)-**3a** with ethyl acetate in the presence of lipase from *Burkhoderia cepacia* (PSL-IM) yielded a mixture of optically active product (1*R*,2*S*)-**7a** and remaining substrate (1*S*,2*R*)-**3a** (Scheme 3.2.1.4). Both compounds were separated by a simple acid-base extraction and their *ee* measured by chiral-HPLC.

[138] www.entrechem.com



Scheme 3.2.1.1.1. Enzymatic hydrolysis of *cis*-(±)-2-(benzyloxy)cyclopentanamine

Although the (1*R*,2*S*) configuration for the produced acetamide **7a** could be assigned on the basis of the *R*-stereoselectivity commonly shown by this lipase in the aminolysis of other structurally related primary amines,[139] we also carried out the assignation of the (1*S*,2*R*) configuration to the remaining substrate by applying the Mosher's method.[33] Thus, treatment of *cis*-(−)-**3a** with (*S*)-MTPA-Cl gave the (*R*)-MTPA-amide derivative **8**, whose ^{1}H-NMR spectrum ($CDCl_3$, 600 MHz) is included in Figure 3.2.1.2.

[139] R. J. Kazlauskas, A. N. E. Weissfloch, A. T. Rappaport, L. A. Cuccia, *J. Org. Chem.*, **1991**, *56*, 2656–2665.

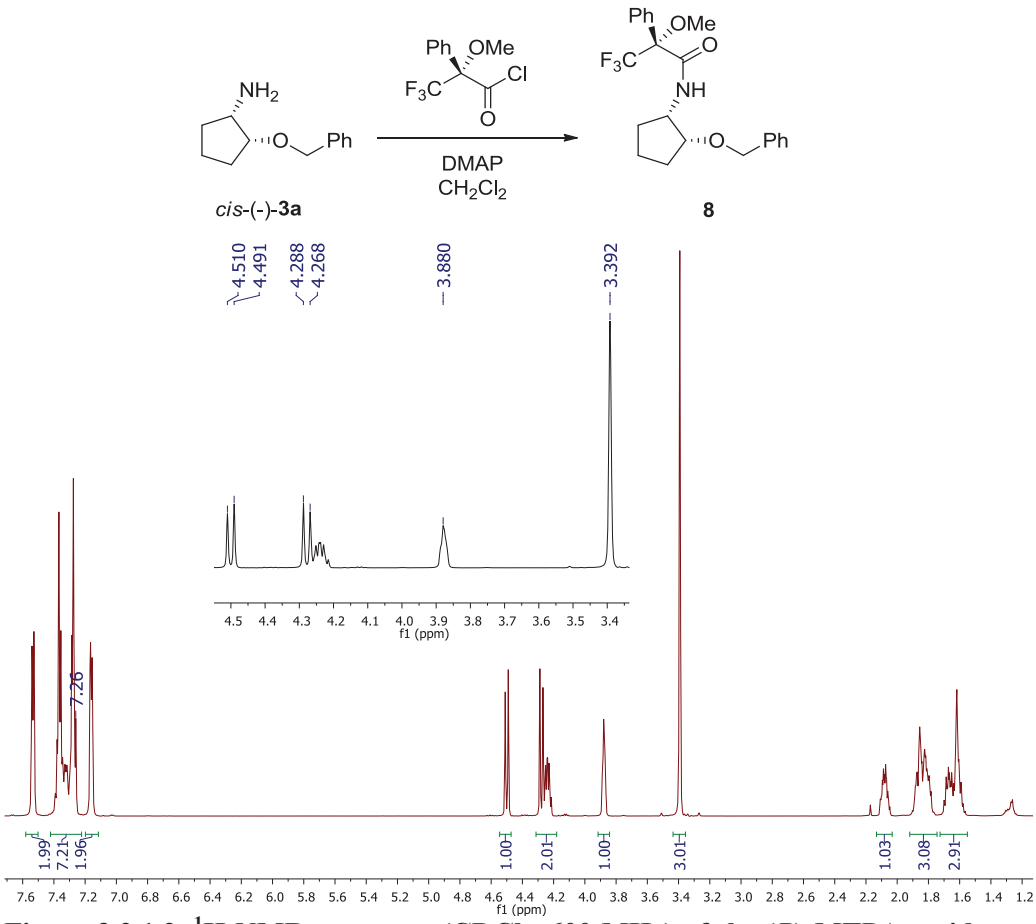


Figure 3.2.1.2. ^{1}H-NMR spectrum ($CDCl_3$, 600 MHz) of the (*R*)-MTPA amide 8 obtained from *cis*-(–)-3a

Similarly, a sample of *cis*-(±)-**3a** was allowed to react with (*S*)-MTPA-Cl to give the mixture of distereomers **8** (Figure 3.2.1.3). Taking the usual working models for these diastereomeric (*R*)-MTPA amides **8** into account,[33] and analyzing the chemical shifts for both diastereotopic protons in C*H*$_2$Ph, as well as for H-2 and OMe of each diastereomer, we have assigned the lowest δ values for the protons C*H*$_2$Ph and for H-2 of the diastereomer named as (*R*)-(1*S*,2*R*)-**8** due to the shielding effect that the phenyl group of the Mosher moiety exerts on these *syn* placed protons. In addition, we have assigned the lowest δ value for OMe of the diastereomer

(*R*)-(1*R,*2*S*)-**8** due to the shielding effect that the phenyl ring of the benzyl group of the amine moiety exerts on the *syn* placed OMe group.

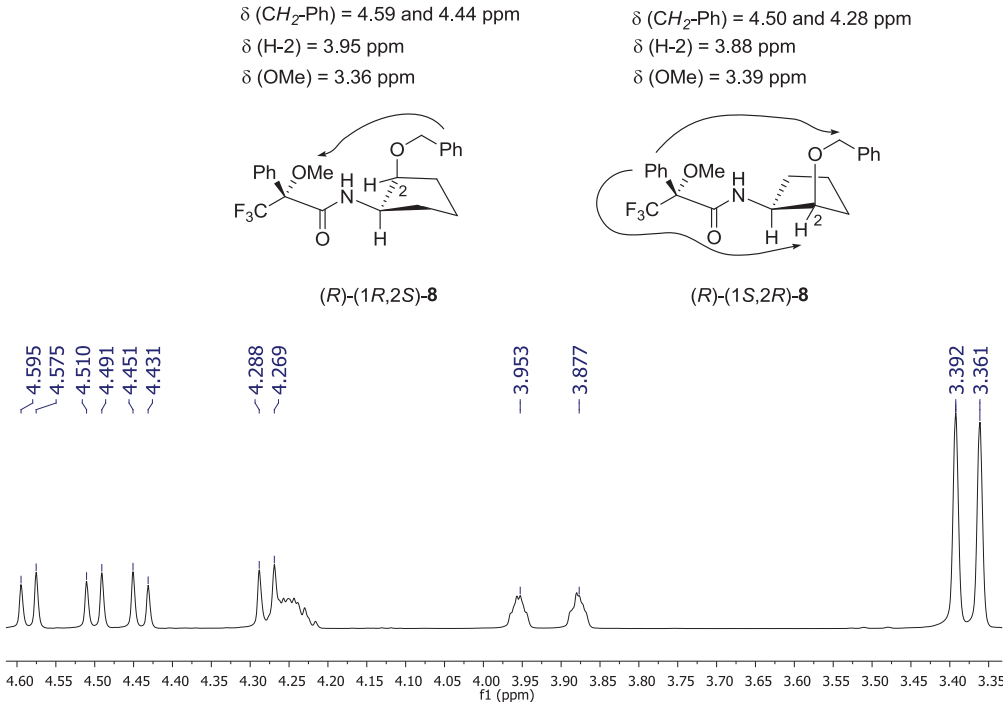


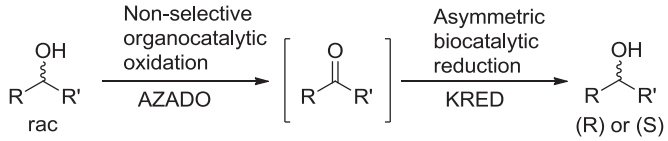
Figure 3.2.1.3. Working models for the diastereomeric (*R*)-MTPA amide derivatives and partial ^{1}H-NMR spectrum ($CDCl_3$, 600 MHz). Arrows in the figure indicate the shielding effects

From the comparison of both spectra, the configuration (1*S*,2*R*) was assigned to the remaining amine *cis*-(–)-**3a**. This also corroborated the (1*R*,2*S*) configuration for the enzymatically produced **7a** and thus, the *R*-preference of the lipase.

Once the absolute configuration of the remaining amine isolated in the lipase-catalyzed process was established as (1*S*,2*R*), the configuration of the optically active *cis*-**3a** obtained in the omega-TA-catalyzed transamination was established by comparison of the chiral-HPLC chromatograms of its acetamide derivative.

3.2.2. Deracemization of *sec*-alcohols

Inspired by the promising results of the previously described methodology, we envisaged to develop a multi-catalytic deracemization protocol of secondary alcohols in which the non-selective AZADO-catalysed oxidation could be conveniently coupled with a stereoselective reduction of the intermediate ketone mediated by a ketoreductase (Scheme 3.2.2.1).



Scheme 3.2.2.1. Proposal of deracemization via a catalytic oxidation-reduction sequence

A wide series of secondary alcohols was selected in this study, as shown in Figure 3.2.2.1.

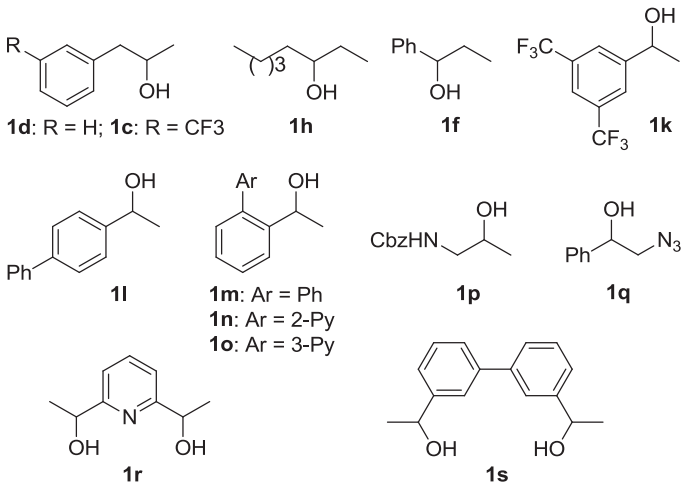


Figure 3.2.2.1. Racemic secondary alcohols of this study

Non-benzylic alcohols **1d** and **1c** are precursors of pharmacologically active amines such as amphetamine and fenfluramine,[140] respectively, and **1h** is a representative example of linear secondary alcohol. Among benzylic alcohols, we selected **1f** and **1k**, this last being a precursor of the orally active NK1 receptor antagonist aprepitant,[141] which is used as antiemetic drug[142] and has promising antitumor activity.[143] In addition, the *para*-fusionated biaryl alcohol **1l**, as a model of biarylic compound, and the *ortho*-fusionated **1m-1o** were also selected. The biaryl moiety forms the basic structure of many biologically active compounds[144] and is also found in electroluminiscent polymers[145] and chiral ligands.[146] The deracemization of the *ortho*-biarylic alcohols **1m-1o** has an extra added-value since previous approaches based on a lipase-catalysed kinetic resolution rendered very low yields due to the high steric congestion near to the reactive centre.[147] Furthermore, the amino alcohol derivative **1p** and the azido alcohol **1q** were also incorporated in our study as representative bifunctionalised compounds. Lastly, the viability of the method to convert the three-stereosiomers mixture of diols **1r** and **1s** into one single stereoisomer was also investigated. Enantiopure C_2-symmetric diols such as **1r** and **1s** are key compounds for the synthesis of chiral ligands,[146, 148] and

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[140] B. Goument, L. Duhamel, R. Mauge, *Bull. Soc. Chim. Fr.*, **1993**, *130*, 450–458.
[141] K. M. J. Brands, J. F. Payack, J. D. Rosen, T. D. Nelson, A. Candelario, M. A. Huffman, M. M. Zhao, J. Li, B. Craig, Z. J. Song, D. M. Tschaen, K. Hansen, P. N. Devine, P. J. Pye, K. Rossen, P. G. Dormer, R. A. Reamer, C. J. Welch, D. J. Mathre, N. N. Tsou, J. M. McNamara, P. J. Reider, *J. Am. Chem. Soc*., **2003**, *125*, 2129–2135.
[142] M. Aapro, A. Carides, B. L. Rapoport, H. J. Schmoll, L. Zhang, D. Warr, *Oncologist*, **2015**, *20*, 450–458.
[143] M. Muñoz, R. Coveñas, F. Esteban, M. Redondo, *J. Biosci*., **2015**, *40*, 441–463.
[144] D. G. Brown, J. Boström, *J. Med. Chem*., **2016**, *59*, 4443–4458.
[145] A. Kraft, A. C. Grimsdale, A. B. Holmes, *Angew. Chem. Int. Ed*., **1998**, *37*, 402–428.
[146] J. M. Longmire, G. Zhu, X. Zhang, *Tetrahedron Lett*., **1997**, *38*, 375–378.
[147] R. Kourist, J. González-Sabín, R. Liz, F. Rebolledo, *Adv. Synth. Catal*., **2005**, *347*, 695–702.
[148] a) Q. Jiang, D. V. Plew, S. Murtuza, X. Zhang, *Tetrahedron Lett*., **1996**, *37*, 797–800; b) R. Sablong, C. Newton, P. Dierkes, J. A. Osborn, *Tetrahedron Lett*., **1996**, *37*, 4933–4936; c) H. C. Brown, G. M. Chen, P. V. Ramachandran, *Chirality*, **1997**, *9*, 506–511.

1r has been also used for the preparation of optically active crown ethers[149] and other complex molecules.[150]

Considering the catalytic potential of AZADO to promote the oxidation of structurally hindered secondary alcohols, some of the aforementioned substrates, as the hindered biarylic derivatives, have been selected to extend the applicability of the previously developed oxidation methodology.

Similarly to the reaction conditions indicated in the Section 3.2.1, the alcohol oxidation step was planned by using a 0.40 M aqueous solution of sodium hypochlorite at pH 8.9, and AZADO (1.0 mol%) as the catalyst, but the co-solvent, $PhCF_3$, was changed to acetonitrile. The idea of employing this water-miscible co-solvent is to suppress or significantly lower the amount of DMSO that would be used as co-solvent in the second step if $PhCF_3$ is employed.

The use of acetonitrile (5% v/v) in the oxidation step only required a slight excess of NaOCl (400 mM, 1.3 equiv) and the same amount of AZADO (1.0 mol%). Under these conditions, all the starting materials were totally converted into the corresponding ketones or diketones **2** after 1.5 h of reaction, and all products were isolated in pure form by a simple extraction with ethyl acetate.

On the other hand, before carrying out the step-wise protocol, a complete screening of KREDs (from Codexis) with the ketones **2** was performed using a range of 10-20 mM substrate concentration. Bioreductions were conducted at 30-40 ºC in 125 mM phosphate buffer (pH 7.0), with $NADP^+$ (1.0 mM) and isopropyl alcohol (IPA, 15% v/v). The most significant results are shown in Table 3.2.2.1. (for ketones) and Table 3.2.2.2 (for diketones).

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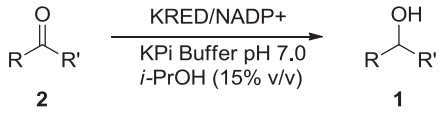
[149] a) Y. Habata, J. S. Bradshaw, J. J. Young, S. L. Castle, P. Huszthy, T. Pyo, M. L. Lee, R. M. Izatt, *J. Org. Chem*., **1996**, *61*, 2413–2415; b) G. M. Chen, H. C. Brown, P. V. Ramachandran, *J. Org. Chem*., **1999**, *64*, 721–725.

[150] J. Uenishi, S. Aburatani, T. Takami, *J. Org. Chem.,* **2007**, *72*, 132–138.

In all the cases, at least one KRED was found to effectively catalyze the asymmetric reduction of the starting ketones (**2d, 2c, 2h, 2f,** and **2k-q**), achieving the enantiopure alcohols with $C > 99\%$. Similarly, one KRED also catalyzed the reduction of each diketone (**2r** and **2s**) with excellent diastereoselectivity and total enantioselectivity.

Table 3.2.2.1. Bioreduction of ketones 2

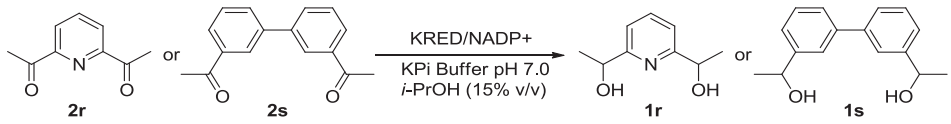


2 **1**

Ketone[a]	Conc (mM)	KRED	T (°C)	*C* (%)	*ee* of 1 (%)
2d	20	P2-H07	30	>99	>99 (*R*)
2d	20	P3-B03	30	>99	96 (*S*)
2c	20	P1-B10	30	>99	>99 (*R*)
2c	20	P3-B03	30	>99	>99 (*S*)
2h	20	P1-A04	30	>99	>99 (*R*)
2h	20	P3-G09	30	>99	>99 (*S*)
2f	20	P1-A04	30	>99	>99 (*R*)
2k	20	P1-B05	30	>99	>99 (*R*)
2l	10	P2-G03	40	97	>99 (*R*)
2l	10	P3-B03	40	>99	>99 (*S*)
2m	10	P1-B05	40	>99	>99 (*S*)
2n	20	P1-B05	40	>99	>99 (*S*)
2o	20	P1-B02	40	>99	>99 (*S*)
2p	20	P1-A04	30	>99	>99 (*R*)
2p	20	P3-G09	30	>99	>99 (*S*)
2q	20	P3-B03	30	>99	97 (*R*)
2q	20	P1-A04	30	>99	>99 (*S*)

[a] For the structure of the ketone, see the alcohol derivative in Figure 3.2.2.1. For biarylic compounds DMSO (10 % v/v) was added.

Table 3.2.2.2. Bioreduction of diketones 2r and 2s



Ketone[a]	Conc (mM)	KRED	T (°C)	*C* (%)	*dr*	*ee* for 1 (%)[b]
2r	20	P1-A04	30	>99	>99:<1	>99 (*R*,*R*)
2r	20	P3-H12	30	>99	96:4	92 (*S*,*S*)
2s	10	P1-A12	40	>99	99:1	>99 (*R*,*R*)
2s	10	P1-B05	40	>99	73:26	94 (*S*,*S*)

[a] DMSO (10 % v/v) was used for **2s**. [b] *ee* and configuration (between brackets) of the major stereoisomer.

At this point, both steps have been independently studied and the conditions to develop a one-pot process should be found. We focused our attention to a sequential process, being aware that the excess of oxidant of the first step should be eliminated before adding the cofactor and enzyme, because both the reduced cofactor NADPH as the resulting optically active alcohol could be oxidized. This issue was easily solved, because the hydrogen donor –isopropyl alcohol– used in great excess in the second step quenches the remaining NaOCl in the reaction medium. Nevertheless, a compatibility proof was carried out to demonstrate that either isopropyl alcohol is an effective scavenger of NaOCl and AZADO did not affect the enzymatic activity.

Furthermore, we posed as a major achievement carrying out the biocatalytic step up to 100 mM substrate concentration, which would be significantly higher than that used in previously reported methodologies.[126,128] As the dilution from 250 to 100 mM has to be achieved by adding phosphate buffer, and as the first step is already

conducted with a high salt concentration, the feasibility of the bioreduction in such a highly saline medium should be assessed. For this compatibility test, the deracemization of **1c** was chosen as benchmark reaction.

In the first attempt of oxidation-reduction sequence, and after carrying out the oxidation of **1c** (250 mM) during 1.5 h in the above mentioned conditions, IPA (15% v/v) was added and the reaction mixture stirred during five minutes. Then, each component of the second step [buffer phosphate (125 mM; pH 7.0), $MgSO_4$ (1.25 mM), and $NADP^+$ (1.0 mM)] was added to reach the target concentration of the intermediate **2c** (100 mM). In these conditions, KRED-P3-B03 retained its stereoselective activity catalysing the reduction of the ketone with total selectivity (*ee* >99%) while the conversion into (*S*)-**1c** was 79% after 24 h, and did not evolve further. The incomplete reduction of **2c** may result from the lower solubility of this ketone in the highly saline reaction medium.

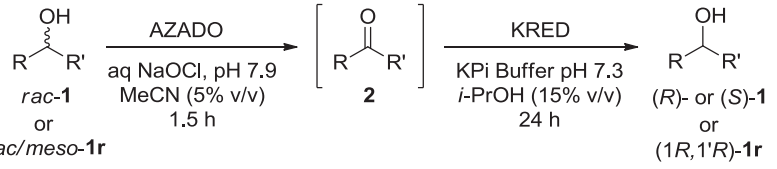
In the first step, the NaOCl solution and the $NaHCO_3$ used to reduce the pH from 9.5 to 8.9, determine a saline concentration of 160 and 190 mM, respectively. As a consequence, the second step is carried out in a saline concentration near to 500 mM. Accordingly, other reaction conditions were tested to lower the salt content in the bioreduction step. We decided to test if the addiction of phosphoric acid in the second step could eliminate or reduce the carbonate species as carbonic acid, thus decreasing the saline concentration. Gratifyingly, a better conversion value was registered (87%), but it did not evolve until completeness.

Finally, we decided to suppress $NaHCO_3$ in the first step and lower the pH of the NaOCl solution by using of KH_2PO_4 for a final concentration of 125 mM, as that required in the second step. Under these conditions, the resulting 400 mM aqueous solution of NaOCl exhibited pH 7.9 and the AZADO-catalysed oxidation of **1c** happened with total conversion after the same reaction time (1.5 h). Then, IPA (15% v/v), 125 mM phosphate buffer at pH 7.0 (containing $MgSO_4$ 2.5 mM), the KRED, and $NADP^+$ (1.0 mM) were successively added to reach a final 100 mM substrate concentration

and pH 7.3. Upon these conditions the degree of conversion into (*S*)-**1c** (*ee* >99%) was complete after 24 h.

Once an efficient process was validated, we extended the study to the selected racemic alcohols to have a broader overview of the potential of this oxidizing-reducing protocol. Therefore, after the oxidation took place, the optimized experimental procedure for the bioreduction step was applied with a selected KRED, at 100 mM substrate concentration, and 30 or 40 ºC depending on the substrate. Interestingly the protocol worked perfectly for a large variety of alcohols, obtaining the desired enantiopure products with complete (>99%) or very high conversion (90-93%) for ten of the thirteen substrates. Thus, the enantiopure alcohols (*R*)-**1d**, (*S*)-**1c**, and (*R*)-**1k**, which are precursors of valuable biologically active compounds, were isolated with very high yields (90-98%). Also remarkable were the results obtained for the bifunctional amino alcohol derivative **1p** and the *ortho*-byarilic alcohols **1n** and **1o**. In the last two cases, the pyridine moiety contributed to increase the solubility of these alcohols in the reaction medium, thereby allowing a 100 mM concentration in the bioreduction step. However, with the analogous biphenylic alcohols **1l** and **1m** as well as with the *meta*-biphenylic diol **1s** very low degree of conversions (<10%) were attained upon analogous reaction conditions. Regarding the pyridine-2,6-diethanol **1r**, the reaction happened with total conversion at 100 mM and KRED-P1-A04 displayed a perfect stereoselectivity to provide the enantiopure diol (1*R*,1'*R*)-**1r** as the sole product with >99:<1 *dr* (Table 3.2.2.3).

Table 3.2.2.3. One-pot deracemization process of 1[a]

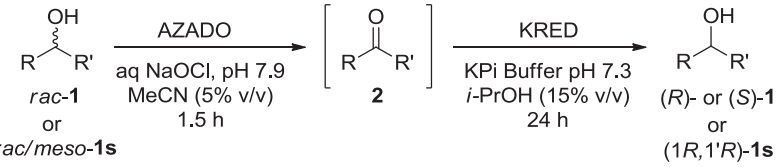


Substrate	KRED	*C* (%)	*ee* (%)	yield (%)
1d	P2-H07	>99	>99 (*R*)	93
1c	P3-B03	>99	>99 (*S*)	90
1h	P1-A04	92	>99 (*R*)	76
1f	P1-A04	90	>99 (*R*)	87
1k	P1-B05	>99	>99 (*R*)	98
1n	P1-B05	>99	>99 (*S*)	92
1o	P1-B02	>99	>99 (*S*)	90
1p	P1-A04	>99	>99 (*R*)	90
1q	P1-A04	93	>99 (*S*)	82
Diol 1r	P1-A04	>99	>99 (*R*,*R*)	95

[a] Reaction conditions. Oxidation step: AZADO (1.0 mol%), 250 mM substrate concentration, except for **1r** (130 mM), room temperature except for **1d** (5 ºC); reduction step was performed at 100 mM substrate concentration, at 30 ºC except for reactions starting from **1n** and **1o** (40 ºC).

In order to improve the unsuccessful results achieved in some cases and reach perfect conversion for the bioreduction of the intermediate ketones, we decided to lower the concentration of the second step at 50 mM, also reducing the amount of $NADP^+$ to 0.50 mM. Gratifyingly, the reactions using racemic **1h**, **1f**, and **1q** proceeded effectively and the corresponding enantiopure alcohols were achieved with complete conversion (Table 3.2.2.4). On the contrary the biarylic alcohols **1l**, **1m**, and diol **1s** remained challenging, despite a slight improvement in the conversion values (up to 65% for **1l**).

Table 3.2.2.4. One-pot deracemization process of 1[a]



Substrate	Conc. (mM)[b]	KRED	*C* (%)	*ee* (%)	yield (%)
1h	50	P1-A04	>99	>99 (*R*)	87
1f	50	P1-A04	>99	>99 (*R*)	92
1l	50	P3-B03	65	>99 (*S*)	61
1l[c]	25	P3-B03	>99	>99 (*S*)	90
1m[c]	25	P1-B05	>99	>99 (*S*)	92
1q	50	P1-A04	>99	>99 (*S*)	90
Diol 1s[c]	25	P1-A12	>99	>99 (*R,R*)	95

[a] See Table 3.2.2.3 for oxidation conditions. [b] Substrate concentration of the reduction step, which was carried out at 30 ºC (for **1h**, **1f**, and **1q**) or 40 ºC (for **1l**, **1m**, and **1s**). [c] DMSO (10% v/v) was added in the second step.

Pleasantly, complete conversion of these substrates could be finally reached by lowering the concentration to 25 mM and adding DMSO (10% v/v) as co-solvent. It should be noted that, despite the low concentration of the bioreduction step, the enantiopure alcohols were isolated in very high yields (90-95%) and the diol (1*R*,1'*R*)-**1s** was converted with >99:<1 *dr* and >99% *ee*.

3.3. Conclusions

In conclusion, two new straightforward multi-catalytic processes have been developed in which racemic secondary alcohols have been transformed into optically active amines or converted into the enantiopure compounds (deracemization), the first step –common for both processes– being an organocatalyzed alcohol oxidation.

A catalytic amount of the organocatalyst AZADO (1 mol%) in combination with an aqueous solution of NaOCl allowed the oxidation of a wide variety of secondary alcohols –benzylic, non-benzylic, functionalized, or hindered– achieving the ketones with very high yields.

The organocatalyzed oxidation was conveniently coupled to an enantioselective bioamination of the intermediate ketone in the presence of a omega-transaminase. In all cases, the desired final amines were isolated in high yields and with excellent enantiomeric excesses. When the strategy was applied to *rac*-2-(benzyloxy)cycloakanols, an efficient DKR took place in the bioamination step, and the enantiopure 2-(benzyloxy)cycloalkanamines were obtained with high to excellent diastereomeric ratios.

The organocatalytic oxidation step was also effectively coupled with a ketoreductase-catalyzed reduction of the intermediate ketone, the last step being carried out up to 100 mM substrate concentration. Fine tuning the oxidation conditions and adequate biocatalyst selection provided a set of synthetically and pharmacologically interesting alcohols with high overall yields (up to 98%) and >99% *ee*. The efficiency of the strategy was also demonstrated for the conversion of *rac*/*meso*-diols into only one enantiomer.

These methodologies, which exploit the advantages of merging organo- and enzymatic catalysis, could be robust and sustainable alternatives to the existing methodologies, with proven efficacy for a broad variety of secondary alcohols.

3.4. Experimental Section

3.4.1. Hybrid organo- and biocatalytic process for the asymmetric transformation of alcohols into amines

3.4.1.1. General information

Enzymes

Codex® ATA Screening Kit was purchased from Codexis.

Transaminases from *Chromobacterium violaceum* (Cv), (*R*)-*Arthrobacter* (ArR) and (*S*)-*Arthrobacter* (ArS) were over expressed in *E.coli* and used and lyophilized cells, as well as the evolved ArRmut11 which was purified by a heat protocol.

Laccase from *Trametes versicolor* (0.5 U/mg), *Coriolus versicolor* (0.3 U/mg), *Rhus vernicifera* (50 U/mg) and *Agaricus bisporus* (4 U/mg) were purchased from Sigma-Aldrich.

Immobilized lipase from *Burkholderia cepacia* (PSL-IM, 816 U/g), which previously was classified as *Pseudomonas cepacia*, was purchased from Amano Pharmaceutical Co.

General procedure for the synthesis of racemic trans-*2-(benzyloxy)cycloalkanols **1a** and **1b***

To a solution of the corresponding cycloalkene oxide (25 mmol) in benzyl alcohol (75 mmol) three drops of conc. sulfuric acid were added and the solution was heated at 95 °C and stirred during 1 h (for **1a**) or 8 h (for **1b**). Then, the reaction mixture was allowed to attain the room temperature and the corresponding product purified by distillation. Yields: 78% (for **1a**) and 89% (for **1b**).

Preparation of 0.40 M aqueous solution of NaOCl, pH = 8.9

It was prepared just before use from the commercially available aqueous solution of NaOCl (1.95 M, determined by reaction with potassium

iodide in acetic acid and then, titration of the iodine liberated with sodium thiosulfate[132]) by adding of solid $NaHCO_3$ and distillated water until a pH value of 8.9.

Racemic amines

Amines (±)-**3e-g** were commercially available. A sample of every diastereomerically pure *trans*-(±)- and *cis*-(±)-2-(benzyloxy)cycloalkanamines **3a,b** was supplied by EntreChem SL.[138]

Amines (±)-**3c,d,h** were prepared by conventional reductive amination procedure of the corresponding ketone (ammonium formate, MeOH, Pd/C).[151]

Enzymatic aminolysis of cis-(±)-2-(benzyloxy)cyclopentanamine

cis-(±)-2-(Benzyloxy)cyclopentanamine (100 mg, 53.0 mmol) was dissolved in ethyl acetate (3.0 mL) and the solution added to a Erlenmeyer containing the lipase from *Burkhoderia cepacia* (PSL-IM, 53 mg) under a nitrogen atmosphere. Then, the mixture was shaken at 28 ºC and 200 rpm during 24 h. After this time, the enzyme was filtered and thoroughly washed with ethyl acetate. The organic solution was extracted with aq 3 M HCl (3 × 7 mL). The organic phase was successively washed with water and brine, dried with Na_2SO_4 and concentrated in *vacuo* to give the optically active amide (1*R*,2*S*)-**7a** ($[\alpha]_D^{20}$ +51.6 (*c* 1.0, $CHCl_3$), *ee* = 80%). On the other hand, the acid aqueous phase containing the remaining amine (1*S*,2*R*)-**3a** was basified (pH = 12) with aq 6 M NaOH and subsequently extracted with ethyl acetate. The typical work-up of this new organic phase gave the corresponding amine (1*S*,2*R*)-**3a** [$[\alpha]_D^{20}$ −19.1 (*c* 0.66, $CHCl_3$), enantiopure]. The *ee* of both remaining substrate **3a** and product (**7a**) was determined by HPLC analysis.

[151] J. González-Sabín, V. Gotor, F. Rebolledo, *Tetrahedron: Asymmetry*, **2002**, *13*, 1315–1320.

*3.4.1.2. Screening of the oxidation conditions using (±)-**1b** as a model substrate*

3.4.1.2.1. Employ of laccase, AZADO, and O_2 as the oxidant reagent.

Substrate (±)-**1b** (11 mg, 50 μmol) and AZADO were dissolved in $PhCF_3$ (150 μL) in a 10 mL microwave vial. Then, laccase and water (1.0 mL) or a 200 mM acetate buffer (pH 4.5, 1.0 mL) were added and the reaction stirred under an oxygen atmosphere (balloon). After 24 h, the mixture was extracted with ethyl acetate (3 × 2.0 mL), the organic layers were combined, dried over Na_2SO_4 and evaporated under vacuum. The degree of conversion (*C*) of each reaction was determined from the ^{1}H-NMR analysis of the crude material.

3.4.1.2.2. Employ of an aqueous solution of NaOCl, and AZADO.

Aqueous NaOCl (pH = 8.9) was added to a solution of alcohol (±)-**1b** (11 mg, 50 μmol) and the corresponding amount of AZADO in the organic co-solvent. The reaction mixture was vigorously stirred (magnetic stirring) and, after the time collected in Table S2, extracted with ethyl acetate (2 × 400 μL). The organic layers were combined, dried over Na_2SO_4 and evaporated under vacuum. The degree of conversion (*C*) of each reaction was determined from the ^{1}H-NMR analysis of the crude material.

*3.4.1.3. Typical procedure for oxidation of alcohols **1***

To a solution of racemic alcohol (150 μmol) and AZADO (1.5 μmol) in $PhCF_3$ (150 μL), a 0.40 M solution of NaOCl (pH = 8.9; 1.0-1.4 equiv) was added. The mixture was vigorously stirred (magnetic stirring) at room temperature in all cases except for reaction with **1d**, which was conducted at 5 ºC. Once the starting material was disappeared (1 h, TLC control using hexane-ethyl acetate 3:1 as eluent), the reaction mixture was extracted with ethyl acetate (3 × 600 μL). The organic layers were combined, dried over Na_2SO_4 and evaporated under vacuum. The ^{1}H-NMR analysis of the crude product (92 - >95% yield) showed the corresponding ketone in pure state for synthetic purposes.

3.4.1.4. Spectral data of ketones

*3.4.1.4.1. (±)-2-(Benzyloxy)cyclopentanone (**2a**)*

It was prepared from *trans*-(±)-2-(benzyloxy)cyclopentanol (50 mmol). Compound **2a** was isolated with >99% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.70-1.90 (m, 2H), 1.98-2.09 (m, 1H), 2.21-2.30 (m, 3H), 3.80 (dd, 1H, *J* 7.3 and 9.4 Hz, H-2), 4.68 and 4.83 (AB system, 2H, $|J_{A,B}|$ = 12.0 Hz, C*H*$_2$Ph), 7.28-7.39 (m, 5H); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 17.43 (CH_2), 29.68 (CH_2), 35.58 (CH_2), 72.07 (CH_2), 80.19 (CH), 127.92 (CH), 128.04 (2 CH), 128.52 (2 CH), 137.80 (C), 216.51 (CO). These spectroscopic data are in good agreement with those previously published.[152]

*3.4.1.4.2. (±)-2-(Benzyloxy)cyclohexanone (**2b**)*

Application of the general procedure to *trans*-(±)-2-(benzyloxy)cyclohexanol (30 mmol) gave **2b** with >99% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.61-1.97 (m, 5H), 2.15-2.32 (m, 2H), 2.50-2.58 (m, 1H), 3.89 (ddd, 1H, *J* 0.9, 5.4, and 9.9 Hz, H-2), 4.47 and 4.76 (AB system, 2H, $|J_{A,B}|$ = 12.0 Hz, C*H*$_2$Ph), 7.28-7.38 (m, 5H); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 23.24 (CH_2), 27.76 (CH_2), 34.69 (CH_2), 40.77 (CH_2), 71.75 (CH_2), 81.80 (CH), 127.82 (CH), 127.87 (2 CH), 128.51 (2 CH), 138.06 (C), 210.29 (CO). These spectroscopic data are in good agreement with those previously published.[153]

3.4.1.4.3. Spectral data for ketones **2c-2j** are in good agreement with those obtained for the corresponding commercially available sample.

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[152] N. Jarkas, R. J. Voll, V. M. Camp, M. M. Goodman, *J. Med. Chem*., **2010**, *53*, 6603–6607.

[153] R. Dalpozzo, A. De Nino, L. Maiuolo, N. Nardi, A. Procopio, A. Tagarelli, *Synthesis*, **2004**, 496–498.

*3.4.1.5. Screening of enzymatic transamination of ketones **2***

3.4.1.5.1. General method for reactions carried out at pH 7.5.

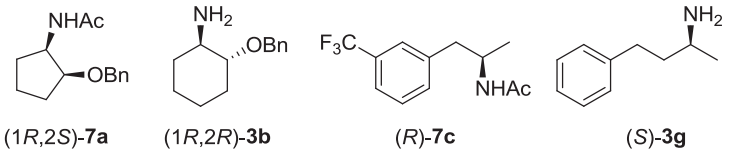
Reactions were carried out in a 2.0 mL eppendorf tube. The corresponding ATA (2.0 mg) was added to 0.95 mL of 100 mM phosphate buffer pH 7.5 containing propan-2-amine (1.0 M) and the cofactor PLP (1.0 mM). Then, a solution of the corresponding ketone (1.5 – 2.0 mg) in DMSO (50 μL) was added and the resulting 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 or GC analysis. On the other hand, aqueous 10 M 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 corresponding amine was determined by chiral HPLC after the conventional derivatization of the sample using acetic anhydride (2 μL / mg of substrate), di-*tert*-butyl dicarbonate (50 μL of a 100 mM solution in ethyl acetate / mg of substrate) or benzyl chloroformate (2 μL / mg of substrate).

3.4.1.5.2. General method for reactions carried out at other pH values (8.5, 10 and 11.5)

The same general method of the previous section was applied, previously adjusting the pH value of the 100 mM phosphate solution (containing 1.0 M propan-2-amine) to the required pH value.

3.4.1.6. General procedure for the oxidation–transamination cascade process and characterization of optically active compounds

For a better overview, a selection of compounds included in this section is reported in the following Figure 3.4.1.6.1.



(1*R*,2*S*)-**7a** (1*R*,2*R*)-**3b** (*R*)-**7c** (*S*)-**3g**

Fig. 3.4.1.6.1. A selection optically active compounds

3.4.1.6.1. Typical procedure for a cascade reaction in a 150 μmol scale

Reactions were carried out in a 50 mL falcon centrifuge tube using 150 μmol of the starting alcohol **1**. Firstly, the oxidation was carried out. Once the oxidation was completed, DMSO [1.4 mL (10% v/v)], the corresponding ATA (15 mg), and 100 mM buffer solution at pH 7.5 or 11.5 (14.4 mL) containing propan-2-amine (1.0 M) and the cofactor PLP (1.0 mM) were added. The reaction mixture was incubated during 24 h at 30 ºC and 250 rpm. After this time, the reaction was stopped by addition of aq 10 M NaOH (1.0 mL). Then, the mixture was extracted with ethyl acetate (2 × 15 mL), the organic layers were separated by centrifugation (90 s, 13000 rpm), combined, and finally dried over Na_2SO_4. Evaporation of the solvent yielded the crude amine which was isolated in pure state. In the case of amine **3c**, due to its volatility, the crude amine was isolated after its transformation into acetamide by reaction with acetic anhydride (5.0 equiv) and triethylamine (1.5 equiv).

*3.4.1.6.1.1. (1R,2S)-2-(Benzyloxy)cylopentanamine [(1R,2S)-**3a**]*

The typical procedure was applied to 20.0 mg (104 μmol) of alcohol **1a** using phosphate buffer 100 mM pH 11.5, DMSO (15% v/v), and ATA-025 as the catalyst of the transamination step. After the described work-up, amine **3a** was isolated with 97% yield (19.2 mg). The HPLC analysis of this sample shown (1*R*,2*S*)-**3a** as the major estereisomer, which was obtained with *ee* > 99% and *dr* = 83:17.

*3.4.1.6.1.2. (1R,2S)-N-[2-(Benzyloxy)cylopentyl]acetamide [(1R,2S)-**7a**]*

A solution of the above 83:17 *cis-trans* distereomeric mixture of **3a** (19 mg, 99 μmol) in CH_2Cl_2 (2.0 mL) was treated with Ac_2O (47 μL, 0.50 mmol) and Et_3N (21 μL, 0.15 mmol). After 2 h of reaction at room temperature, CH_2Cl_2 (8.0 mL) was added and the organic solution was successively washed with aq saturated $NaHCO_3$ (5.0 mL) and NaCl (5.0 mL). Evaporation of the organic phase yielded the mixture of diastereomeric acetamides **7a**, which was submitted to flash column chromatography (hexane-ethyl acetate 2:1 as the eluent) to give pure the first eluted *cis*-diastereomer (1*R*,2*S*)-**7a**. Yield: 18 mg (78%). $[\alpha]_D^{20}$ +58.8 (*c* 1.0, $CHCl_3$), *ee* >99%. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.45-1.65 (m, 2H), 1.70-1.90 (m, 3H), 1.90-2.10 [m +s, 4H, singlet centered at 1.95 ppm (CH_3)], 3.85-3.92 (m, 1H, H-2), 4.11-4.24 (m, 1H, H-1), 4.39 and 4.59 (AB system, 2H, $|J_{A,B}|$ = 11.8 Hz, C*H*$_2$Ph), 6.02 (brs, 1H, NH), 7.26-7.41 (m, 5H, Ph); ^{13}C-NMR (300 MHz, $CDCl_3$) δ (ppm): 20.64 (CH_2), 23.58 (CH_3), 29.36 (CH_2), 30.05 (CH_2), 52.95 (CH), 71.00 (CH_2), 80.01 (CH), 127.75 (2 CH), 127.89 (CH), 128.62 (2 CH), 138.50 (C), 196.94 (CO). HRMS (ESI^+): *m/z* $[M+Na]^+$ found: 256.1306. $C_{14}H_{19}NNaO_2$ requires 256.1308.

*3.4.1.6.1.3. (1R,2R)-2-(Benzyloxy)cylopentanamine [(1R,2R)-**3a**]*

The typical procedure was applied to 10 mg (52 μmol) of alcohol **1a** using buffer phosphate pH 11.5, DMSO (15% v/v), and ATA-303 as the catalyst of the transamination step. After the described work-up, amine (1*R*,2*R*)-**3a** was isolated with *ee* > 99%, *dr* = 89:11, and 90% yield (9.0 mg).

*3.4.1.6.1.4. (1R,2R)-2-(Benzyloxy)cylohexanamine [(1R,2R)-**3b**]*

Starting from 150 μmol (31 mg) of **1b** and using DMSO (15% v/v), 100 mM phosphate buffer (pH = 11.5), and ATA-303 in the transamination step, amine (1*R*,2*R*)-**3b** was isolated in 94% yield (29 mg). $[\alpha]_D^{20}$ –79.0 (*c*

1.0, $CHCl_3$), *ee* >99%, *dr* 96:4.[154] ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.05-1.82 (m, 4H), 1.60-1.67 (m, 3H), 1.72-1.76 (m, 1H), 1.81-1.88 (m, 1H), 2.09-2.16 (m, 1H), 2.62-2.70 (ddd, 1H, *J* 4.2, 9.0, and 11.1 Hz), 2.96-3.03 [td, 1H, *J* 4.5 (d) and 9.3 (t) Hz], 4.44 and 4.66 (AB system, 2H, $|J_{A,B}|$ = 11.4 Hz, C*H*$_2$Ph), 7.22-7.35 (m, 5H); ^{13}C-NMR (300 MHz, $CDCl_3$) δ (ppm): 24.64 (2 CH_2), 29.84 (CH_2), 33.63 (CH_2), 55.16 (CH), 70.78 (CH_2), 84.61 (CH), 127.55 (CH), 127.77 (2 CH), 128.39 (2 CH), 138.90 (C).

*3.4.1.6.1.5. (R)-N-{1-[3-(Trifluoromethyl)phenyl]propan-2 yl}acetamide [(R)-**7c**]*

The typical procedure was applied to 31 mg (152 μmol) of alcohol **1c** using phosphate buffer 100 mM pH 7.5, and ATA-P2-B01 as the catalyst of the transamination step. Once the reaction was stopped and the produced amine (*R*)-**3c** extracted with ethyl acetate, the organic phase was dried with Na_2SO_4, filtered, and the volume was reduced under vacuum until 1.5 mL. Then, acetic anhydride (5.0 equiv) and triethylamine (1.5 equiv) were added and the solution stirred during 2 h. After the usual work-up, acetamide (*R*)-**7c** was isolated with 91% yield (34 mg); $[\alpha]_D^{20}$ +28.9 (*c* 1.2, $CHCl_3$), *ee* >99%. Lit.[155] for (*S*)-**7c**: $[\alpha]_D^{20}$ –31.4 (*c* 0.50, $CHCl_3$), *ee* = 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.10 (d, 3H, *J* 6.6 Hz, CH_3), 1.92 (s, 3H, Ac), 2.76 (dd, 1H, *J* 7.2 and 13.5 Hz, C*H*H), 2.90 (dd, 1H, *J* 6.0 and 13.5 Hz, CH*H*), 4.17-4.31 (m, 1H, CH), 5.45 (brs, 1H, NH), 7.35-7.49 (m, 5H, Ph); ^{13}C-NMR (300 MHz, $CDCl_3$) δ (ppm): 19.97 (CH_3), 23.48 (CH_3), 42.31 (CH_2), 46.21 (CH), 123.46 (q, $^3J_{C,F}$ = 3.8 Hz, CH), 124.26 (q, $^1J_{C,F}$ = 272.5 Hz, CF_3), 126.22 (q, $^3J_{C,F}$ = 3.8 Hz, CH), 128.94 (CH), 130.75 (q, $^2J_{C,F}$ = 32.5 Hz, C), 132.88 (CH), 139.14 (C), 169.54 (CO). These spectroscopic data are in agreement with published data.[155]

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[154] The optical rotation for a diastereomerically pure sample (1*R*,2*R*)-**3b** (Aldrich catalogue) is: $[\alpha]_D^{20}$ –82.0 (*c* 1.0, $CHCl_3$), *ee* >99%.

[155] S. Zhu, T. Liu, S. Yang, S. Song, Q. L. Zhou, *Tetrahedron*, **2012**, *68*, 7685–7690.

*3.4.1.6.1.6. (*S*)-4-Phenylbutan-2-amine [(*S*)-**3g**]*

The typical procedure was applied to 10 mg (67 μmol) of alcohol **1g** using phosphate buffer 100 mM pH 7.5, and ATA-P1-A06 as the catalyst of the transamination step. Amine (*S*)-**3g** was isolated with 90% yield (9.0 mg); $[\alpha]_D^{20}$ +5.9 (*c* 0.50, $CHCl_3$), *ee* >99%. Lit.[151] for (*S*)-**3g**: $[\alpha]_D^{20}$ +6.4 (*c* 0.47, $CHCl_3$), *ee* = 98%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.13 (d, 3H, *J* 6.3 Hz, CH_3), 1.60-1.85 (m + b s, 4H, CH_2 + NH_2), 2.58-2.75 (m, 2H, CH_2), 2.87-3.01 (m, 1H, CH), 7.15-7.30 (m, 5H, Ph); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm): 24.05 (CH_3), 32.96 (CH_2), 41.87 (CH_2), 46.72 (CH), 125.88 (CH), 128.46 (2 CH), 128.50 (2 CH), 142.39 (C). These spectroscopic data are in agreement with published data.[151]

3.4.1.6.2. General procedure for the oxidation-transamination cascade in an analytical 10 μmol scale.

Firstly, oxidation reaction was carried starting from 50 μmol of alcohol **1**. After 1 h of reaction, an aliquot containing 10 μmol of ketone was placed in an Eppendorf tube. Then, DMSO (100 μL), the corresponding ATA (1.0 mg), and 1.0 mL of the 100 mM buffer phosphate pH 7.5 (for **1d, 1e, 1f, 1h**) or 10 (for **1a**) (1.0 M in propan-2-amine and 1.0 mM in PLP) were added. After 24 h of reaction, the methods indicated in section 8 were followed in order to determine the degree of conversion (*C*) and the *ee* of the produced optically active amine.

*3.4.1.6.2.1. Reaction starting from 2-(benzyloxy)cyclopentanol (**1a**)*

The general procedure was followed using ATA-P2-A01 for the enzymatic step. Thus, (1*R*,2*S*)-2-(benzyloxy)cyclopentanamine was formed with *C* >99%, 95:5 *dr*, and *ee* >99%.

*3.4.1.6.2.2. Reaction starting from 1-phenylpropan-2-ol (**1d**)*

The general procedure was followed using ATA-251 for the enzymatic step. The degree of conversion was 96% and (*S*)-1-phenylpropan-2-amine was formed with *ee* >99%.

*3.4.1.6.2.3. Reaction starting from 1-phenylethanol (**1e**)*

Following the general procedure and using ATA-P1-A06 for the enzymatic step, the corresponding amine (*S*)-**3e** was obtained with *C* = 80% and *ee* >99%.

*3.4.1.6.2.4. Reaction starting from 1-phenylpropan-1-ol (**1f**)*

The general procedure was followed using ATA-033 for the enzymatic step. Thus, the corresponding amine (*R*)-**3f** was obtained with *C* = 87% and *ee* >99%.

*3.4.1.6.2.5. Reaction starting from 3-octanol (**1h**)*

The general procedure was followed using ATA-033 for the enzymatic step. Thus, the corresponding amine (*R*)-**3h** was obtained with *C* = 85% and *ee* = 97%.

*3.4.1.7. Experimental procedures for the selective transformation of optically active **3b***

*3.4.1.7.1. Palladium-catalyzed debenzylation of (1R,2R)-2-(benzyloxy)cyclopentanamine (**3b**)*

A suspension of (*1R,2R*)-**3b** (20 mg, 97 μmol) and Pd-C (10%, 5 mg), in deoxygenated methanol (2.0 mL) was stirred for 12 h at room temperature under a hydrogen atmosphere. The reaction mixture was filtered through Celite© and the filtrate was concentrated in *vacuo* to yield pure (*1R,2R*)-**4b** in quantitative yield.

*3.4.1.7.2. N-Boc protection of (1R,2R)-2-(benzyloxy)cyclopentanamine (**3b**)*

A solution of (*1R,2R*)-**3b** (20 mg, 97 μmol) in dichloromethane (2.0 mL) was treated with di-*tert*-butyl dicarbonate (1.0 equiv) and then stirred for 1 h at room temperature. Then the reaction mixture was concentrated in *vacuo* to yield pure (*1R,2R*)-**5b** in quantitative yield.

3.4.1.7.3. Palladium-catalyzed debenzylation of (*1R,2R*)-***5b***

The previous (*1R,2R*)-**5b** (97 μmol) was debenzylated following the procedure already described, yielding pure (*1R,2R*)-**6b** in quantitative yield.

*3.4.1.7.4. Simultaneous debenzylation and N-Boc protection of (1R,2R)-**3b***

A suspension of (*1R,2R*)-**3b** (20 mg, 97 μmol), di-*tert*-butyl dicarbonate (1 equiv) and Pd-C (10%, 5 mg), in deoxygenated methanol (2.0 mL) was stirred for 12 h at room temperature under a hydrogen atmosphere. The reaction mixture was filtered through Celite© and the filtrate was concentrated in *vacuo* to yield pure (*1R,2R*)-**6b** in quantitative yield.

The identity of the resulting (*1R,2R*)-**4b**, (*1R,2R*)-**5b** and (*1R,2R*)-**6b** was confirmed by comparison with commercially available analytical standards of EntreChem SL.[138]

3.4.1.8. HPLC and GC analytical data for C, ee, and dr determinations

3.4.1.8.1. Analytical data for the determination of the degree of conversion (C) of the ATA-catalyzed reactions and cascade processes

HPLC Method: HPLC analyses were carried out using a reversed phase column (Zorbax Eclipse XDB-C18, RR, 18μm, 4.6 x 50 mm, Agilent) with acetonitrile and water (0.1% TFA) as solvent. 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 2.0 ml/min. Detection of peaks (UV absorption) were performed at 220 and 324 nm.

GC Method: The following column was used: Hewlett Packard HP-1 (30 m × 0.32 mm × 0.25μm, 12.2 psi N_2). Temperature program: 60/3/10/180 (initial T (ºC) / time (min) / ramp (ºC/min) / final T (ºC).

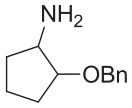
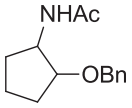
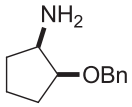
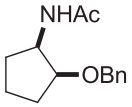
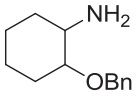
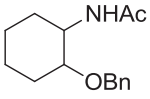
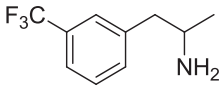
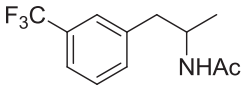
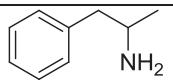
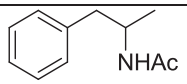
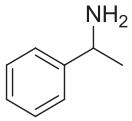
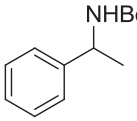
Table 3.4.1.8.1.1. Analytical data for determination of *C* in omega-TA-catalyzed transamination of ketones and in the oxidation–transamination cascade

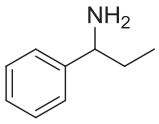
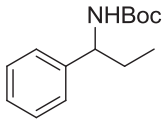
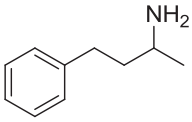
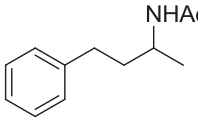
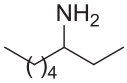
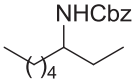
Method	Retention time (t_R, min)					
	Alcohol	t_R	ketone	t_R	amine	t_R
HPLC	**1a**	3.6	**2a**	4.1	**3a**	2.2 (*trans*), 2.3 (*cis*)
HPLC	**1b**	4.3	**2b**	4.5	**3b**	2.6 (*trans*), 2.8 (*cis*)
HPLC	**1c**	4.5	**2c**	4.7	**3c**	2.6
HPLC	**1d**	2.9	**2d**	3.2	**3d**	1.4
HPLC	**1e**	2.3	**2e**	2.9	**3e**	0.8
HPLC	**1f**	3.2	**2f**	4.1	**3f**	1.3
HPLC	**1g**	3.7	**2g**	4.1	**3g**	1.9
GC	**1h**	4.5	**2h**	4.4	**3h**	4.8

3.4.1.8.2. Analytical data for ee and dr (if applicable) determinations

For the determination of the *ee* and *dr* (when applicable) of each amine, this was previously converted into the acetamide, the *tert*-butyl or the benzylcarbamate and then, this derivative analyzed by chiral-HPLC. Each amine beside its derivative as well as all data corresponding to the HPLC analyses using a chiral column is shown in the following Table 3.4.1.8.2.1. Assignment of the configuration for every peak is also included.

Table 3.4.1.8.2.1. HPLC analyses data of the derivatives 7, 8, or 9 prepared from chiral amines 3a-3h

Amine	Derivative	Conditions	Retention time (min)
 **3a**	 **7a**	Chiralpack IC, Hexane:IPA 80:20, 30 ºC, 0.8 mL/min	9.23 (1*S*,2*S*) and 11.11 (1*R*,2*R*); R_S = 2.1 12.78 (1*R*,2*S*) and 13.59 (1*S*,2*R*); R_S = 1.0
 *cis*-**3a**	 *cis*-**7a**	Chiralcel OD, Hexane:IPA 90:10, 30°C 0.8mL/min	8.24 (1*S*,2*R*) and 9.45 (1*R*,2*S*); *R*s = 1.4
 **3b**	 **7b**	Chiralpak IA, Hexane:IPA 95:5, 30 ºC, 0.8 mL/min	11.91 (1*R*,2*S*) and 13.18 (1*S*,2*R*); R_S = 1.5 21.16 (1*R*,2*R*) and 30.18 (1*S*,2*S*); R_S = 4.0
 **3c**	 **7c**	Chiralcel OB-J, Hexane:IPA 90:10, 40 ºC, 0.8 mL/min	6.98 (*S*) and 8.77 (*R*); R_S = 2.0
 **3d**	 **7d**	Chiralcel OD, Hexane:IPA 96:4, 40 ºC, 0.8 mL/min	21.73 (*S*) and 23.85 (*R*); R_S = 1.2
 **3e**	 **8e**	Chiralcel OJ-H, Hexane:IPA 95:5, 30 ºC, 0.8 mL/min	7.74 (*S*) and 9.44 (*R*); R_S = 3.8

 **3f**	 **8f**	Chiralcel OJ-H, Hexane:IPA 90:10, 30 ºC, 0.8 mL/min	5.32 (*S*) and 6.11 (*R*); $R_S = 2.6$
 **3g**	 **7g**	Chiralpak AD-H, Hexane:IPA 98:2, 40 ºC, 0.8 mL/min	31.17 (*R*) and 33.94 (*S*); $R_S = 1.7$
 **3h**	 **9h**	Chiralcel OJ-H, Hexane:IPA 97:3, 30 ºC, 0.8 mL/min	8.13 (*S*) and 10.01 (*R*); $R_S = 4.6$

3.4.1.9. CD Support

In the CD support, detailed additional information about the experimental of this Chapter is included. A complete section of enzymatic screening, copy of GC-HPLC chromatograms and NMR spectra for all the synthesized optically pure compounds will be found.

3.4.2. Deracemization of sec*-alcohols*

3.4.2.1. General Information

Enzymes

Codex® KRED Screening Kit was purchased from Codexis.

Racemic substrates

Alcohols **1d, 1h,** and **1f** were commercially available. Alcohols **1c**, **1k-o**, **1q**, and **1s** were prepared by conventional reduction of the corresponding ketones with $NaBH_4$ (4.0 equiv) in MeOH (0.10 M). Alcohol **1p** was obtained by conventional reaction of (±)-1-aminopropan-2-ol (1.0 equiv) with benzyl chloroformate (1.0 equiv). Yield: 97%.

No commercially available ketones

No commercially available ketones were prepared as previously described: **2q**,[156] **2l-o**.[147] Ketone **2p** was obtained by oxidation of racemic alcohol **1p**. Ketones **2d, c, h, f, k** and **2r** were commercially available.

The spectroscopic data of the synthesised substrates (alcohols and ketones) were in good agreement with those previously published.

*3.4.2.2. Typical procedure for oxidation of alcohols **1***

To a solution of racemic alcohol (150 μmol) and AZADO (1.5 μmol) in MeCN (150 μL), a 0.40 M solution of NaOCl (pH = 7.9 by adding KH_2PO_4; 1.3 equiv) was added (for compounds **1n-o** 1.2 equiv were used). The mixture was vigorously stirred (magnetic stirring) at room temperature in all cases except for reaction with **1d**, which was conducted at 5 ºC. Once the starting material disappeared (1.5 h, TLC control using hexane-ethyl acetate 3:1 as eluent), the reaction mixture was extracted with ethyl acetate (3 × 600 μL). The organic layers were combined, dried over Na_2SO_4 and

[156] F. R. Bisogno, I. Lavandera, W. Kroutil, V. Gotor, *J. Org. Chem.*, **2009**, *74*, 1730–1732.

evaporated under vacuum. The ^{1}H-NMR analysis of the crude product (>95% yield) showed the corresponding ketone in pure state for synthetic purposes.

*3.4.2.3. General procedure for the bioreduction of ketones and diketones **2***

In a 2.0 mL eppendorf tube, KRED (the same weight as the ketone), ketone (20 mM, except for ketones **2l**, 2**m**, and diketone **2s**), *i*-PrOH (150 μL) were added to 900 μL of 125 mM phosphate buffer, pH 7.0. This buffer also contains $MgSO_4$ (1.25 mM) and the cofactor $NADP^+$ (1.0 mM). For ketones **2l**, **2m**, and diketone **2s**, the reaction was conducted at 10 mM concentration. For ketones **2l-o,** and diketone **2s**, also DMSO (100 μL) was added. The resulting reaction mixture was shaken at 250 rpm and 30°C for 24 h, except for ketones **2l-o** and diketone **2s**, those reactions were carried out at 40°C. After this time, a 10 μL aliquot was removed by the determination of the degree of conversion by HPLC or GC analysis. Then, the mixture 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 diastereomeric ratio (if applicable) and enantiomeric excess of the corresponding alcohol was determined by chiral HPLC or GC.

3.4.2.4. Oxidation-bioreduction process: general procedure and characterization of optically active compounds.

For a better overview, the compounds included in this section are reported in the following Figure 3.4.2.4.1.

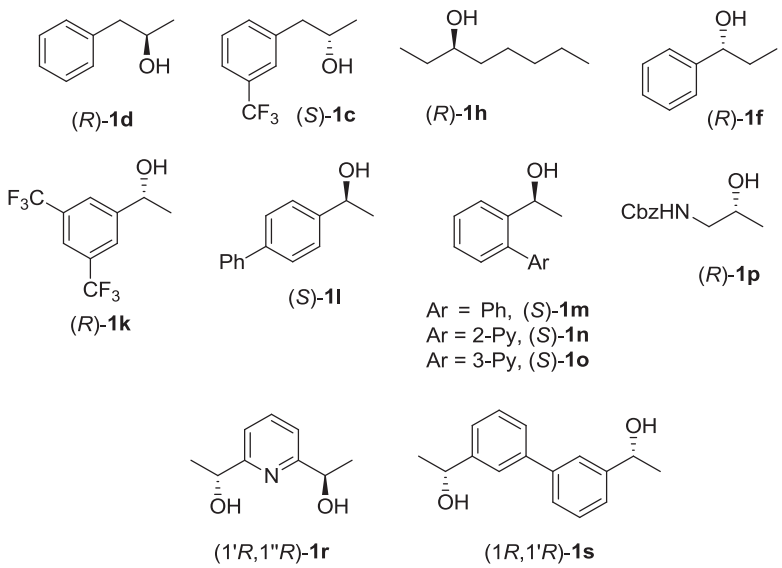


Fig. 3.4.2.4.1. Optically active compounds

3.4.2.4.1 General procedure for the one-pot two-step process at 100 mM

Reactions were carried out in a 2.0 mL eppendorf tube using 10.0 mg of the starting alcohol **1 (d, c, h, f,** and **n-r)**. For compound **1k** the reaction was conducted at 50.0 mg scale. Firstly, the oxidation was carried out. Once the oxidation was complete (1.5 h), isopropyl alcohol (15% v/v), 125 mM phosphate buffer at pH 7.0 (containing $MgSO_4$ 2.5 mM), the corresponding KRED (the same weight as the alcohol), and the cofactor $NADP^+$ (1.0 mM) were added for a final concentration of 100 mM. The reaction mixture was incubated during 24 h at 30 or 40°C and 250 rpm. After this time, the mixture was extracted with ethyl acetate (2 × 1.0 mL), the organic layers

separated by centrifugation (90 s, 13000 rpm), combined, and finally dried over Na_2SO_4. Evaporation of the solvent yielded the crude alcohol.

*3.4.2.4.1.1. (R)-1-Phenylpropan-2-ol **1d***

The typical procedure was applied to 10.0 mg (73 μmol) of the alcohol **1d** using KRED-P2-H07 as the catalyst for the bioreduction step. The crude alcohol was purified by flash column chromatography (hexane-ethyl acetate 5:1 as eluent) to give pure (*R*)-**1d** with 93% yield (9.3 mg); $[\alpha]_D^{25}$ -36.5 (*c* 0.47, $CHCl_3$), *ee* >99%. Lit.[157] for (*R*)-**1d**: $[\alpha]_D^{25}$ -35.4 (*c* 0.47, $CHCl_3$), *ee* >99%. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.25 (d, *J* 6.3 Hz, 3H), 1.72 (b s, 1H), 2.66-2.82 (2H, AB signals of an ABX system, $^3J_{A,X}$ 7.8 Hz, $^3J_{B,X}$ 5.0 Hz, and $|^2J_{A,B}|$ 13.4 Hz), 3.97-4.07 (m, 1H), 7.27-7.35 (m, 5H). ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ (ppm): 22.90 (CH_3), 45.90 (CH_2), 68.99 (CH), 126.59 (CH), 128.65 (CH), 129.51 (CH), 138.63 (C).

*3.4.2.4.1.2. (S)-{1-[3-(Trifluoromethyl)phenyl]}propan-2-ol **1c***

The typical procedure was applied to 10.0 mg (49 μmol) of the alcohol **1c** using KRED-P3-B03 as the catalyst for the bioreduction step. The crude alcohol was purified by flash column chromatography (hexane-ethyl acetate 5:1 as eluent) to give pure (*S*)-**1c** with 90% yield (9.0 mg); $[\alpha]_D^{25}$ +23.3 (*c* 0.45, EtOH), *ee* >99%. Lit.[158] for (*S*)-**1c**: $[\alpha]_D^{25}$ +24.5 (*c* 1.1, EtOH), *ee* >99%. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.25 (d, *J* 6.3 Hz, 3H), 1.61 (b s, 1H), 2.73-2.87 (2H, AB signals of an ABX system, $^3J_{A,X}$ 7.6 Hz, $^3J_{B,X}$ 5.1 Hz, and $|^2J_{A,B}|$ 13.6 Hz), 4.00-4.10 (m, 1H), 7.41-7.51 (m, 4H). ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ (ppm): 23.15 (CH_3), 45.50 (CH_2), 68.80 (CH), 123.46 (q, *J* 3.8 Hz, CH), 126.19 (q, *J* 3.8 Hz, CH), 124.34 (q, *J* 270.8 Hz, CF_3), 129.00 (CH), 130.90 (q, *J* 31.5 Hz, C), 132.97 (CH), 139.70 (C).

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[157] M. Veguillas, R. Solá, M. A. Fernández-Ibáñez, B. Maciá, *Tetrahedron: Asymmetry,* **2016**, *27*, 643–648.

[158] X. Wu, X. Li, A. Zanotti-Gerosa, A. Pettman, J. Liu, A. J. Mills, J. Xiao, *Chem. Eur. J.,* **2008**, *14*, 2209–2222.

*3.4.2.4.1.3. (R)-1-[3',5'-Bis(trifluoromethyl)phenyl]ethanol **1k***

The typical procedure was applied to 50.0 mg (194 μmol) of the alcohol **1k** using KRED-P1-B05 as the catalyst for the bioreduction step. The crude alcohol was purified by flash column chromatography (hexane-ethyl acetate 5:1 as eluent) to give pure (*R*)-**1k** with 98% yield (49.0 mg); $[\alpha]_D^{26}$ +24.3 (*c* 0.40, $CHCl_3$), *ee* >99%. Lit.[159] for (*R*)-**1k**: $[\alpha]_D^{20}$ +22.9 (*c* 1.0, $CHCl_3$), *ee* 99%. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.55 (d, *J* 6.6 Hz, 3H), 1.57 (b s, 1H), 5.01-5.08 (q, *J* 6.3 Hz, 1H), 7.79-7.84 (m, 3H). ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ (ppm): 25.72 (CH_3), 69.42 (CH), 121.45 (m, CH), 123.50 (q, *J* 270.7 Hz, CF_3), 125.78 (CH), 131.88 (q, *J* 33.2 Hz, C), 148.35 (C).

*3.4.2.4.1.4. (S)-1-[2-(2-Pyridyl)phenyl]ethanol **1n***

The typical procedure was followed using 10.0 mg (50 μmol) of the alcohol **1n** using KRED-P1-B05 as the catalyst for the bioreduction step. The crude alcohol was purified by flash column chromatography (hexane-ethyl acetate 2:1 as eluent) to give pure (*S*)-**1n** with 92% yield (9.2 mg); $[\alpha]_D^{26}$ –14.3 (*c* 0.40, $CHCl_3$), *ee* >99%. Lit.[147] for (*S*)-**1n**: $[\alpha]_D^{20}$ –6.4 (*c* 1.10, $CHCl_3$), *ee* 24%. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.50 (d, *J* 6.6 Hz, 3H), 4.72-4.79 (q, *J* 6.6 Hz, 1H), 7.31-7.48 (m, 4H), 7.57-7.62 (m, 2H), 7.83-7.89 (td, *J* 1.8 Hz (d), *J* 7.8 Hz (t), 1H), 8.62-8.65 (m, 1H). ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ (ppm): 20.54 (CH_3), 66.89 (CH), 122.33 (CH), 124.42 (CH), 126.63 (CH), 127.72 (CH), 129.34 (CH), 130.76 (CH), 137.75 (CH), 139.57 (C), 143.56 (C), 147.94 (CH), 159.93 (C).

*3.4.2.4.1.5. (S)-1-[2-(3-Pyridyl)phenyl]ethanol **1o***

The typical procedure was followed using 10.0 mg (50 μmol) of the alcohol **1o** using KRED-P1-B02 as the catalyst for the bioreduction step. The crude alcohol was purified by flash column chromatography (hexane-ethyl acetate 2:1 as eluent) to give pure (*S*)-**1o** with 90% yield (9.0 mg);

[159] R. Bigler, A. Mezzetti, *Org. Process Res. Dev.,* **2016**, *20*, 253–261.

$[\alpha]_D^{26}$ –25.2 (*c* 0.45, $CHCl_3$), *ee* >99%. Lit.[147] for (*S*)-**1o**: $[\alpha]_D^{20}$ –19.9 (*c* 1.83, $CHCl_3$), *ee* 67%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.39 (d, *J* 6.3 Hz, 3H), 2.76 (b s, 1H), 4.83-4.89 (q, *J* 6.3 Hz, 1H), 7.11-7.14 (dd, *J* 1.5 Hz, *J* 7.5 Hz, 1H), 7.29-7.35 (m, 2H), 7.42-7.47 [td, *J* 1.5 Hz (d), *J* 6.0 Hz (t), 1H], 7.60-7.64 [dt, *J* 2.1 Hz (t), *J* 7.8 Hz (d), 1H], 7.71-7.74 (dd, *J* 1.5 Hz, *J* 8.1 Hz, 1H), 8.40 (m, 1H), 8.45-8.47 (dd, *J* 1.8 Hz, *J* 5.1 Hz, 1H). ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ (ppm): 25.17 (CH_3), 66.09 (CH), 123.20 (CH), 126.00 (CH), 127.39 (CH), 128.95 (CH), 130.03 (CH), 136.26 (C), 136.88 (C), 137.01 (CH), 143.85 (C), 148.09 (CH), 149.58 (CH).

*3.4.2.4.1.6. Benzyl (R)-(2-hydroxypropyl)carbamate **1p***

The typical procedure was followed using 10.0 mg (48 μmol) of the alcohol **1p** using KRED-P1-A04 as the catalyst for the bioreduction step. The crude alcohol was purified by flash column chromatography (hexane-ethyl acetate 3:1 as eluent) to give pure (*R*)-**1p** with 90% yield (9.0 mg); $[\alpha]_D^{20}$ –21.1 (*c* 0.35, $CHCl_3$), *ee* >99%. Lit.[160] for (*R*)-**1p**: $[\alpha]_D^{20}$ –16.4 (*c* 1.04, $CHCl_3$), *ee* >99%. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.18 (d, *J* 6.3 Hz, 3H), 1.83 (b s, 1H), 3.04-3.10 (m, 1H), 3.30-3.38 (m, 1H), 3.91-3.94 (m, 1H), 5.10 (s, 2H), 5.18 (b s, 1H), 7.31-7.36 (m, 5H). ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ (ppm): 20.82 (CH_3), 48.41 (CH_2), 67.06 (CH_2), 67.56 (CH), 128.26 (CH), 128.31 (CH), 128.68 (CH), 136.51 (C), 157.27 (C=O).

*3.4.2.4.1.7. (1'R,1''R)-2,6-Bis(1-hydroxyethyl)pyridine **1r***

The typical procedure was applied to 10.0 mg (60 μmol) of the diol **1r** using KRED-P1-A04 as the catalyst for the bioreduction step. The crude alcohol was purified by flash column chromatography (ethyl acetate as eluent) to give pure (*R,R*)-**1r** with 95% yield (9.5 mg); $[\alpha]_D^{20}$ +22.2 (*c* 0.50, $CHCl_3$), *dr* >99:<1, *ee* >99%. Lit.[161] for (*S,S*)-**1r**: $[\alpha]_D^{29}$ –25.0 (*c* 1.0, $CHCl_3$), *ee* >99%. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.51 (d, *J* 6.6 Hz,

[160] C. Cassani, G. Bergonzini, C. J. Wallentin, *Org. Lett.,* **2014**, *16*, 4228–4231.
[161] J. Uenishi, S. Aburatani, T. Takami, *J. Org. Chem.,* **2007**, *72*, 132–138.

6H), 3.62 (b s, 2H), 4.87-4.94 (q, *J* 6.6 Hz, 2H), 7.20-7.22 (d, *J* 7.8 Hz, 2H), 7.70 (t, *J* 7.5 Hz, 1H). ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ (ppm): 24.27 (CH_3), 69.29 (CH), 118.52 (CH), 137.94 (CH), 162.11 (C).

3.4.2.4.2. General procedure for the one-pot two-step process at 50 mM

Reactions were carried out in a 2.0 mL eppendorf tube using 10.0 mg of the starting alcohol **1**. Firstly, the oxidation was carried out following the general procedure. Once the oxidation was complete (1.5 h), isopropyl alcohol (15% v/v), 125 mM phosphate buffer at pH 7.0 (containing $MgSO_4$ 2.5 mM), the corresponding KRED (10.0 mg), and the cofactor $NADP^+$ (0.5 mM) were added for a final concentration of 50 mM. The reaction mixture was incubated during 24 h at 30°C and 250 rpm. After this time, the described work-up was followed.

*3.4.2.4.2.1. (R)-3-Octanol **1h***

The typical procedure was applied to 10.0 mg (77 μmol) of the alcohol **1h** using KRED-P1-A04 as the catalyst for the bioreduction step. The crude alcohol was purified by flash column chromatography (hexane-ethyl acetate 3:1 as eluent) to give pure (*R*)-**1h** with 87% yield (8.7 mg); $[\alpha]_D^{20}$ –10.2 (*c* 0.47, $CHCl_3$), *ee* >99%. Lit.[53] for (*R*)-**1h**: $[\alpha]_D^{25}$ –10.2 (*c* 0.50, $CHCl_3$), *ee* >99%. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 0.86-0.95 (m, 6H), 1.27-1.54 (m, 11H), 3.47-3.55 (m, 1H). ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ (ppm): 10.00 (CH_3), 14.18 (CH_3), 22.79, 25.47, 30.26, 32.06, 37.04 (CH_2), 73.46 (CH).

*3.4.2.4.2.2. (R)-1-Phenylpropan-1-ol **1f***

The typical procedure was applied to 10.0 mg (74 μmol) of the alcohol **1f** using KRED-P1-A04 as the catalyst for the bioreduction step. The crude alcohol was purified by flash column chromatography (hexane-ethyl acetate 5:1 as eluent) to give pure (*R*)-**1f** with 92% yield (9.2 mg); $[\alpha]_D^{20}$ +4.78 (*c* 0.60, $CHCl_3$), *ee* >99%. Lit.[53] for (*R*)-**1f**: $[\alpha]_D^{20}$ +4.90 (*c*

0.50, $CHCl_3$), *ee* >99%. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 0.92 (t, *J* 7.2 Hz 3H), 1.68-1.88 (m, 3H), 4.58-4.62 (m, 1H), 7.27-7.35 (m, 5H). ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ (ppm): 10.29 (CH_3), 32.03 (CH_2), 76.18 (CH), 126.10 (CH), 127.64 (CH), 128.54 (CH), 144.71 (C).

*3.4.2.4.2.3. (S)-2-Azido-1-phenylethanol **1q***

The typical procedure was applied to 10.0 mg (61 μmol) of the alcohol **1q** using KRED-P1-A04 as the catalyst for the bioreduction step. The crude alcohol was purified by flash column chromatography (hexane-ethyl acetate 8:1 as eluent) to give pure (*S*)-**1q** with 90% yield (9.0 mg); $[\alpha]_D^{19}$ +80.1 (*c* 0.30, $CHCl_3$), *ee* >99%. Lit.[162] for (*R*)-**1q**: $[\alpha]_D^{25}$ -80.1 (*c* 1.0, $CHCl_3$), *ee* >99%. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.68 (b s, 1H), 3.41-3.53 (m, 2H), 4.87-4.91 (dd, *J* 4.2 Hz, *J* 7.5 Hz, 1H), 7.35-7.39 (m, 5H). ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ (ppm): 58.25 (CH_2), 73.58 (CH), 126.05 (CH), 128.53 (CH), 128.85 (CH), 140.67 (C).

3.4.2.4.3. General procedure for the one-pot two-step process at 25 mM

Reactions were carried out in a 15.0 mL falcon centrifuge tube using 10.0 mg of the starting alcohol **1**. Once the oxidation was complete (1.5 h), DMSO (10 % v/v), isopropyl alcohol (15 % v/v), 125 mM phosphate buffer at pH 7.0 (containing $MgSO_4$ 2.5 mM), the corresponding KRED (10.0 mg), and the cofactor $NADP^+$ (0.5 mM) were added for a final concentration of 25 mM. The reaction mixture was incubated during 24 h at 40°C and 250 rpm. After this time, the mixture was extracted with ethyl acetate (2 × 2.0 mL), the organic layers separated by centrifugation (90 s, 13000 rpm), combined, washed with brine (2 × 2.0 mL) and finally dried over Na_2SO_4. Evaporation of the solvent yielded the crude alcohol.

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[162] C. Aguirre-Pranzoni, F. R. Bisogno, A. A. Orden, M. Kurina-Sanza, *J. Mol. Catal. B: Enzym.*, **2015**, *114*, 19–24.

*3.4.2.4.3.1. (S)-1-(Biphenyl-4-yl)ethanol **1l***

The typical procedure was applied to 10.0 mg (50 μmol) of the alcohol **1l** using KRED-P3-B03 as the catalyst for the bioreduction step. The crude alcohol was purified by flash column chromatography (hexane-ethyl acetate 5:1 as eluent) to give pure (*S*)-**1l** with 90% yield (9.0 mg); $[\alpha]_D^{22}$ –43.3 (*c* 0.50, $CHCl_3$), *ee* >99%. Lit.[147] for (*S*)-**1l**: $[\alpha]_D^{20}$ –44.1 (*c* 0.97, $CHCl_3$), *ee* >99%. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.55 (d, *J* 6.6 Hz, 3H), 1.87 (b s, 1H), 4.93-4.99 (q, *J* 6.3 Hz, 1H), 7.33-7.38 (m, 1H), 7.43-7.48 (m, 4H), 7.58-7.62 (m, 4H). ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ (ppm): 25.29 (CH_3), 70.30 (CH), 125.98 (CH), 127.21 (CH), 127.39 (CH), 128.90 (CH), 140.58 (C), 140.98 (C), 144.94 (C).

*3.4.2.4.3.2. (S)-1-(Biphenyl-2-yl)ethanol **1m***

The typical procedure was applied to 10.0 mg (50 μmol) of the alcohol **1m** using KRED-P1-B05 as the catalyst for the bioreduction step. The crude alcohol was purified by flash column chromatography (hexane-ethyl acetate 5:1 as eluent) to give pure (*S*)-**1m** with 92% yield (9.2 mg); $[\alpha]_D^{22}$ –30.3 (*c* 0.50, $CHCl_3$), *ee* >99%. Lit.[147] for (*S*)-**1m**: $[\alpha]_D^{20}$ –26.5 (*c* 1.00, $CHCl_3$), *ee* 45%. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.41 (d, *J* 6.3 Hz, 3H), 1.68 (b s, 1H), 4.95-5.02 (q, *J* 6.3 Hz, 1H), 7.19-7.22 (dd, *J* 1.5 Hz, *J* 7.5 Hz, 1H), 7.29-7.45 (m, 7H), 7.66-7.69 (dd, *J* 1.5 Hz, *J* 7.8 Hz, 1H). ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ (ppm): 25.04 (CH_3), 66.61 (CH), 125.48 (CH), 127.26 (CH), 128.13 (CH), 128.30 (CH), 129.40 (CH), 130.09 (CH), 140.48 (C), 141.04 (C), 143.18 (C).

*3.4.2.4.3.3. (1R,1'R)-1,1'-[(1,1'-biphenyl)-3,3'-diyl]diethanol **1s***

The typical procedure was applied to 10.0 mg (41 μmol) of the diol **1s** using KRED-P1-A12 as the catalyst. The crude alcohol was purified by flash column chromatography (hexane-ethyl acetate 3:1 as eluent) to give pure (*R,R*)-**1s** with 90% yield (9.0 mg); $[\alpha]_D^{19}$ +60.4 (*c* 0.42, $CHCl_3$), *dr*

>99:<1, *ee* >99%. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.55 (d, *J* 6.3 Hz, 6H), 1.85 (b s, 2H), 4.94-5.01 (q, *J* 6.6 Hz, 1H), 7.35-7.37 (m, 2H), 7.43 (t, *J* 7.5 Hz, 2H), 7.49-7.53 [dt, *J* 1.5 Hz (t), *J* 7.5 Hz (d), 2H], 7.61 (m, 2H). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ (ppm): 25.44 (CH_3), 70.62 (CH), 124.40 (CH), 124.55 (CH), 126.49 (CH), 129.10 (CH), 141.51 (C), 146.52 (C).

3.4.2.5. HPLC and CG analytical data for C, dr and ee determination

3.4.2.5.1. Reactions involving alcohols ***1d, 1c, 1h, 1f, 1p, and 1q***

3.4.2.5.1.1 Methods and analytical data for the determination of the degree of conversion (C) of the KRED-catalyzed reactions and sequential oxidation-reduction processes

HPLC Method:

HPLC analyses were carried out using a reversed phase column (Zorbax Eclipse XDB-C18, RR, 18μm, 4.6 x 50 mm, Agilent) with acetonitrile and water (0.1% TFA) 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 2.0 ml/min. Detection of peaks (UV absorption) was performed at 220 and 324 nm.

GC Method:

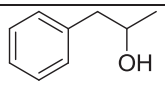
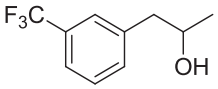
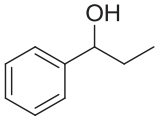
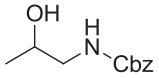
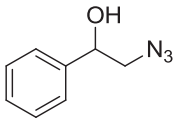
RT®-βdexe (30m × 0.25 mm × 0.25μm, 12.2 psi N_2). Program: 90/5/2.5/105/10/160/20/180 [initial T (ºC) / time (min) / ramp (ºC/min) / T (°C) / ramp (°C/min) / T (°C) / ramp (°C/min) / final T (ºC)].

Table 3.4.2.5.1.1.1. Analytical data for determination of *C*

Method	Retention time (t_R, min)			
	Alcohol	t_R	**ketone**	t_R
HPLC	**1d**	2.9	**2d**	3.2
HPLC	**1c**	4.5	**2c**	4.7
GC	**1h**	10.2	**2h**	8.2
HPLC	**1f**	3.1	**2f**	4.0
HPLC	**1p**	2.7	**2p**	3.0
HPLC	**1q**	3.2	**2q**	4.0

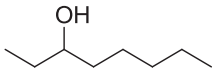
3.4.2.5.1.2 HPLC and GC analytical data for ee determinations

Table 3.4.2.5.1.2.1. HPLC analyses data

Alcohol	Conditions[a]	Retention time (min)
 **1d**	Chiralpak AD-H, Hexane:IPA 99:1, 40 ºC, 0.8 mL/min	10.89 (*S*), 11.58 (*R*); R_S = 1.5
 **1c**	Chiralpak AD-H, Hexane:IPA 99:1, 40 ºC, 0.8 mL/min	11.14 (*S*), 11.80 (*R*); R_S = 1.1
 **1f**	Chiralcel OJ-H, Hexane:IPA 90:10, 40 ºC, 0.8 mL/min	7.60 (*S*), 8.00 (*R*); *R*s = 2.8
 **1p**	Chiralpak ID, Hexane:IPA 90:10, 40 ºC, 0.8 mL/min	17.59 (*S*), 18.41 (*R*); R_S = 3.4
 **1q**	Chiralcel OJ-H, Hexane:IPA 90:10, 30°C 0.8mL/min	13.75 (*R*), 15.77 (*S*); *R*s = 6.4

[a] Detection of peaks (UV absorption) was performed at λ = 216 nm.

Table 3.4.2.5.1.2.2. GC analyses data for compound 1h

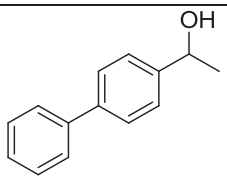
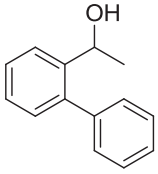
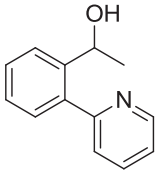
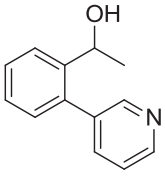
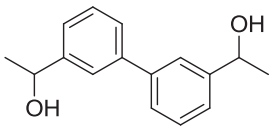
Alcohol	Conditions (for the acetyl derivative)	Retention time (min) (for the acetyl derivative)
 **1h**[a]	RT-β-dexe 90/5/2.5/105/8/20/180	11.4 (*S*), 12.4 (*R*)

[a] Previously this alcohol was derivatised with acetic anhydride.

*3.4.2.5.2. Reactions involving alcohols **1k-o, 1r,and 1s**. HPLC and GC analytical data for C, dr (if applicable) and ee determination*

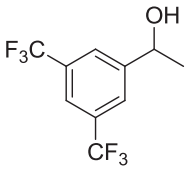
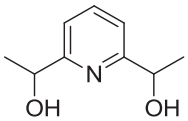
Conversion degree, *dr* (if applicable), and *ee* have been determinated in the same analysis. In all cases, the factor (*f*) for area normalization was calculated. The conversion degree is then calculated applying this factor *f* to the area of the ketone peak.

Table 3.4.2.5.2.1. HPLC analyses data

Alcohol	Conditions[a]	Retention time (min)
 **1l**	Chiralcel OD, Hexane:IPA 95:5, 25 ºC, 0.8 mL/min	14.97 (*S*), 16.93 (*R*); R_S = 7.4 Ketone = 9.52 *f* = 1.17
 **1m**	Chiralcel OD, Hexane:IPA 99:1, 20 ºC, 0.8 mL/min	12.11 (*S*), 13.75 (*R*); R_S = 5.1 Ketone = 8.58 *f* = 1.07
 **1n**	Chiralcel OD, Hexane:IPA 90:10, 20 ºC, 0.8 mL/min	10.24 (*S*), 14.32 (*R*); R_S = 18 Ketone = 28.56 *f* = 1.08
 **1o**	Chiralcel OD, Hexane:IPA 90:10, 20 ºC, 0.8 mL/min	9.70 (*S*), 12.28 (*R*); R_S = 7.4 Ketone = 17.71 *f* = 1.10
 **1s**	Chiralcel OD, Hexane:IPA 75:25, 20 ºC, 0.8 mL/min	7.85 (*R*,*R*), 9.96 (*S*,*S*)[b] 14.40 (*meso*). Ketone = 11.31 *f* = 1.44

[a] Detection of peaks (UV adsorption) at λ = 216 nm. [b] *R*s = 9.3 (for the racemic mixture).

Table 3.4.2.5.2.2. GC analyses data

Alcohol	Conditions	Retention time (min)
 **1e**	RT-β-dexe 100/0/1/118/20/180[a]	14.63 (*S*), 16.02 (*R*) Ketone = 5.81 *f* = 2.01
 **1l**	CP-ChiraSil 90/5/3/160/2/3/165/20/180[b]	27.63 (*S*,*S*), 29.39 (*R*,*R*); 28.39 (*meso*) Ketone = 17.91 *f* = 1.50

Temperature program: [a] (initial T (ºC) / time (min) / ramp (ºC/min) / T (ºC) / ramp (°C/min) / final T (°C); [b] (initial T (ºC) / time (min) / ramp (ºC/min) / T (ºC) / time (min) / ramp (°C/min) / T (°C) / ramp (°C/min) / final T (°C).

3.4.2.6. Assignment of the absolute configuration to the optically active alcohols

The absolute configuration of the isolated optically active alcohols was assigned after comparison of the sign of the optical rotation of each sample with reported data. In addition, the absolute configuration of optically active alcohols was assigned by comparison of the chiral-HPLC chromatogram with that obtained from a commercially available optically active sample (**1d**) or by comparison with the previously published chiral-HPLC chromatogram (**1h, 1f**,[53] **1l-o**[147]). In all cases these data were in good agreement with the stereopreference indicated in the specifications of the used KREDs.

For alcohol **1s**, since the optical rotation was not reported in literature, the sign was compared with the unreacted alcohol of a lipase-catalyzed reaction employing CAL-B, whose stereopreference is known to be (*R*).

3.4.2.7. CD Support

In the CD support, detailed additional information about the experimental of this Chapter is included. A complete section of enzymatic screening, copy of GC-HPLC chromatograms and NMR spectra for all the synthesized optically pure compounds will be found.

Conclusions

In conclusion, different multi-catalytic methodologies have been developed for the efficient synthesis of optically pure building blocks. The efficiency of these processes relies on the possibility to perform the reactions in a one-pot concurrent or sequential fashion.

In Chapter 1, purified enzymes as ketoreductases and whole cells of *Rhodococcus rhodochrous* have been used in a genuine cascade process for the synthesis of optically pure 2-hydroxycycloalkanecarboxylic acids. In all the cases, the final compounds have been obtained with excellent enantiomeric excess, excellent diastereomeric ratio and yield. A new protocol for the synthesis of optically pure 2-hydroxycyclohexanecarboxyamide has also been determined, but an incomplete inhibition of the amidase activity resulted in a detraction of the final yield.

In order to overcome this limitation, in Chapter 2 we developed a multi-catalytic methodology for the synthesis of optically pure β-hydroxy amides starting from easily accessible and commercially available β-ketonitriles. Thus, the tandem action of a Ru(IV) catalyst and ketoreductases allowed the synthesis of the desired compounds in excellent yields and enantiomeric excesses. Also β-ketonitriles bearing a chiral centre in the α-position have been investigated and an efficient dynamic reductive kinetic resolution yielded the final products with very high diastereomeric ratios.

This contribution represents a valid example of compatibility between metals and enzymes in water, without need of separation or compartmentalization.

Finally, in Chapter 3, new hybrid methodologies for the synthesis of interesting amino alcohols, amines, alcohols and diols have been investigated. The core of this project was the development of a NaOCl/AZADO-catalyzed unselective oxidation in water for the quantitative conversion of easily available racemic alcohols into prochiral ketones. Thus, once the prochiral intermediate was obtained, the subsequent action of a transaminase or a ketoreductase allowed the synthesis of optically active amines or alcohols, respectively, with excellent enantiomeric excess, yields and diastereomeric ratios (when applicable).

To conclude, this Thesis underlines the importance of biocatalysis for the synthesis of optically pure building blocks for pharmaceuticals. Additionally, as more and more different types of enzymes become available, more cascades become feasible, leading to an exponential growth of cascade designs and synthesis of optically pure molecules.

Conclusiones

En conclusión, diferentes estrategias multicatalíticas han permitido la síntesis de compuestos ópticamente activos. La eficiencia de estos procesos se basa en la posibilidad de realizar las reacciones de manera concurrente o secuencial.

En el primer capítulo, enzimas purificadas como cetoreductasas y células enteras de *Rhodococcus rhodochrous* se han utilizado en un proceso de cascada concurrente para la síntesis de ácidos 2-hidroxicicloalcanocarboxílicos con excelente exceso enantiomérico, relación diastereomérica y rendimiento. También se ha desarrollado un nuevo protocolo para la síntesis de 2-hidroxiciclohexanocarboxamida enantioméricamente pura, cuyo rendimiento final resultó empobrecido debido a una inhibición incompleta de la actividad de la amidasa del microorganismo.

Para superar esta limitación, en el capítulo 2 se ha desarrollado una metodología multicatalítica para la síntesis de 2-hidroxiamidas a partir de β-cetonitrilos fácilmente accesibles y disponibles comercialmente. Así, la acción en tándem de un catalizador de Ru(IV) y cetoreductasas permitió la síntesis de los compuestos deseados con redimientos excelentes y muy altos excesos enantioméricos. También se han estudiado β-cetonitrilos con un centro quiral en la posición α y, como consecuencia de una eficiente resolución cinética dinámica, los productos finales se han obtenido con

relaciones distereoméricas muy altas. Esta contribución representa un ejemplo válido de compatibilidad entre metales y enzimas en agua, sin necesidad de separación o compartimentación.

Finalmente, en el capítulo 3, se han descrito nuevas metodologías híbridas para la síntesis de amino alcoholes, aminas, alcoholes y dioles. El núcleo de este proyecto ha sido el desarrollo de una oxidación no selectiva catalizada por NaOCl/AZADO en medio acuoso para la obtención de cetonas prochirales. De este modo, una vez obtenido el intermedio, la acción subsiguiente de una transaminasa o una cetoreductasa permitió la síntesis de aminas o alocholes ópticamente activos con rendimientos, excesos enantioméricos y relaciones diastereoméricas excelentes.

Para concluir, esta tesis subraya la importancia de la biocatálisis para la síntesis de intermedios farmacéuticos. Además, con el descubrimiento de nuevos tipos de enzimas, más cascadas se vuelven factibles, lo que lleva a un crecimiento exponencial de los diseños en cascada y de la síntesis de compuestos ópticamente activos.

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